Hyperhomocysteinaemia, hyperlipidaemia and risk of venous thromboembolism in Shiraz

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فرط هوموسيستئين الدم وفرط شحميات الدم واختطار الانضمام الوريدي الخثاري في شيراز، الجمهورية الإيرانية الإسلامية

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الخلاصة: لتقييم اختطار الانصمام الوريدي الخُثاري المرافق لفرط هوموسيستئين الدم وفرط شحميات الدم، أجرينا دراسة للحالات والشواهد، وقمنا بقياس مستوى الهوموسيستئين والغليسريدات الثلاثية والكوليستيرول لدى 43 مريضاً مصاباً بانصمام وريدي خُثاري ولدى 43 مريضاً شاهداً. وقد وُجد أن وسطي مستوى الهوموسيستئين كان أعلى بشكل واضح لدى مجموعة الاختبار، وقد كانت نسبة الأرجحية للإصابة بالانصمام الوريدي الخُثاري في المصابين بفرط هوموسيستئين الدم 2.7، مع ترابط بشكل أوضح لدى النساء ولدى الذين تقل أعمارهم عن 50 عاماً. أما نسبة الأرجحية لمن كان لديهن فرط والغليسريدات الثلاثية وفرط الكوليستيرول، فقد كان أكبر بشكل ملحوظ لدى مَنْ هم دون 50 عاماً. يتبيّن من ذلك أن فرط هوموسيستئين الدم من عوامل الاختطار الهامة بين الإيرانيين، وأنه قد لوحظ ازدياد في اختطار الخُثار الوريدي بين من تقل أعمارهم عن 50 عاماً ممّن يعانون من نوعَيْ اضطرابات الشحميَّات معاً (أي فرط الكوليستيرول والغليسريدات).

ABSTRACT To assess the risk of venous thromboembolism (VTE) associated with hyperhomocysteinaemia (hyper-Hcy) and hyperlipidaemia, we performed a case–control study. Fasting total homocysteine (Hcy), triglyceride and cholesterol levels were assessed in 43 patients with VTE and 43 controls. Mean Hcy level was significantly higher in the test group. Odds ratio (OR) for VTE in patients with hyper-Hcy was 2.7, with the association stronger in women and those under 50. The OR for those with both hypertriglyceridaemia and hypercholesterolaemia was significantly greater in those under 50. Increased risk for venous thrombosis was found among those under 50 having both lipid abnormalities.

L'hyperhomocystéinémie, l'hyperlipidémie et le risque de thromboembolie veineuse à Chiraz (République islamique d'Iran)

RESUME Nous avons réalisé une étude cas-témoins afin d'évaluer le risque de thromboembolie veineuse associé à l'hyperhomocystéinémie et à l'hyperlipidémie. Les taux d'homocystéine totale, de triglycérides et de cholestérol à jeun ont été mesurés chez 43 patients présentant une thromboembolie veineuse et chez 43 témoins. Le taux moyen d'homocystéine était significativement plus élevé dans le groupe expérimental. Le risque relatif de thromboembolie veineuse chez les patients atteints d'hyperhomocystéinémie était de 2,7 ; l'association était plus forte chez les femmes et les personnes de moins de 50 ans. Le risque relatif pour ceux qui présentaient à la fois une hypertriglycéridémie et une hypercholestérolémie était significativement plus élevé chez les moins de 50 ans. Un risque accru de thrombose veineuse a été constaté chez les moins de 50 ans porteurs des deux anomalies lipidiques.

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Introduction

Most case–control studies have suggested that hyperhomocysteinaemia (hyper-Hcy) is a risk factor for venous thromboembolism. Also, hyperlipidaemia is implicated in the development of venous thrombosis. To assess the risk of venous thromboembolism (VTE) associated with these factors in our population, we performed a case–control study.

The development of venous thrombosis is a dynamic and multifactorial process. Conventional risk factors such as surgery do not account for all cases, and only a fraction of those with these risk factors develop VTE [*1–3*]. There is considerable epidemiological evidence from retrospective studies linking mild to moderate hyper-Hcy and VTE, however, no prospective study has demonstrated such a relationship except for idiopathic VTE in men [*4*]. The controversy surrounding the role of hyperlipidaemia in the development of VTE is even more prominent [*5*], and may be related to the small number of studies that have been carried out up to now.

