Plasma Total Homocysteine Levels in Patients With Early-Onset Coronary Heart Disease and a Low Cardiovascular Risk Profile

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Mild hyperhomocysteinemia has been associated with an increased risk to develop premature coronary heart disease. Recently, the homocysteine concentration has been positively correlated with several main cardiovascular risk factors. We addressed the issue as to whether patients with coronary heart disease and a low cardiovascular risk profile also have a higher prevalence of hyperhomocysteinemia than matched controls. Ninety-five patients (aged 50.5 ± 6.6 years) and 34 controls (50.0 ± 6.7 years) less than 60 years of age were selected from a sample of patients after coronary angiography. Subjects with hypertension, diabetes, and moderate or severe hyperlipidemia were excluded. We determined plasma aminothiols (total homocysteine, cysteine, and glutathione), lipoprotein fractions, fibrinogen, and uric acid, the body mass index (weight in kilograms divided by height in meters squared), and the waist to hip ratio. Furthermore, 37 healthy subjects aged 30.8 ± 7.5 years underwent aminothiol determinations. Patients and controls were similar with regard to age and primary cardiovascular risk factors. Total homocysteine concentrations in the patient group (9.2 ± 2.4 μmol/L) were significantly higher than in the healthy subjects (8.0 ± 2.0 μmol/L). However, they did not differ from the levels in the age-matched controls (9.3 ± 3.0 μmol/L). Neither total cysteine nor glutathione concentrations were significantly different between patients and controls. Male patients (n = 85) had higher mean very-low-density lipoprotein (VLDL) triglycerides (1.36 ± 0.90 mmol/L) and lower high-density lipoprotein 3 (HDL_3) cholesterol (0.75 ± 0.21 mmol/L) than male controls (n = 28; 1.01 ± 0.62 and 0.88 ± 0.26 mmol/L, respectively). Female patients did not have any significant differences in lipoprotein concentrations versus the controls. Among further cardiovascular risk factors, we found a higher prevalence of central obesity in male patients.

In conclusion, there was not a higher incidence of hyperhomocysteinemia among patients with premature coronary heart disease and a low cardiovascular risk profile. The higher prevalence of hyperhomocysteinemia found in other studies may be related to the primary risk factors seen in these populations, and may therefore be an indicator of the global cardiovascular risk.

HOMOCYSTINURIA, first described in 1962,[1] is a rare genetic disorder of methionine metabolism.[2] Due to the homozygous deficiency of cystathionine β-synthase,[3] further breakdown of homocysteine along the transsulfuration pathway is greatly impaired. Thus, the affected patients accumulate homocysteine in excess of 400 μmol/L in plasma.[4] Atherosclerosis and thromboembolism may occur at an early age,[5,6] and account for a high morbidity and mortality.[7] Endothelial damage,[8,9] activation of factor V,[10] increased thrombocyte aggregation,[9,11] increased binding of lipoprotein (a) [Lp(a)] to fibrin,[12] and enhanced lipid peroxidation[13] have been discussed in homocysteine-mediated vascular injury.

In normal subjects, the homocysteine level has a range of 6 to 14 μmol/L.[14,15] Apart from homozygous homocystinuria, hyperhomocysteinemia today is known to be present in various disease states, among them renal failure and deficiencies of vitamin B_6, vitamin B_{12}, and folic acid.[14-18] In recent years, there is growing evidence that mild to moderate hyperhomocysteinemia may be associated with cardiovascular atherosclerotic disease. In the majority of studies,[19-30] albeit not all,[31-33] significantly higher homocysteine concentrations have been detected in patients with coronary heart disease than in their respective controls.

