

*Original Article*

## **Difference in the homocysteine-lowering effect of folic acid in haemodialysis patients with and without occlusive vascular disease**

Eric Descombes<sup>1</sup>, Olivier Boulat<sup>2</sup>, Louis-Félix Bersier<sup>3</sup> and Gilbert Fellay<sup>1</sup>

<sup>1</sup>Dialysis Unit, Department of Internal Medicine, Hôpital Cantonal, Fribourg, <sup>2</sup>Clinical Chemistry Laboratory, University Hospital, Lausanne and <sup>3</sup>Institute of Zoology, University of Neuchâtel, Neuchâtel, Switzerland

### **Abstract**

**Background.** Hyperhomocysteinaemia has been identified as an independent cardiovascular risk factor and is found in more than 85% of patients on maintenance haemodialysis. Previous studies have shown that folic acid can lower circulating homocysteine in dialysis patients. We evaluated prospectively the effect of increasing the folic acid dosage from 1 to 6 mg per dialysis on plasma total homocysteine levels of haemodialysis patients with and without a history of occlusive vascular artery disease (OVD).

**Methods.** Thirty-nine stable patients on high-flux dialysis were studied. Their mean age was  $63 \pm 11$  years and 17 (43%) had a history of OVD, either coronary and/or cerebral and/or peripheral occlusive disease. For several years prior to the study, the patients had received an oral post-dialysis multivitamin supplement including 1 mg of folic acid per dialysis. After baseline determinations, the folic acid dose was increased from 1 to 6 mg/dialysis for 3 months.

**Results.** After 3 months, plasma homocysteine had decreased significantly by  $\approx 23\%$  from  $31.1 \pm 12.7$  to  $24.5 \pm 9$   $\mu\text{mol/l}$  ( $P=0.0005$ ), while folic acid concentrations had increased from  $6.5 \pm 2.5$  to  $14.4 \pm 2.5$   $\mu\text{g/l}$  ( $P<0.0001$ ). However, the decrease of homocysteine was quite different in patients with and in those without OVD. In patients with OVD, homocysteine decreased only marginally by  $\approx 2.5\%$  (from  $29.0 \pm 10.3$  to  $28.3 \pm 8.4$   $\mu\text{mol/l}$ ,  $P=0.74$ ), whereas in patients without OVD there was a significant reduction of  $\approx 34\%$  (from  $32.7 \pm 14.4$  to  $21.6 \pm 8.6$   $\mu\text{mol/l}$ ,  $P=0.0008$ ). Plasma homocysteine levels were reduced by  $>15\%$  in three patients (18%) in the group with OVD compared with 19 (86%) in the group without OVD ( $P=0.001$ ), and by  $>30\%$  in none of the patients (0%)

in the former group compared with 13 (59%) in the latter ( $P=0.001$ ).

**Conclusions.** These results indicate that the homocysteine-lowering effect of folic acid administration appears to be less effective in haemodialysis patients having occlusive vascular disease than in those without evidence of such disease.

**Keywords:** cardiovascular disease; folic acid; haemodialysis; homocysteine; vitamins

### **Introduction**

In recent years, several studies have shown that hyperhomocysteinaemia is an independent cardiovascular risk factor in patients both with and without renal failure [1–5]. Hyperhomocysteinaemia seems to promote atherosclerosis by endothelial dysfunction and injury, followed by platelet activation and thrombus formation [1]. According to previous reports, more than 85% of patients on maintenance haemodialysis (HD) have a slight-to-moderate hyperhomocysteinaemia [2–13]. A few studies have suggested that folic acid administration, either alone or together with vitamins B<sub>6</sub> or B<sub>12</sub>, can lower homocysteine in dialysis patients [2–7]. As cardiovascular disease is a major cause of morbidity and mortality among HD patients, a reduction in homocysteine concentrations may have an important potential benefit [2–5].

For several years the patients dialysed at our centre have received a low-dose supplement of 1 mg of folic acid per dialysis, which corresponds roughly to the recommended daily allowance (RDA). The purpose of the present study was to evaluate prospectively the effect of an increase of the folic acid supplement from 1 to 6 mg per dialysis on plasma total homocysteine

Correspondence and offprint requests to: Dr med. Eric Descombes, Dialysis Unit, Department of Internal Medicine, Hôpital Cantonal, CH-1700 Fribourg, Switzerland.