In our study, we evaluated the relationship between mild to moderate hyper-Hcy and VTE in hospitalized patients, considering the possible genetic differences between the general population of the Islamic Republic of Iran and other populations. We also investigated the relationship between hyperlipidaemia and VTE.

Methods

In a case–control study, all patients diagnosed with VTE and subsequently admitted to hospitals affiliated to the University of Shiraz were invited to take part in this study, which was conducted from May to September 2001. Overt cancer, history of atherosclerosis, known inherited or acquired hypercoagulable states and abnormal renal function tests were exclusion criteria. None of the cases were using vitamin supplements.

VTE was diagnosed by the following methods:

- for deep vein thrombosis, duplex ultrasonography;
- for pulmonary embolism, combination of compatible medical presentation, echocardiography and ventilation-perfusion scan;
- for clot in the inferior vena cava, Doppler sonography or computed tomography with venous phase contrast.

We matched each of the 43 cases in the study group to one control according to both sex and age within 5 years. Controls were randomly selected from a large sample of healthy adults who were participating in an ongoing national osteoporosis risk survey in Shiraz, Iran. No specific medical history, including history of VTE or vitamin supplementation was available for controls. After fasting for 10 hours, two separate blood samples were collected from each subject, one of them anticoagulated with EDTA. The plasma and serum were separated within one hour of venepuncture and frozen and stored at –20 °C until time of analysis.

Plasma total homocysteine (Hcy) was assessed by enzyme immunoassay (DRG Instruments GmbH, Marburg, Germany) essentially according to the method of Frantzen et al [*6*]. Serum triglycerides and cholesterol were measured by an enzymatic colorimetric method [*7,8*].

We used the *t*-test for comparing the mean values of plasma total Hcy, serum triglycerides and cholesterol of patients and controls. A two-sided *P*-value of less than 5% was considered statistically significant. The cut-off point for defining hyper-Hcy

was the upper limit of the laboratory reference range (15 µmol/L). Hypertriglyceridaemia and hypercholesterolaemia are defined as a fasting serum level ≥ 200 mg/dL [*9*]. Conditional and unconditional logistic regression analyses for matched and unmatched data respectively were used for calculating the crude odds ratio (OR) as estimates of relative risk. Data were analysed using *SPSS*, version 9 and *Egret* software (Egret Software Corporation, Seattle, Washington).

Results

The characteristics of the patients and controls are shown in Table 1. The mean ages of the patients and the controls were 47.1 (range 16–89) years and 45.2 (range 19–86) years respectively. Sex distribution was equal in both groups.

Most cases had deep-vein thrombosis of the lower extremities (88.4%). A minority of them had pulmonary embolism (4.7%), deep-vein thrombosis of upper extremities (4.7%) or clot in the inferior vena cava (2.3%). For the majority of cases (79.1%) we found one or more predisposing factors, including minor events such as a prolonged trip and minor surgery [*10*].

In the 43 cases, the mean (± standard deviation) total Hcy level was significantly higher than for the controls (20.2 ± 6.3 µmol/L versus 17.0 ± 5.7 µmol/L respectively, $P = 0.015$). The difference in mean serum triglyceride between cases and controls was not statistically significant (144.5 ±76.5 mg/dL versus 161.6 ± 90.5 mg/dL respectively, $P = 0.29$). The cholesterol level was significantly higher in the control group compared with the patient group (205.3 ± 46.6 mg/dL versus 182.1 ± 55.1 mg/dL respectively, $P = 0.026$) (Table 1).

Thirty-four patients (79.1%) had fasting total Hcy level above the cut-off point of 15 µmol/L compared with 25 (58.1%) in the control group (OR 2.70, 95% CI: 1.10–7.10). For patients aged below fifty years the OR was 3.60 (95% CI: 1.10–11.25) while for those aged 50 and over it was lower (OR = 1.60, 95% CI: 0.20–11.30). The OR for women was higher than for men (4.20, 95% CI: 1.00–17.30 versus 1.90, 95% CI: 0.50–6.97). The calculated ORs for these and other subgroups are shown in Table 2.