The homocysteine concentration has been related to the cholesterol level, hypertension, and smoking.[34] This raises the question of whether mild hyperhomocysteinemia may occur secondary to primary cardiovascular risk factors and act instead as an indicator of the cardiovascular risk. Elevated cysteine concentrations have been observed in subjects with cerebral infarction,[35] but the significance for the pathogenesis of coronary artery disease remains to be investigated. In vitro, cysteine has been shown to induce superoxide production and subsequent oxidation of low-density lipoprotein (LDL).[13] Decreased cysteine concentrations may be found in patients with reduced activity of the transsulfuration pathway.[2] Since cysteine has been considered rate-limiting for glutathione biosynthesis,[36] a shortage of this amino acid, eg, in cystathionine β-synthase deficiency, may impair the production of glutathione. Inhibition of glutathione synthesis in vitro has been shown to increase the toxicity of oxidized LDL to monocytes and macrophages, thus promoting foam cell formation.[37]

In the present study, we therefore determined the concentrations of total plasma cysteine, homocysteine, and glutathione in a population with premature coronary heart disease and a low cardiovascular risk profile. All commonly known cardiovascular risk factors were either excluded or matched for in the respective groups.

SUBJECTS AND METHODS

Patients With Coronary Heart Disease and Controls

A sample of 95 patients with coronary heart disease and 34 controls, as defined by coronary angiography, aged less than 60 years were studied. They were selected from a large sample of patients admitted for cardiological investigation on the basis of the following inclusion criteria. Since there were only a few premenopausal women in each group, only postmenopausal women were included in the calculations.

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None of the participants had an acute or malignant illness. Patients with a myocardial infarction did not enter the study until 3 weeks after the event. All subjects had normal hepatic and renal function (pseudocholinesterase, 3,000 to 8,000 U/L; creatinine, <106 μmol/L in males and <88 μmol/L in females). Patients with diabetes (defined by history, use of antidiabetic drugs, or fasting glucose >6.6 mmol/L), hypertension (defined by history, medication, and actual blood pressure), and moderate, severe, or drug-treated hyperlipoproteinemia (LDL cholesterol >4.92 mmol/L and triglycerides >3.42 mmol/L on dietary therapy) were excluded to minimize the influence of other cardiovascular risk factors. After reviewing the inclusion and exclusion criteria, informed consent was obtained from all patients. The results of the history (including medication, diet, smoking habits, history of angina pectoris, myocardial infarction, stroke, peripheral vascular occlusive disease, or thromboembolism, and family history), physical examination (body mass index calculated as weight in kilograms divided by height in meters squared, and waist to hip ratio), and coronary angiography were recorded. Subjects were classified as smokers, former smokers (if stopped for ≥3 weeks), and nonsmokers. After an overnight fast, blood samples were drawn to determine serum creatinine and uric acid, homocysteine, cysteine, glutathione, lipoproteins, and fibrinogen. The tubes used for sampling lipoproteins and aminothiols contained EDTA. The latter were placed on ice immediately after tapping. Blood for determination of fibrinogen was collected in heparin-containing tubes. All samples were centrifuged within 2 hours (2,000 × *g* for 10 minutes), and the supernatant was stored until analysis. Plasma for determination of aminothiols and fibrinogen was stored at −70°C until analysis, and the remaining plasma was kept at 4°C and analyzed within 1 week.

Healthy Subjects

To enable the correlation of homocysteine concentrations with age, we also studied young healthy subjects. None of these subjects had an acute or chronic disease, and none took any medication other than contraceptives (n = 4) or smoked. After obtaining informed consent, one fasting blood specimen was drawn for analysis of aminothiols, lipoproteins, and safety parameters. All participants had normal renal and hepatic function as documented by serum creatinine, γ-glutamyltransferase, alanine aminotransferase, and pseudocholinesterase levels. The samples were treated as already described.

