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Hyperhomocysteinemia in Patients Operated for Lower Extremity Ischaemia Below the Age of 50—Effect of Smoking and Extent of Disease*

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Moderate hyperhomocysteinemia may be a risk factor for atherosclerotic peripheral vascular disease (PVD). In order to develop PVD at an early age risk factors are more strongly expressed and hyperhomocysteinemia may be one such factor. Homocysteine is derived from methionine and is metabolised by cystathionine-synthase to cystathionine or remethylated to methionine. Cystathionine-synthase activity is dependent on vitamin B6 while the remethylation of homocysteine is dependent on vitamin B12 and folate. The present study analyses homocysteine in patients operated on for lower extremity ischaemia before the age of 50. Homocysteine before and after loading with methionine, vitamin B6, B12 and folate were measured at follow-up. The patients were compared to age- and sex-matched controls. Significantly more patients than controls had hyperhomocysteinemia, 16/58 vs. 4/65, defined as fasting total homocysteine above 18.6 μmol/l. Loading with methionine did not further discriminate between patients and controls. Smoking patients had higher levels of homocysteine than non-smoking patients or smoking and non-smoking controls. Smoking patients also had lower levels of vitamin B6. When comparing patients with suprainguinal, infrainguinal and multilevel disease the highest homocysteine levels were seen in the latter group. Also, in this group smoking patients had higher homocysteine levels. Multivariate analysis revealed that homocysteine was associated with low levels of vitamin B12, folate and smoking. Smoking therefore seems to be connected to increased homocysteine levels in patients with early development of atherosclerosis, partly explained by decreased levels of B6, B12 and folate.

Key Words: Homocysteine; Smoking; Peripheral vascular disease.

Introduction

Atherosclerosis is a disease of advanced age and the majority of patients operated for peripheral vascular disease (PVD) are consequently above the age of 60. Some patients, however, develop PVD severe enough to require vascular reconstruction at an earlier age and it is usually reported that a few per cent of the patients operated for PVD are below the age of 50.[1] It is highly likely that individuals who develop PVD at an early age have a stronger expression of risk factors. Identification of such risk factors is important not only for the future management of these patients but may also give clues to the pathogenetic mechanisms of atherosclerosis development.

Homocysteine has been proposed to be an atherogenic agent based on the finding that patients suffering from the inborn error homocystinuria have a severe form of premature atherosclerosis.[2] These patients have very high levels of plasma homocysteine but clinical studies including approximately 1600 patients suggest that moderate elevation of plasma homocysteine is also an isolated risk factor for vascular disease.[3]

Homocysteine is a sulphur containing amino acid derived from methionine by demethylation. It can be further metabolised by condensation with serine to yield cystathionine, a reaction catalysed by cystathione β synthase (CBS). Deficiency of this enzyme is the common cause for homocystinuria. The activity of CBS is dependent on vitamin B6. Homocysteine, however, also can be remethylated to methionine and this reaction is dependent on vitamin B12 and folate.[4]

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In patients with moderate hyperhomocysteinaemia the research interest has mainly focused on individuals heterozygous for deficiency of CBS. There are, however, as described above, alternative pathways for the metabolism of homocysteine and therefore many mechanisms exist, including B 12, folate and B 6 deficiency, that may lead to moderate hyperhomocysteinemia.

The purpose of the present study was to evaluate the influence of moderate hyperhomocysteinemia in patients undergoing vascular reconstruction for lower extremity ischaemia before the age of 50. Both fasting total homocysteine levels and those achieved after loading with methionine were measured and the influence of smoking, vitamin B 6, vitamin B 12 and folate were especially examined.

Materials and Methods

Patients

During the 12 year period from 1979–1990, 82 patients had vascular reconstructions for PVD before the age of 50 at the Departments of Surgery of Karolinska Hospital and St Görans Hospital, excluding patients operated for trauma and emboli. Of the total patients, 66 were available for follow-up. Nine had died, all from myocardial infarction or other vascular causes, three had emigrated, three refused to take part in the investigation and one could not be traced. Four patients operated on for abdominal aortic aneurysms (AAA), two for renal artery stenosis and two for symptomatic carotid stenosis were not included in this study.

Fifty-eight patients, 29 males and 29 females, were operated on for lower extremity ischaemia and constitute the basis of this report. They were investigated in a case-control study and compared with a randomly selected age- and sex-matched control person, selected from the population register. Clinical examination and blood sampling of all patients was performed 0–11 years postoperatively (mean 6 years). History including smoking habits and preoperative angiograms were also evaluated. Blood samples were obtained from controls who also filled in a health declaration.