In the control group we determined the 90th percentile of distribution of Hcy measurements as 24.2 µmol/L, and redefined hyper-Hcy as anything above this. From the analysis of data using this cut-off point we obtained an OR of 2.53 (95% CI: 0.73–9.14) (Table 2).

When we stratified the Hcy measurements of both patients and controls into quartiles and calculated OR for thrombosis in the patients at the three highest levels as compared with those at the lowest level [*11*], the risk of thrombosis increased gradually with increasing Hcy concentrations (Table 3).

Eleven (25.6%) patients had hypertriglyceridaemia compared with 9 (20.9%) in the control group. The OR was 1.30 (95% CI: 0.47–3.55). Although the calculated ORs for women (2.46, 95% CI: 0.51–11.80) and those < 50 years (2.42, 95% CI: 0.63–9.30) showed a trend toward increased risk, they were not statistically significant (Table 4).

Hypercholesterolaemia was seen in 16 (37.2%) patients but in 23 (53.5%) controls. The computed OR was 0.52 (95% CI: 0.22–1.22). In subgroup analysis, no significantly increased risk was detected (Table 4).

Table 1 **Characteristics of cases and controls**

Characteristic	Cases (*n* = 43)	Controls (*n* = 43)
Age, years (mean ± *s*)	47.1 ± 18.1	45.2 ± 16.9
Age range, years	16–89	19–86
Female:male ratio	24:19	24:19
Recurrent venous thromboembolism, No. (%)	7 (16.3)	NA
Predisposing factors for venous thromboembolism, No. (%)	34 (79.1)	NA
Trauma, No. (%)	7 (16.3)	NA
Immobilization, No. (%)	9 (20.9)	NA
Estrogen related[a], No. (%)	6 (14.0)	NA
Recent surgery, No. (%)	1 (2.3)	NA
Previous deep-vein thrombosis, No. (%)	2 (4.7)	NA
Minor events,[b] No. (%)	9 (20.9)	NA
Fasting plasma total homocysteine, µmol/L (mean ± *s*)	20.2 ± 6.3	17.0 ± 5.7
Fasting serum triglycerides, mg/dL (mean ± *s*)	144.5 ± 76.5	161.6 ± 90.5
Fasting serum cholesterol, mg/dL (mean ± *s*)	182.1 ± 55.1	205.3 ± 46.6

[a]*Using oral contraceptive pill or estrogen, pregnancy and postpartum period.*
[b]*Minor surgery, prolonged trip or sitting.*
NA = not applicable.
s = *standard deviation.*

Finally, 10 (23.3%) patients had both abnormally high levels of triglyceride and cholesterol compared with 7 (16.3%) of the 43 controls with OR of 1.60 (95% CI: 0.50–4.60). In subgroup analysis OR of 5.30 (95% CI: 1.004–27.70) for those < 50 years and 2.46 (95% CI: 0.50–11.80) for female patients were computed (Table 4).

Discussion

This case–control study identified hyper-Hcy as a risk factor for VTE in the general Iranian population, a finding seen when we used either the upper reference laboratory value or the 90th percentile of the control group as the cut-off point. The prevalence of mild to moderate hyper-Hcy is estimated to be 5%–7% in the general population [*12–14*] and even higher in the older population [*15*]. These facts, in addition to a high prevalence of VTE, its lethality and the magnitude of resources needed for detection, treatment and prevention of VTE, make our finding (OR = 2.6–2.7) more clinically significant.

In addition, we found a higher OR for those in the younger age group compared

Table 2 **Odds ratios for thrombosis associated with hyperhomocysteinaemia, according to age and sex**

Age group	Odds ratio (95% CI) Hcy level > 15.00 µmol/L[a]	Hcy level > 24.20 µmol/L[b]
< 50 years		
Men	1.87 (0.39–9.01)	1.64 (0.23–11.70)
Women	7.50 (1.31–43.03)	1.65 (0.23–11.99)
Both sexes	3.60 (1.10–11.25)	1.64 (0.40–6.63)
≥ 50 years		
Men	2.25 (0.20–29.77)	NC
Women	1.00 (0.05–20.83)	NC
Both sexes	1.60 (0.20–11.30)	NC
All ages		
Men	1.90 (0.50–6.97)	2.89 (0.50–16.67)
Women	4.20 (1.00–17.30)	2.27 (0.36–14.18)
Both sexes	2.70 (1.10–7.10)	2.58 (0.73–9.14)

[a]*Upper limit of laboratory reference range.*
[b]*90th percentile of control distribution.*
CI = confidence interval.
NC = could not be calculated due to missing data.

with the older individuals. This was compatible with observations of the other studies generally [*1*], and specifically with the fact that homocystinuria presents with early onset thromboembolic events [*12*].