Laboratory Methods

For determination of plasma total homocysteine, cysteine, and glutathione, we used a specific thiol derivatization with ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate and a high-performance liquid chromatography (HPLC) method first described by Araki and Sako,[38] with minor modifications. Reverse-phase HPLC (Ultrasphere ODS column, 4.6 mm × 25 cm, Beckman, Fullerton, CA; pump model 480, Gynkothek, Germering, Germany) at room temperature was used for separation. Detection was performed fluorometrically (Spektralfluorometer SFM 23, excitation at 385 nm and emission at 515 nm; Kontron, Neufahrn, Germany), and the signal was recorded by an integrator (Chromatopac CR 6A; Shimadzu, Osaka, Japan). For elution, we used a linear gradient from buffer A (0.1 mol/L acetate buffer, pH 4.0, containing 2% methanol) to buffer B (0.1 mol/L phosphate buffer, pH 6.0, containing 10% methanol) in 10 minutes. The retention time for cysteine, homocysteine, and glutathione was 5.19 ± 0.09, 6.78 ± 0.17, and 9.98 ± 0.26 minutes, respectively; the intraassay coefficient of variation was 2.70%, 2.57%, and 4.04%. The fourth peak in the chromatogram represents cysteinylglycine, which was not quantified. Mercaptopropionylglycine was used as an internal standard. A chromatogram of the aminothiols from a healthy control subject is shown in Fig 1.

Total cholesterol and triglyceride levels were measured enzymatically (Monotest Cholesterol and MRP 2 Triglycerides GPO-PAP; Boehringer, Mannheim, Germany) using an automated analyzer (Epos Analyzer 5060; Eppendorf, Hamburg, Germany). Separation of very–low-density lipoprotein (VLDL) was achieved by ultracentrifugation. Three milliliters of plasma was overlayered with 2 mL NaCl solution of density 1.006 kg/L (NaCl/L 11.04 g/L) and centrifuged at 220,000 × *g* and 4°C for 24 hours (model L5-75, rotor Ti 50; Beckman, Palo Alto, CA). Then, the upper 2 cm of the tube was sliced, and VLDL cholesterol and triglyceride levels were measured enzymatically in this plasma fraction. For determination of HDL cholesterol, the apolipoprotein (Apo) B–containing lipoproteins in the infranatant were precipitated with $MgCl_2$ and heparin. After centrifugation (10 minutes at 2,700 × *g*), high-density lipoprotein (HDL) cholesterol was determined enzymatically in the supernatant. The concentration of LDL cholesterol was calculated by subtracting HDL cholesterol from total cholesterol in the infranatant after ultracentrifugation.

HDL_2 and HDL_3 cholesterol levels were measured enzymatically after precipitating the defined lipoprotein fractions with different concentrations of polyethyleneglycol (Quantolip HDL (HDL_2/HDL_3) cholesterol precipitation reagent; Immuno, Vienna, Austria). Lp(a) was assayed radioimmunologically (Apo(a) RIA; Mercodia, Uppsala, Sweden). Fibrinogen was determined by immunonephelometry (Nephelometer 100; Behringwerke, Marburg, Germany). All other laboratory tests, including uric acid and liver and renal function tests, were performed using standard laboratory techniques.

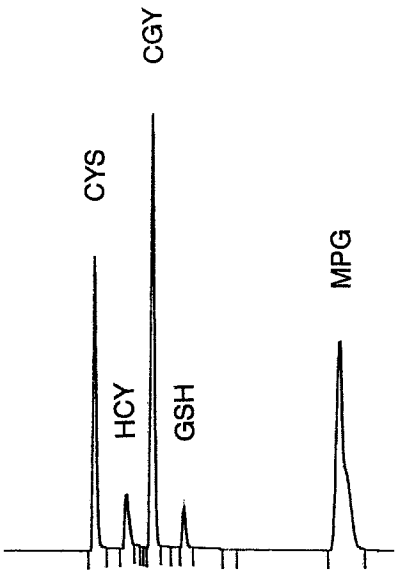

Fig 1. Chromatogram showing retention times and concentrations of aminothiols for a young healthy subject (male aged 37 years). CYS, cysteine (5.74 minutes, 172 μmol/L); HCY, homocysteine (7.80 minutes, 10.3 μmol/L); CGY, cysteinylglycine (9.41 minutes); GSH, glutathione (11.48 minutes, 5.93 μmol/L); MPG, mercaptopropionylglycine (21.45 minutes).