Methods

Total homocysteine was measured in the fasting state and 4 h after an oral intake of methionine (0.1 g/kg) with a high performance liquid chromatography (HPLC)/fluorescence-method as previously described.[5]

Vitamin B 12, S-folate and S-creatinine were measured with standard methods. Vitamin B 6 was measured as pyridamol-phosphate (PLP) with an apotyrosine decarboxylase HLPC-method.[6] Reference values: B 12 (110–700 pmol/l), S-folate (5–30 nmol/l), S-creatinine (<120 micromol/l) and B 6 (20–60 mmol/l).

Pathologically elevated fasting levels of homocysteine and increase after loading with methionine were defined as values exceeding the mean of the controls by more than 2 SD. This implies that fasting values >18.6 μmol/l and an increase >36.8 μmol/l after loading with methionine were considered pathological.

Statistical methods

Homocysteine values were expressed as means with confidence intervals and differences between groups were tested with Wilcoxon's/Kruskal-Wallis test. Chi square with Yates' correction was used when comparing the frequency of smokers among patients and controls. Multiple regression analysis was also performed with fasting homocysteine as responder (JMP software, SAS Institute).

Results

There were 58 patients, 29 males and 29 females. The mean age at onset of symptoms was 40 years, at surgery 44 and at follow-up 49. Seventeen had infrainguinal lesions, 28 suprainguinal and 13 multilevel disease at the time of surgery. Multilevel disease was defined as a suprainguinal lesion and occlusion of at least one superficial femoral artery. All but two patients, one with severe diabetes and one with Takayashus disease, were smokers at the time of surgery. The mean daily cigarette consumption was 18 cigarettes for 20 years. At follow-up, 31/58 patients (53%) admitted to smoking but in the control group 19/58 (33%) were smokers ($p = 0.039$).

Sixteen patients (28%) had elevated fasting homocysteine values compared to only three (5%) in the control group. The corresponding figures for homocysteine increase after loading with methionine were 10/58 (17%) for patients and 3/58 (5%) for controls. Only three patients had both increased fasting and post-load values.

Of the 16 patients with high fasting levels of homocysteine a large majority (12) were current smokers but this effect of smoking was not seen in the control group. All three controls with high homocysteine values were non-smokers. Homocysteine levels were significantly higher in smoking patients than in non-smoking patients. Homocysteine was also significantly higher in smoking patients than in smoking controls (Fig. 1).

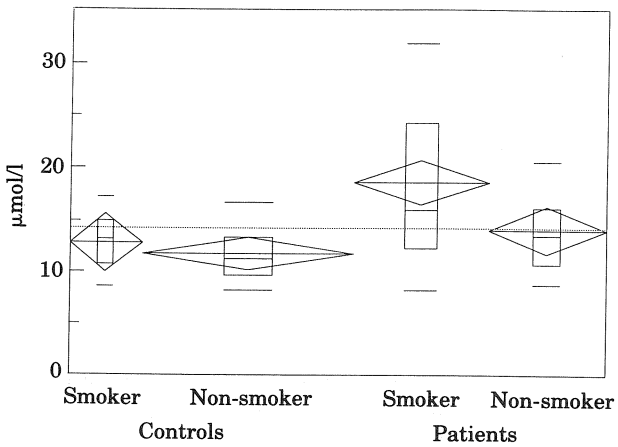

Fig. 1. Box plot representation of homocysteine in smoking and non-smoking patients and controls. There is a significant difference between smoking and non-smoking patients ($p < 0.05$) and between smoking patients and smoking controls ($p < 0.05$). The lower end of the "box" represents the 25th percentile and the upper end of the box the 75th. The line within the box represents the 50th percentile (median value). The horizontal lines below and above the boxes represent the 10th and 90th percentiles, respectively. The diamond shaped figure represents the 95% confidence interval and the horizontal line the mean value. The width of the box and the diamond are proportional to the number of observations. The dotted line represents the mean value for the total material.

Values for vitamin B 6 (PLP), B 12, folate, creatinine and homocysteine before and after loading with methionine are given in Table 1. In order to evaluate factors that might influence fasting homocysteine the following parameters were included in a multiple regression analysis: age, sex, patient/control, smoking habits, creatinine, B 6, B 12, folate and interaction components of these factors. The following parameters were found to be of significant influence for fasting homocysteine levels: folate, B 12, interaction B 12 folate, being a patient and smoking habits (Table 2). The influence of B 6 is probably partly hidden by the effect of smoking, since there is a significant effect of smoking on B 6 values for both patients and controls (Fig. 2).