(tHcy) levels in patients with and without a history of occlusive vascular artery disease (OVD).

## Patients and methods

All the stable chronic HD patients attending our centre who gave their informed consent were included. Thirty-nine Caucasian patients were evaluated. The mean age was  $63 \pm 11$  years and they had been on dialysis for  $5 \pm 5$  years. The causes of renal failure were chronic glomerulonephritis ( $n=9$ ), analgesic nephropathy ( $n=7$ ), polycystic kidney disease ( $n=7$ ), hypertensive nephropathy ( $n=4$ ), diabetic nephropathy ( $n=3$ ), and miscellaneous ( $n=9$ ). They were dialysed by means of short high-efficiency HD with high-flux hollow-fibre dialysers having surface areas ranging from 1.1 to 1.9 m<sup>2</sup>. For several years prior to the study, all the patients had received an oral post-dialysis multivitamin supplement containing folic acid 1 mg, thiamine hydrochloride 100 mg, riboflavin 20 mg, pyridoxine hydrochloride 50 mg, and ascorbic acid 500 mg (Dialvit<sup>®</sup>, Bichsel AG, Interlaken, Switzerland). These vitamins were given at the end of dialysis by the nurses and thus complete compliance was ensured.

Seventeen of the 39 patients (43%) had a history of OVD. Thirteen patients had coronary artery disease with either a history of myocardial infarction ( $n=4$ ) or of angina with diagnostic coronarography ( $n=8$ ) or thallium scintigraphy ( $n=1$ ). Thirteen patients had a history of peripheral occlusive artery disease, confirmed by arteriography ( $n=10$ ) or Doppler examination ( $n=3$ ). Two patients had a history of non-embolic ischaemic stroke with diagnostic confirmation by cerebral CT scanning. Nine patients had a combination of at least two form of OVD.

The other 22 patients had no symptoms of OVD. In 13 of them a complete pre-transplantation work-up was normal (including normal coronarography in four, normal thallium scintigraphy in four, and normal treadmill test in five). Of the remaining nine patients (including one diabetic), none had symptoms or signs of OVD. Strictly speaking, we cannot exclude that one of these latter patients may have asymptomatic OVD. However, none of them developed symptoms or

signs of OVD during the year following the study (or until death).

Baseline plasma tHcy was determined while patients were receiving the multivitamin supplement indicated above. Thereafter, the dose of folic acid was increased from 1 to 6 mg per dialysis (by adding 1 tablet of 'acidum folicum' 5 mg per dialysis) and a new determination of homocysteine was made 3 months later. For homocysteine determinations, pre-dialysis blood samples were centrifuged within 15 min after being drawn and the plasma (EDTA) was stored at  $-70^{\circ}\text{C}$  until analysis. Total homocysteine was determined by high-performance liquid chromatography with fluorimetric detection according to Verster and Rasmussen [14] by the Amino Acid Laboratory of the University Hospital of Lausanne (Switzerland). It is to be noted that not all the patients underwent fasting homocysteine determinations. However, Hultberg *et al.* [10] showed that there are no significant changes of the homocysteine levels during the day in HD patients, even after a meat-rich meal. Pre-dialysis determinations of plasma folic acid and vitamin B<sub>12</sub> and of the pyridoxine-dependent erythrocyte glutamate-oxaloacetate transaminase activity (EGOT<sub>o</sub> and alpha-EGOT index) were also performed as previously described [15].

Results are given as mean  $\pm$  standard deviation (SD). Paired data were compared with the Wilcoxon signed-rank test, unpaired data with the Mann-Whitney U test, and categorical data with the chi-square test. Significance was considered at  $P < 0.05$ .