Statistical Analysis

For statistical analysis, we used the program SPSS/PC+ (Statistical Package for the Social Sciences, Version 4.0; SPSS Inc, Chicago, IL). Before applying the tests for statistical significance, the variables were checked for normal distribution (Shapiro-Wilks test; Lilliefors test if N ≥ 50). If the variables were normally distributed, we used the *T* test for statistical significance. In the case of a nonnormal distribution, the Mann-Whitney *U* test was applied. Chi-square statistics were used for nonparametric variables. *P* values less than .05 were regarded as significant.

RESULTS

Table 1 shows clinical characteristics of the patients, controls, and healthy subjects. The age distribution was equal in the patients and control group. There also was no difference in the prevalence of smokers, former smokers, and nonsmokers. Among the smokers, we found a higher, albeit nonsignificant, number of pack-years in the control group, whereas among former smokers, the patient group had a significantly higher number of pack-years. In former smokers, the median time from quitting smoking to inclusion in the study was 50 ± 368 weeks in patients and 208 ± 371 weeks in controls. Sixty-one (83.6%) former smokers had quit for at least 8 weeks. Further, differences regarding the occurrence of myocardial infarction and the family history of noncoronary atherosclerosis were found to be significant between patients and controls. In patients with a previous myocardial infarction, the mean time between the event and inclusion in the study was 78 ± 129 weeks. In 57 patients (78.0%), at least 8 weeks had passed since the event. There were no significant differences regarding the sex distribution, prevalence of stroke, peripheral arterial occlusive disease, or thromboembolism, family history of myocardial infarction, and family history of hyperlipoproteinemia. Among the controls, there were two patients with a history of thromboembolism, one of which was followed by a pulmonary embolism. One previous stroke in the control group was due to an embolic vascular occlusion. Thirty-four subjects in the patient group (35.1%) and 10 controls (26.3%) adhered to a lipid-lowering diet. None of the subjects were taking any vitamin supplements, nor were any on a drug or diet that could influence plasma homocysteine or vitamin levels. In the group of young healthy subjects, the sex distribution was well balanced. None had a history of vascular disease, nor were there any smokers in this group.

The mean concentrations of the different aminothiols are depicted in Table 2. Total cysteine and homocysteine were significantly higher and total glutathione was significantly lower in the patients and controls compared with the young healthy subjects. The patients and control group did not differ significantly for any of the three aminothiols. Five patients (5.3%) with coronary heart disease had a cysteine, one (1.1%) homocysteine, and four (4.2%) glutathione concentration higher or lower than (glutathione) the mean ± 2 SD of the controls. The ratio of homocysteine to cysteine as a measure of activity of the sulfhydryl pathway was similar in all three groups. If the 95th percentile (5th percentile for glutathione) value of the young healthy subjects was selected as a cutoff value, 32 (33.7%) patients with coronary heart disease would have a cysteine level and 10 (10.5%) a homocysteine level higher than the normal range, and 39 (41.1%) would have a glutathione level less than the normal range.

In the male subgroups, significant differences between patients and normal subjects were found for cysteine and glutathione. In the female subgroups, two patients and one control received hormone replacement therapy and four young healthy subjects were on oral contraceptives. Since omitting these subjects would not have affected any of the results, they were included in the calculations. Mean cysteine concentrations were significantly higher in postmenopausal patients compared with premenopausal healthy subjects; however, there were no significant differences compared with the controls. Glutathione levels were lower both in patients and in controls compared with healthy subjects. Mean homocysteine concentrations did not differ significantly in either of the three groups.