Table 2. Factors with significant influence on homocysteine levels in a multiple regression analysis. Responder: total fasting homocysteine

Significant regressors	*p*-value
Patient *vs.* control subject	<0.001
Smoking	0.03
S-folate	<0.001
B 12	<0.001
Interaction folate/B 12	<0.001

When looking at the influence of homocysteine on the level of disease it was found that 13 of the 16 patients with high fasting homocysteine levels had suprainguinal or multilevel disease but only three patients with infrainguinal lesions were found in this

Table 1. Fasting and post-load increase of homocysteine and co-factors influencing these levels in smoking and non-smoking patients and controls. Mean values and 95% confidence interval

	Patients			Controls		
	Smokers		Non/ex-smokers	Smokers		Non/ex-smokers
PLP (B 6) (nmol/l)	26.5 (21–31)	*	34.13 (28–39)	34.83 (28–41)	*	42.33 (39–46)
B 12 (pmol/l)	470 (344–598)	NS	393 (276–510)	350 (268–431)	NS	364 (321–402)
S-folate (nmol/l)	11.15 (8–15)	*	15.21 (11–19)	12.78 (10–15)	NS	13.33 (11–15)
Fasting homocysteine (µmol/l)	18.79 (16–22)	*	14.05 (11–17)	12.85 (11–14)	NS	11.79 (11–13)
Post-load increase homocysteine (µmol/l)	24.28 (20–28)	NS	23.88 (19–28)	22.27 (17–28)	NS	18.82 (17–21)
S-creatinine (µmol/l)	101 (52–101)	NS	96 (87–106)	78 (71–84)	NS	90 (84–96)

* $p < 0.05$, NS = non-significant.

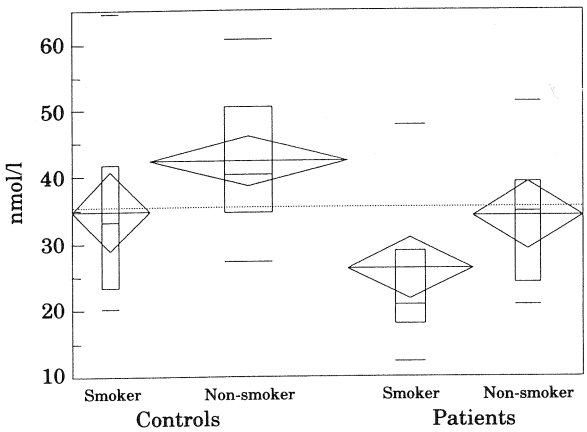

Fig. 2. Box plot representation of levels of vitamin B 6 in smoking and non-smoking patients and controls. There is a significant difference between smoking and non-smoking patients ($p < 0.05$) and between smoking and non-smoking controls ($p < 0.05$). For explanation of symbols see legend to Fig. 1.

group. The highest levels of homocysteine were found in patients who were current smokers with multilevel disease (Fig. 3).

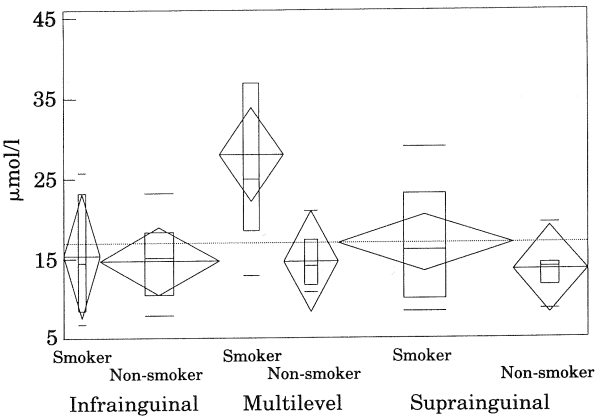

Fig. 3. Box plot representation of homocysteine levels in smoking and non-smoking patients with different levels and extent of disease. Smoking patients with multilevel disease had higher homocysteine levels than the other groups. For explanation of symbols see Fig. 1.

There was a significantly larger increase in homocysteine after loading with methionine in patients compared to controls. This difference was explained solely by the values obtained in men since there was a significant difference between male patients and male controls but no such difference among women (Fig. 4). Gender, however, did not influence fasting homocysteine levels. There was no influence of smoking on post load homocysteine levels.