## Results

Table 1 compares the clinical and laboratory characteristics in patients with and without OVD. The patients with a history of OVD were significantly older (by an average of 10 years) and tended to have been on dialysis for a longer period of time. Traditional cardiovascular risk factors (hypertension, smoking, hyperlipidaemia, obesity, and diabetes) tended to be more frequent in the group with OVD but without reaching

**Table 1.** Baseline characteristics of the groups with (OVD+) and without (OVD-) occlusive vascular disease

	OVD+ <i>n</i> = 17	OVD- <i>n</i> = 22	<i>P</i> value
Age (years)	68 $\pm$ 8	58 $\pm$ 11	0.01
Time on dialysis (months)	52 $\pm$ 68	46 $\pm$ 65	0.13
Gender (male), <i>n</i> (%)	11 (65)	12 (54)	0.45
Hypertension, <i>n</i> (%)	16 (94)	16 (73)	0.07
Ever smokers, <i>n</i> (%)	12 (71)	11 (50)	0.18
Present smokers, <i>n</i> (%)	3 (18)	6 (27)	0.52
Obesity, <i>n</i> (%)	5 (29)	3 (14)	0.17
Diabetes mellitus, <i>n</i> (%)	2 (12)	2 (9)	0.85
On lipid-lowering therapy, <i>n</i> (%)	4 (24)	2 (9)	0.18
Total cholesterol (mmol/l)	5.1 $\pm$ 1.3	5.0 $\pm$ 1.4	0.77
Triglycerides (mmol/l)	2.2 $\pm$ 0.86	2.0 $\pm$ 0.81	0.49
HDL cholesterol (mmol/l)	0.97 $\pm$ 0.35	1.05 $\pm$ 0.42	0.71
LDL cholesterol (mmol/l)	3.1 $\pm$ 1.2	3.0 $\pm$ 1.2	0.88
Total/HDL cholesterol > 5, <i>n</i> (%)	10 (59)	10 (45)	0.40
Folic acid (normal > 3.0 $\mu\text{g/l}$ )	6.1 $\pm$ 1.2	6.8 $\pm$ 3.2	0.95
Vitamin B <sub>12</sub> (normal > 200 ng/l)	424 $\pm$ 134	416 $\pm$ 197	0.52
EGOT <sub>o</sub> (normal > 228 U/l)	381 $\pm$ 114	351 $\pm$ 93	0.60
alpha-EGOT index (normal < 1.8)	1.33 $\pm$ 0.13	1.44 $\pm$ 0.29	0.29

statistical significance, perhaps due to the small size of the groups considered. However, on average, the patients with OVD had significantly more cardiovascular risk factors than those without ( $2.5 \pm 0.8$  risk factors per patient *vs*  $1.7 \pm 1.0$ ,  $P=0.033$ ). Thus, greater age and the higher prevalence of traditional risk factors can, to some extent, explain why the patients of the OVD-positive group developed cardiovascular disease while having similar pre-study plasma tHcy levels to those who did not (see Table 2). Table 1 also shows that the baseline levels of folic acid, vitamin B<sub>12</sub>, and pyridoxine-dependent EGOT activity were similar in the two groups.

Table 2 shows the homocysteine and folic acid levels at baseline and at month 3. At baseline, the mean tHcy among all patients was  $31.1 \pm 12.7$   $\mu\text{mol/l}$  and only one patient (3%) had normal values ( $<15$   $\mu\text{mol/l}$ ). At baseline the OVD-negative group had slightly higher mean tHcy levels and this was partly related to the presence in this group of the only patient having very high tHcy levels ( $=84.1$   $\mu\text{mol/l}$ ). After the 3 months during which the folic acid dose was increased from 1 to 6 mg/dialysis, tHcy significantly decreased by  $\approx 23\%$ , resulting in normal values in seven patients of 39 (18%). However, if we compare the patients with and without OVD, the reduction in tHcy levels was quite different. Homocysteine decreased only marginally in patients with OVD, by an average of  $\approx 2.5\%$ . On the other hand, tHcy decreased significantly by an average of  $\approx 34\%$  in the group without OVD. Table 3 shows that the homocysteine levels were reduced by  $>15\%$  in only three patients (18%) in the group with OVD compared with 19 (86%) in the group without ( $P=0.001$ ), and by  $>30\%$  in none of the patients (0%) in the former group compared with 13 (59%) in the latter ( $P=0.001$ ). At month 3, the tHcy levels were significantly lower in the group without OVD, compared with the group with OVD ( $P=0.008$ ) (Table 2), and six patients (27%) in the former group had normal tHcy levels compared with only one (6%) in the latter ( $P=0.08$ ).