To explore the possibility of a dose–response relationship, we stratified the patients and controls according to their Hcy concentrations. An increasing OR with increasing Hcy concentration was observed. As with cholesterol in the pathogenesis of atherosclerosis, a graded response rather than a threshold effect may also be possible for hyper-Hcy in the genesis of arterial and venous thrombosis, [*16–19*] although at least one study showed a different inference [*20*].

Table 3 **Odds ratios for thrombosis according to plasma homocysteine level**

Homocysteine level (µmol/L)	Cases No. (*n* = 43)	Controls No. (*n* = 43)	Odds ratio (95% CI)
< 15	9	18	1[a]
15–19	12	14	1.71 (0.56–5.20)
20–23	11	6	3.67 (1.02–13.10)
> 23	11	5	4.40 (1.17–16.57)

[a]*Reference category, odds ratio = 1.*

Table 4 **Odds ratios for thrombosis associated with different classes of hyperlipidaemia according to age and sex**

Age group	Odds ratio (95% CI)		
	Hypercholesterolaemia[a]	Hypertriglyceridaemia[b]	Both[c]
< 50 years			
Men	0.74 (0.16–3.39)	1.00 (0.16–6.10)	3.50 (0.30–39.10)
Women	1.00 (0.20–4.67)	7.50 (0.73–76.80)	7.50 (0.70–76.80)
Both sexes	0.86 (0.29–2.51)	2.42 (0.63–9.30)	5.30 (1.004–27.70)
≥ 50 years			
Men	0.07 (0.01–0.80)	0.58 (0.07–4.60)	0.26 (0.02–3.06)
Women	0.50 (0.05–5.15)	0.40 (0.02–6.17)	0.40 (0.03–6.20)
Both sexes	0.20 (0.04–0.91)	0.50 (0.09–2.62)	0.30 (0.05–1.90)
All ages			
Men	0.33 (0.10–1.13)	0.79 (0.20–3.04)	1 (0.22–4.56)
Women	0.81 (0.23–2.90)	2.46 (0.51–11.80)	2.46 (0.50–11.80)
Both sexes	0.52 (0.22–1.22)	1.30 (0.47–3.55)	1.60 (0.50–4.60)

[a]*Cholesterol ≥ 200 mg/dL.*
[b]*Triglycerides ≥ 200 mg/dL.*
[c]*Cholesterol ≥ 200 mg/dL and triglycerides ≥ 200 mg/dL.*
CI = confidence interval.

It is also important to address the potential strengths and limitations of our study. We used enzyme immunoassay (EIA) for determining the Hcy level instead of high-pressure liquid chromatography (HPLC). Although HPLC is a very precise method, it is expensive, time consuming and needs trained staff and special facilities which are not available everywhere. Moreover, recent studies have confirmed the precision and reliability of EIA for measuring Hcy levels [*6,21,22*], with some studies favouring EIA over HPLC for use in the clinical laboratory [*21*]. So our results are comparable with those that would be obtained by HPLC. We could not exclude the possibility of the presence of other established inherited thrombophilia and calculate the risk in their absence or presence to explore the independence of hyper-Hcy as a causative factor in VTE. We did not take into account vitamin deficiency or vitamin supplementation, factors known to affect Hcy levels. Vitamin supplementation is not frequently used in our country, fruits and vegetables are preferred, however, lack of vitamin supplementation may explain the higher level of Hcy found in our healthy control group compared with those obtained for the United States of America and Western European countries [*12,13*]. In our study, confounding variables such as the presence of overt malignancy or renal failure were excluded, a correction which has not been done in most studies.

We did not have any information on the occurrence of a previous VTE or atherosclerotic event in our control group or their renal function status. The presence of any such factors would lead to an underestimation of true risks.