Discussion

This report demonstrates that moderate hyperhomocysteinemia is present in a large proportion of patients operated for lower extremity ischaemia before the age of 50. The finding is in agreement with several other studies of patients with either lower extremity ischaemia[7] or PVD at other locations.[8]

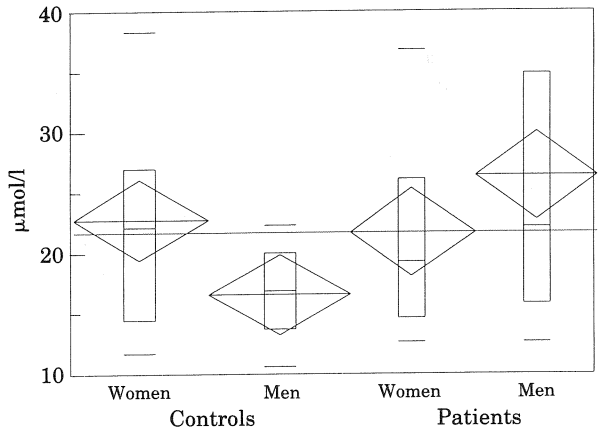

Fig. 4. Box plot representation of increase in homocysteine after loading with methionine in male and female controls and patients. There is a significant difference between male controls and male patients ($p < 0.05$). For explanation of symbols see Fig. 1.

Elevated levels of homocysteine after loading with methionine may be explained by defective CBS activity in individuals heterozygous for homocystinuria, but high fasting values are probably not fully explained by insufficient catabolism.[3] The finding that 5% of the controls had high fasting levels of homocysteine makes it less probable that this is determined only by defective CBS, since the frequency of this autosomal recessive deficiency is reported not to exceed 2% in a healthy population. The prevalence of 5% hyperhomocysteinemia in healthy individuals in the present series agrees with a previous report.[9] A strong genetic influence on fasting total homocysteine has been demonstrated in studies of healthy twins and siblings of patients with early coronary artery disease.[10–12] The finding that homocysteine levels after loading with methionine did not further discriminate between patients and controls further emphasises that defective CBS is not the only determinant for hyperhomocysteinemia. Thus, the genetic factors that lead to a high fasting homocysteine level remain partly unexplained.

Nutritional factors strongly influence homocysteine levels. Even within their "normal range" vitamin B12 and folate influence plasma concentrations of homocysteine.[13,14] Regarding the influence of folate, it has been pointed out that homocysteine is elevated only if folate is below 11 nmol/l.[9] Our results support this observation since a majority of the patients had low folate levels. Only four of 17 patients with hyperhomocysteinemia had folate

values exceeding 11 nmol/l. Defective folate metabolism may be another example of genetically determined hyperhomocysteinemia. Inherited low activity of methylene-tetrathydrofolate reductase, which catalyses the synthesis of 5-methyltetrahydrofolate, the main form of folate, is more common in coronary artery disease patients than in normals.[15]

It has been suggested that homocysteine induces atherosclerosis by formation of free radicals which results in the formation of oxidised low density lipoproteins (LDL). This has been suggested to be a crucial step in the early development of atherosclerosis.[16] Cultured endothelial cells require thiols with free sulphydrylgroups to oxidise LDL.[17] Consequently, there is more than one mechanism that links homocysteine with free radical formation and oxidation of LDL.

Homocysteine was influenced by smoking in the patient group but not in controls. In contrast to our findings, many reports have failed to demonstrate an effect of smoking on homocysteine levels.[7,14] One explanation for this discrepancy is that this study consisted of young patients with a high risk of developing PVD. In another study, also examining patients with premature disease, a correlation between smoking and homocysteine levels was found in siblings of patients with premature coronary disease.[11]

Smoking was shown to influence the levels of B 6 and folate which partly explains the increased levels of homocysteine seen in smoking patients. This is in agreement with previous findings in healthy individuals both regarding B 6[18,19] and folate.[20] These factors cannot, however, as shown in the regression analysis in the present study, fully explain the effect of smoking on homocysteine. No significant effect of smoking on homocysteine levels was seen in the control persons.

The "allergy-to-smoke" concept implies that some individuals have a lower tolerance to smoking because of some smoking induced pathogenic mechanism. Hyperhomocysteinemia may be one such mechanism possibly induced by lowering of folate and B 6 since these changes were more pronounced in smoking patients than in smoking controls. In most previous reports, however, investigating the influence of B 6 on fasting homocysteine levels no relationship between B 6 and homocysteine has been demonstrated.[21]

Increase of plasma homocysteine after loading with methionine has been shown to be more pronounced in men.[22–23] This is in agreement with the present material regarding patients but not regarding controls. If moderate hyperhomocysteinemia is a causal risk factor for atherosclerosis, there is a simple and safe method to lower homocysteine levels by the daily intake of 10–15 mg folate. Whether such medication inhibits the progression of atherosclerotic disease remains, however, to be shown and would require a randomised trial to be demonstrated. This report indicates that an efficient way to lower homocysteine levels in a current smoking younger atherosclerotic patient is to quit the habit.

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