These data indicate that the lowering effect of folic acid on tHcy levels is much less in patients with OVD. One may question, however, whether this may be due to a confounding factor, for example the presence of a cardiovascular risk factor rather than to the presence of cardiovascular disease *per se*. To explore the potential confounding role of factors other than the presence of OVD in the response to folic acid treatment, we compared by one-way analysis of variance (ANOVA) the relative reduction of tHcy levels during treatment ( $(\text{pre-treatment} - \text{post-treatment}) / \text{pre-treatment tHcy levels}$ ) with different potential confounders including age, gender, traditional cardiovascular risk factors, pre-study tHcy, and vitamins levels. The results of this analysis are reported in Table 4, which shows that among the parameters tested the only one significantly linked to the response to folic acid administration is the presence of cardiovascular disease ( $P=0.0002$ ). It should be noted that age, which was significantly different in our two study groups (Table 1), seems not

**Table 2.** Change of plasma total homocysteine (tHcy) and folic acid (FA) levels overall and in the groups with (OVD+) and without (OVD-) occlusive vascular disease

		Baseline	Month 3	P value
Over all (n = 39)	tHcy ( $\mu\text{mol/l}$ )	$31.1 \pm 12.7$	$24.5 \pm 9$	0.0005
	FA ( $\mu\text{g/l}$ )	$6.5 \pm 2.5$	$14.4 \pm 2.5$	$<0.0001$
OVD+ (n = 17)	tHcy ( $\mu\text{mol/l}$ )	$29.0 \pm 10.3$	$28.3 \pm 8.4$	0.74
	FA ( $\mu\text{g/l}$ )	$6.1 \pm 1.2$	$14.1 \pm 3.2$	0.0004
OVD- (n = 22)	tHcy ( $\mu\text{mol/l}$ )	$32.7 \pm 14.4^*$	$21.6 \pm 8.6^{**}$	0.0008
	FA ( $\mu\text{g/l}$ )	$6.8 \pm 3.2$	$14.6 \pm 1.8$	0.0001

\* $P=0.63$  compared to the OVD+ group at baseline; \*\* $P=0.008$  compared to the OVD+ group at month 3.

**Table 3.** Distribution of the percentage reduction of the tHcy levels in patients with (OVD+) and without (OVD-) occlusive vascular disease

Reduction of tHcy (%)	$>15$	$>30$	$>50$
OVD+ (n = 17) (%)	3 (18)	0	0
OVD- (n = 22) (%)	19 (86)	13 (59)	4 (18)

**Table 4.** Statistical analysis of the treatment response in respect to different clinical and biological pre-treatment parameters

Parameter	P value
Age	0.662
Gender	0.376
Hypertension	0.111
Smoking history	0.365
Hyperlipidaemia	0.401
Obesity	0.829
Cardiovascular disease	0.0002
Pre-treatment tHcy	0.193
Pre-treatment folic acid	0.653
Pre-treatment vitamin B <sub>12</sub>	0.145
Pre-treatment EGOT <sub>0</sub>	0.924
Pre-treatment alpha-EGOT index	0.773

to be a significant determinant of the response to folic acid treatment.

## Discussion

In the last decade a large amount of data has supported the view that hyperhomocysteinaemia is an independent risk factor for cardiovascular disease in patients with and without renal failure [1–13]. Several studies have shown that a majority of the patients with renal failure have hyperhomocysteinaemia and therefore, as cardiovascular disease is a major cause of morbidity and mortality among HD patients, the lowering of homocysteine concentrations may have an important potential benefit. The hyperhomocysteinaemia observed in dialysis patients is related to various factors, including low renal clearance, altered

metabolism, genetic defects, and/or deficiencies of vitamins B<sub>6</sub> and B<sub>12</sub> or folic acid [2,8,9]. Previous investigators have reported that folic acid administration in doses varying from 1 to 15 mg/day produces, on average, a 20–40% reduction in the tHcy levels in dialysis patients, even in the presence of normal or elevated folic acid concentrations [2–7,11–13]. The homocysteine-lowering effect of folic acid is probably due to an improvement in the re-methylation pathway of homocysteine to methionine [9]. Overall, the ≈23% reduction in homocysteine levels observed in our study is comparable to the results of previous studies in which similar doses of folic acid (1–5 mg/day) had been prescribed [2–7,11,12]. It is interesting to note that two recent studies have reported that folic acid supplements ranging from 1 to 5 mg/day seem to have almost equivalent lowering effects on tHcy levels [11,12].