Several lines of evidence suggest that Hcy level has a cause and effect relationship with venous thrombosis and is not simply a marker for another risk factor

[*12*]. Endothelial dysfunction has been implicated as the major underlying mechanism by which Hcy exerts its deleterious effect [*23*]. It may be caused by oxidative damage promoted by Hcy [*23*], loss of antithrombotic function of endothelium by enhancing the activities of coagulant factors [*24*,*25*] or depressing the levels of heparan sulfate [*26*] and thrombomodulin [*27*] and impairing the production of nitric oxide as a relaxing factor for both conduit and resistance vessels [*28*].

Our findings in this retrospective study do not support the hypothesis that hyperlipidaemia plays a role in the pathogenesis of VTE. No significant difference in the risk of developing the condition was seen between those with and those without hyperlipidaemia, although those who were younger than 50 years showed a significantly increased risk of developing VTE in the presence of both hypercholesterolaemia and hypertriglyceridaemia.

Kawasaki et al., in two separate case–control studies, showed the interrelation between hyperlipidaemia and hypercholesterolaemia among an Asian population [*29*,*30*]. In their prospective study, Goldhaber and colleagues found no associated increased risk for pulmonary embolism in the presence of hypercholesterolaemia [*31*]. Evaluating risk factors for VTE among women, McColl et al. observed a lower cholesterol level among cases versus controls [*32*]. The small number of these studies, in addition to their conflicting results, mandates more deliberation.

There are several sources of bias in the current study, one of them being the collection of specimens from our patients during hospital treatment, as we could not assure continuous follow-up out of hospital. Specimen collection remote from the initial VTE event may be important, since cholesterol is known to decrease in the presence of acute vascular events such as myocardial infarction or VTE [*33*,*34*], potentially introducing a negative confounding effect between VTE and cholesterol. On the other hand, stressful events such as myocardial infarction may increase the triglyceride level [*33*] (even up to 1 year after the event), thus exerting a positive confounding effect between triglyceride and VTE.

In summary, we found that hyper-Hcy may be a risk factor for VTE in the general population of the Islamic Republic of Iran, similar to populations of the United States and Western European countries. A similar finding was not seen for hyperlipidaemia except for a significantly increased risk for younger patients with both lipid abnormalities. Unlike the other thrombophilic disorders, a simple, safe therapy using pyridoxine, folic acid and vitamin B12 is available for hyper-Hcy [*35*]that may prove effective in the primary and secondary prevention of VTE in future studies. Due to the benign nature of this therapy, it can be offered to those with VTE and hyper-Hcy, while waiting for more evidence from further clinical trials.

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References

1. Ray JG. Meta-analysis of hyperhomocysteinaemia as a risk factor for venous thromboembolic disease. *Archives of internal medicine*, 1998, 158:2101–6.

2. den Heijer M et al. Hyperhomocysteinaemia and venous thrombosis: a meta-analysis. *Thrombosis and haemostasis*, 1998, 80(6):874–7.

3. Langman LJ et al. Hyperhomocysteinaemia and the increased risk of venous thromboembolism. *Archives of internal medicine*, 2000, 160: 961–4.

4. Ridker PM et al. Interrelation of hyperhomocyst(e)inemia, factor V Leiden, and risk of future venous thromboembolism. *Circulation*, 1997, 95: 1777–82.

5. Ray JG, Rosendaal FR. The role of dyslipidaemia and statins in venous thromboembolism. *Current controlled trials in cardiovascular medicine*, 2001, 2:165–70.

6. Frantzen F et al. Enzyme conversion immunoassay for determining total homocysteine in plasma or serum. *Clinical chemistry*, 1998, 44(2):311–6.

7. Bucolo G, David H. Quantitative determination of serum triglyceride by the use of enzymes. *Clinical chemistry*, 1973, 19: 476–82.

8. Allain CC et al. Enzymatic determination of total serum cholesterol. *Clinical chemistry*, 1974, 20:470–5.

9. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the national cholesterol education program (NCEP). *Journal of the American Medical Association*, 2001, 285:2486–97.

10. Eekhoff EM, Rosendaal FR, Vandenbroucke JP. Minor events and the risk of deep venous thrombosis. *Thrombosis and haemostasis*, 2000, 83(3):408–11.