The mean concentrations of the different lipid parameters are shown in Table 3. Within the patient group, men showed significantly lower concentrations of HDL (0.98 ± 0.23 *v* 1.32 ± 0.36 mmol/L, $P \leq .002$), HDL_2 (0.23 ± 0.08 *v* 0.34 ± 0.13 mmol/L, $P \leq .008$), and HDL_3 cholesterol (0.75 ± 0.21 *v* 0.90 ± 0.15 mmol/L, $P \leq .02$) than women. Statistical tests for lipoprotein concentrations were therefore performed separately in male and female patients. In male patients, we found significantly lower concentrations of HDL and HDL_3 cholesterol compared with the control levels. VLDL triglycerides were significantly elevated in the patients. Concentrations of total cholesterol, LDL cholesterol, HDL_2 cholesterol, and Lp(a) were not significantly different in the patients and

Table 1. Age, Sex, Smoking Status, Family History, Extracoronary Atherosclerosis, and Distribution of Findings on Coronary Angiogram in Patients With Coronary Heart Disease, Controls, and Healthy Subjects (mean ± SD)

Parameter	Patients (n = 95)		Controls (n = 34)			Healthy Subjects (n = 38)		
	No.	%	No.	%	*P*	No.	%	*P*
Age (yr)	50.5 ± 6.6		50.0 ± 6.7		<.64	30.8 ± 7.5		<.0001
Sex (M, F-pre, F-post)	85, 0, 10	89.4, 0.0, 10.5	29, 0, 6	82.4, 0, 17.6	<.31	18, 20, 0	47.3, 52.6, 0	<.000001
Smoking status (S, FS, NS)	14, 60, 20	14.7, 63.2, 21.1	9, 13, 10	26.5, 38.2, 29.4	<.11	0, 0, 38	0, 0, 100	<.000001
Pack-years								
S	36.3 ± 17.8		44.8 ± 49.4		<.56	—		ND
FS	30.7 ± 19.0		19.8 ± 14.7		<.03	—		ND
Coronary angiogram (0, 1, 2, 3 VD)	0, 43, 27, 22	0, 45.2, 28.4, 23.2	34, 0, 0, 0	100, 0, 0, 0	<.000001	ND		ND
Myocardial infarction	73	76.8	0	0	<.000001	0	0	<.000001
Stroke	3	3.2	1	2.9	<.50	0	0	<.27
PAOD	7	7.4	0	0	<.10	0	0	<.10
Thromboembolism	6	6.3	1	2.9	<.90	0	0	<.11
FH of myocardial infarction	40	42.1	10	29.4	<.13	ND		ND
FH of atherosclerosis (other forms)	61	64.2	16	47.1	<.028	ND		ND

NOTE. *P* values compare the patients with the controls and healthy subjects.

Abbreviations: M, male; F-pre, premenopausal female; F-post, postmenopausal female; S, smokers; FS, former smokers; NS, nonsmokers; VD, vessel disease; PAOD, peripheral arterial occlusive disease; FH, family history; ND, not determined.

Table 2. Plasma Total Cysteine, Homocysteine, and Glutathione Levels in Patients With Coronary Heart Disease, Controls, and Healthy Subjects (mean ± SD)

Parameter	Patients (n = 95)			Controls (n = 34)			Healthy Subjects (n = 38)		
	Total (n = 95)	Male (n = 85)	Female (n = 10)	Total (n = 34)	Male (n = 28)	Female (n = 6)	Total (n = 38)	Male (n = 18)	Female (n = 20)
Total cysteine (μmol/L)	221 ± 37*	220 ± 36‡	225 ± 40§	216 ± 45‖	217 ± 44#	211 ± 47	189 ± 21	193 ± 17	185 ± 24
Homocysteine (μmol/L)	9.2 ± 2.4†	9.5 ± 3.2	9.4 ± 2.7‡	9.3 ± 3.0¶	9.5 ± 3.0	8.3 ± 2.8	8.0 ± 2.0	8.2 ± 1.7	7.9 ± 2.3
Glutathione (μmol/L)	2.48 ± 0.73*	2.52 ± 0.72*	1.99 ± 0.85	2.49 ± 0.80‡	2.50 ± 0.72*	2.16 ± 1.18**	4.22 ± 1.73	4.47 ± 0.90	3.57 ± 0.85
Homocysteine/cysteine ratio (×100%)	4.45 ± 3.01	4.52 ± 3.17	3.98 ± 0.49	4.37 ± 1.52	4.47 ± 1.59	2.67 ± 0.68	5.08 ± 4.99	4.29 ± 0.94	5.81 ± 6.82

NOTE. Statistical values are for comparisons to the healthy subjects.
*$P < .0001$.
†$P < .008$.
‡$P < .0001$.
§$P < .02$.
‖$P < .005$.
¶$P < .05$.
#$P < .01$.
**$P < .004$.

controls. Female patients and controls did not differ significantly for any of the lipoproteins evaluated. Although VLDL triglycerides were higher in the patient group, this did not reach statistical significance.