The main and somewhat unexpected finding of the present study was the fact that the patients with OVD seem to have a much lower response to folic acid administration than those without OVD—at least in the range of doses of folic acid we prescribed. This difference cannot be ascribed to difference in vitamins B<sub>6</sub> or B<sub>12</sub> levels, as they were similar in both study groups. At baseline, the only significant difference between our two study groups was age. Although older patients tend to have slightly higher tHcy concentrations (often in relation to subclinical vitamin deficiency) [16], the statistical analysis of our data failed to identify age as a significant determinant of the response to folic acid treatment. This is in agreement with the results of a previous study in pre-dialysis patients, which showed that the homocysteine-lowering effect of folic acid is similar in both young and old patients [6]. Although data analysis of our results failed to identify a confounding factor, it is clear that our results are obtained from a small self-controlled cohort and need to be confirmed in larger randomized trials.

Previous studies have evaluated the homocysteine-lowering effect of folic acid in groups of unselected dialysis patients, but as yet none has directly investigated whether the response to folic acid is the same in patients with and without OVD. However, Robinson and colleagues [4] reported tHcy levels in 176 dialysis patients, most of whom were receiving a routine multi-vitamin supplement including 1 mg of folic acid per day, a situation not so different from that of our study. These authors report tHcy levels of  $29.9 \pm 1.3$   $\mu\text{mol/l}$  in patients with vascular events and of  $23.9 \pm 1.5$   $\mu\text{mol/l}$  in those without [4], concentrations that are similar to the end-study tHcy levels observed in our patients. Thus, although Robinson *et al.* did not analyse their results specifically with respect to folic acid supplementation, their results may indirectly support the finding of the present study. If patients with OVD respond differently to folic acid, one may question whether the metabolism of homocysteine is different in these patients or whether hyperhomocysteinaemia may, at least to some extent, be a marker of different metabolic patterns that predispose to cardiovascular disease and/or be a marker of cardiovascular disease

itself. These different hypotheses have already been considered and several authors have emphasized that the link between hyperhomocysteinaemia and vascular disease seems to depend on complex genetic–environmental–nutritional interactions [1,17–20]. Concerning genetic factors, it should be noted that recent studies have shown that genetic mutations of some enzymes involved in homocysteine metabolism, such as the methylene tetrahydrofolate reductase gene (MTHFR 677C>T mutation) or the cystathionine beta synthase gene (CBS 699C>T and 1080T>C mutations), can modulate both the homocysteine-lowering effect of folic acid and the risk of developing cardiovascular disease [7,21–24]. For example, Kruger *et al.* [21] have reported that some specific CBS alleles are associated with a higher risk of cardiovascular disease and a decreased homocysteine-lowering response to folic acid. Interestingly, an increase in the doses of folic acid prescribed seems to improve the responsiveness to treatment of some genotypes [7]. Thus, one may speculate whether the polymorphism of the above-mentioned genes may partly explain the findings of the present study.

In conclusion, our data indicate that the administration of folic acid supplements induces a marked reduction of the total homocysteine levels in dialysis patients without OVD but seems to have more limited effects in those with OVD. As stated above, these preliminary findings need to be confirmed in larger randomized studies, but we consider that the possibility that different groups of patients may respond differently to folic acid administration (and/or to different doses of folic acid) should be taken into account in the planning and in the analysis of future studies concerning the treatment of hyperhomocysteinaemia.

*Acknowledgements.* The authors would like to thank Miss Marianne Thalmann and Miss Violette Hasler for their skilful collaboration and Miss Franziska Huppertz for homocysteine determinations.