11. Schlesselman JJ. *Case–control studies. Design, conduct, analysis.* Oxford, Oxford University Press, 1982.

12. Ueland PM, Refsum H. Plasma homocysteine, a risk factor for vascular disease: plasma levels in health, disease and drug therapy. *Journal of laboratory and clinical medicine*, 1989, 114:473–501.

13. Kang SS, Wong PW, Malinow MR. Hyperhomocyst(e)inaemia as a risk factor for occlusive vascular disease. *Annual review of nutrition*, 1992, 12: 279–98.

14. McCully KS. Homocysteine and vascular disease. *Nature medicine*, 1996, 2:386–9.

15. Selhub J et al. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *Journal of the American Medical Association*, 1993, 270:2693–8.

16. den Heijer M et al. Is hyperhomocysteinaemia a risk factor for recurrent venous thrombosis? *Lancet*, 1995, 345: 882–5.

17. Selhub J et al. Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. *New England journal of medicine*, 1995, 332:286–91.

18. Arnesen E et al. Serum total homocysteine and coronary heart disease. *International journal of epidemiology*, 1995, 24:704–9.

19. Malinow MR et al. Carotid artery intimal-medial wall thickening and plasma homocyst(e)ine in asymptomatic adults. *Circulation*, 1993, 87:1107–13.

20. den Heijer M et al. Hyperhomo-cysteinaemia as a risk factor for deep-vein thrombosis. *New England journal of medicine*, 1996, 334:759–62.

21. Marangon K. Validation of an immunoassay for measurement of plasma total homocysteine. *American journal of clinical pathology*, 1999, 112:757–62.

22. Mansoor MA. Comparison of Abbott IMx total homocysteine assay with a high

pressure liquid chromatography method for the measurement of total homocysteine in plasma and serum from a Norwegian population. *Scandinavian journal of clinical and laboratory investigation*, 1999, 59(5):369–74.

23. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. *New England journal of medicine*, 1998, 338: 1042–50.

24. Ratnoff OD. Activation of Hageman factor by L-homocysteine. *Science*, 1968, 162: 1007–9.

25. Rodgers GM, Kane WH. Activation of endogenous factor V by a homocysteine-induced vascular endothelial cell activator. *Journal of clinical investigation*, 1986, 77:1909–16.

26. Nishinaga M, Ozawa T, Shimada K. Homocysteine, a thrombogenic agent, suppresses anticoagulant heparan sulfate expression in cultured porcine aortic endothelial cells. *Journal of clinical investigation*, 1993, 92:1381–6.

27. Lentz SR, Sadler JE. Inhibition of thrombomodulin surface expression and protein C activation by thrombogenic agent homocysteine. *Journal of clinical investigation*, 1991, 88:1906–14.

28. Stamler JS et al. Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. *Journal of clinical investigation,* 1993, 91:308–18.

29. Kawasaki T et al. Hypercholesterolaemia as a risk factor for deep-vein thrombosis. *Thrombosis research*, 1997, 88(1):67–73.

30. Kawasaki T, Kambayashi J, Sakon M. Hyperlipidaemia: a novel etiologic factor in deep vein thrombosis. *Thrombosis research*, 1995, 79(2):145–51.

31. Goldhaber SZ et al. A prospective study of risk factors for pulmonary embolism in women. *Journal of the American Medical Association*, 1997, 277(8):642–5.

32. McColl MD et al. Lipoprotein (a), cholesterol and triglycerides in women with venous thromboembolism. *Blood coagulation & fibrinolysis*, 2000, 111(3):225–9.

33. Bachorik PS, Rifkind BM, Kwiterovich. PO. Lipids and dyslipoproteinemia. In: Henry JB, ed. *Clinical diagnosis and management by laboratory methods*, 19th ed. Philadelphia, WB Saunders Company, 1996, 208–36.

34. Volpato S et al. Acute phase markers are associated with reduced plasma lipid in a population of hospitalized elderly patients. *Gerontology*, 2000, 46:22–7.

35. Franken DG et al. Treatment of mild hyperhomocysteinemia in vascular disease patients. *Arteriosclerosis and thrombosis*, 1994, 14:465–70.