## References

1. Welch GN, Loscalzo J. Homocysteine and atherosclerosis. *N Engl J Med* 1998; 338: 1042–1050
2. Bostom AG, Culleton BF. Hyperhomocysteinemia in chronic renal disease. *J Am Soc Nephrol* 1999; 10: 891–900
3. Makoff R, Dwyer J, Rocco MV. Folic acid, pyridoxine, cobalamin and homocysteine and their relationship to cardiovascular disease in end-stage renal disease. *J Ren Nutr* 1996; 6: 2–11
4. Robinson K, Gupta A, Dennis V *et al.* Hyperhomocysteinemia confers an independent increased risk of atherosclerosis in end-stage renal disease and is closely linked to plasma folate and pyridoxine concentrations. *Circulation* 1996; 94: 2743–2748
5. Massy ZA. Hyperhomocysteinemia in renal failure—what are the implications? *Nephrol Dial Transplant* 1996; 11: 2392–2393
6. Chauveau P, Chadeaux B, Coude M *et al.* Long-term folic acid (but not pyridoxine) supplementation lowers elevated plasma homocysteine level in chronic renal failure. *Miner Electrolyte Metab* 1996; 22: 106–109
7. Trambly R, Bonnardeaux A, Geadah D *et al.* Hyperhomocysteinemia in hemodialysis patients, effects of 12-month supplementation with hydrosoluble vitamins. *Kidney Int* 2000; 58: 851–858



8. Vychytil A, Födinger M, Wöflf G *et al*. Major determinants of hyperhomocysteinemia in peritoneal dialysis patients. *Kidney Int* 1998; 53: 1775–1782
9. Guttormsen AB, Ueland PM, Svarstad E, Refsum H. Kinetic basis of hyperhomocysteinemia in patients with chronic renal failure. *Kidney Int* 1997; 52: 495–502
10. Hultberg B, Andersson A, Sterner G. Plasma homocysteine in renal failure. *Clin Nephrol* 1993; 40: 230–234
11. Dierkes J, Domrose U, Ambrosch A, Bosselmann HP, Neumann KH, Luley C. Response of hyperhomocysteinemia to folic acid supplementation in patients with end-stage renal disease. *Clin Nephrol* 1999; 51: 105–115
12. Spence JD, Cordy P, Kortas C, Freeman D. Effect of the usual doses of folate supplementation on elevated plasma homocyst(e)ine in hemodialysis patients, no difference between 1 and 5 mg daily. *Am J Nephrol* 1999; 19: 405–410
13. Kunz K, Petijean P, Lirsi M *et al*. Cardiovascular morbidity and endothelial dysfunction in chronic haemodialysis patients, is homocyst(e)ine the missing link? *Nephrol Dial Transplant* 1999; 14: 1934–1942
14. Verster B, Rasmussen K. High performance liquid chromatography method for rapid and accurate determination of homocysteine in plasma and in serum. *Eur J Clin Chem Biochem* 1991; 29: 549–554
15. Descombes E, Hanck AB, Fellay G. Water soluble vitamins in hemodialysis patients and need for supplementation. *Kidney Int* 1993; 43: 1319–1328
16. Kark JD, Selhub J, Adler B *et al*. Nonfasting plasma total homocysteine level and mortality in middle-aged and elderly men and women in Jerusalem. *Ann Intern Med* 1999; 131: 321–330
17. Graham I. Homocysteine in health and disease. *Ann Intern Med* 1999; 131: 387–388
18. Siri PW, Verhoef P, Kok FJ. Vitamins B6, B12, and folate, association with plasma total homocysteine and risk of coronary atherosclerosis. *Am Coll Nutr* 1998; 17: 435–441
19. Kang SS, Wong PW. Genetic and nongenetic factors for moderate hyperhomocyst(e)inemia. *Atherosclerosis* 1996; 119: 135–138
20. 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
21. Kruger WD, Evans AA, Wang L *et al*. Polymorphism in the CBS gene associated with decreased risk of coronary artery disease and increased responsiveness to total homocysteine lowering by folic acid. *Mol Gen Metabol* 2000; 70: 53–60
22. Woodside JV, Yarnell JW, McMaster D *et al*. Effect of B-group vitamins and antioxidant vitamins on hyperhomocysteinemia, a double-blind, randomized, factorial-design, controlled trial. *Am J Clin Nutr* 1998; 67: 858–866
23. Födinger M, Mannhalter C, Wöflf G *et al*. Mutation (677 C to T) in the methylenetetrahydrofolate reductase gene aggravates hyperhomocysteinemia in hemodialysis patients. *Kidney Int* 1997; 52: 517–523
24. Malinow MR, Kruger WD, Duell PB *et al*. The effect of folic acid supplementation on plasma total homocysteine are modulated by multivitamin use and methylenetetrahydrofolate reductase genotypes. *Arterioscler Thromb Vasc Biol* 1997; 17: 1157–1162

Received for publication: 29.11.99

Accepted in revised form: 10.10.00