Cardiovascular risk factors investigated in addition to the lipoproteins included uric acid, fibrinogen, body mass index, and waist to hip ratio. We found a significantly higher waist to hip ratio in male patients compared with the controls (0.98 ± 0.06 *v* 0.95 ± 0.06, $P < .05$). Female patients did not have a waist to hip ratio different from that of the controls (0.92 ± 0.11 *v* 0.87 ± 0.08, $P < .48$). There were no differences in the uric acid level (374 ± 83 *v* 380 ± 107 μmol/L), fibrinogen level (2.88 ± 0.90 *v* 2.62 ± 0.87 g/L), and body mass index (26.1 ± 3.0 *v* 25.6 ± 4.0 kg/m^2) between patients and controls.

In the total study population, the homocysteine concentration was positively correlated with cysteine ($r = .56880$, $P < .00001$). This phenomenon was observed in all three groups. In the patient group, we found a positive correlation between cysteine and uric acid ($r = .26641$, $P < .01$), as well as fibrinogen ($r = .31895$, $P < .002$). A negative correlation was observed between the waist to hip ratio and HDL cholesterol ($r = -.32280$, $P < .002$), HDL_2 cholesterol ($r = .25584$, $P < .002$), and HDL_3 cholesterol ($r = -.25168$, $P < .02$). This was true only for the patient group; it did not reach significance in the control group. In none of the groups was there a significant correlation between the concentration of aminothiols and lipids, creatinine, or pseudocholinesterase, the body mass index, or the waist to hip ratio.

DISCUSSION

In evaluating the clinical characteristics of the patients and controls, we found that the groups were similar in age and primary cardiovascular risk factors. Patients with diabetes and hypertension were excluded, and the prevalence of smokers and the LDL cholesterol concentration did not differ significantly. The higher number of pack-years in patients who formerly smoked was outweighed by the lower number of pack-years in the group of smokers with coronary heart disease. Thus, a good comparison of the groups was possible. The patient group may be considered to have a low cardiovascular risk profile. Mean plasma cysteine and homocysteine levels were significantly higher and glutathione levels significantly lower in the patient group compared with the healthy subjects. However, they did

Table 3. Lipid Parameters in Patients With Coronary Heart Disease, Controls, and Healthy Subjects (mean ± SD)

Parameter	Patients (n = 95)		Controls (n = 34)		Healthy Subjects (n = 38)	
	Male (n = 85)	Female (n = 10)	Male (n = (28)	Female (n = 6)	Male (n = 18)	Female (n = 20)
Cholesterol (mmol/L)	5.55 ± 1.08	5.83 ± 1.06	5.47 ± 1.24	5.83 ± 1.08	4.88 ± 0.62	5.05 ± 0.93
Triglycerides (mmol/L)	1.69 ± 1.03§	1.54 ± 0.46**	1.40 ± 0.66	1.35 ± 0.79	1.28 ± 0.62	0.97 ± 0.34
HDL cholesterol (mmol/L)	0.98 ± 0.23*‖	1.32 ± 0.36‖	1.14 ± 0.31	1.24 ± 0.28	1.19 ± 0.23	1.78 ± 0.41
HDL_2 cholesterol (mmol/L)	0.23 ± 0.08§	0.34 ± 0.13‖	0.36 ± 0.10	0.34 ± 0.08	0.52 ± 0.93	0.57 ± 0.23
HDL_3 cholesterol (mmol/L)	0.75 ± 0.21†¶	0.90 ± 0.15**	0.88 ± 0.26	0.90 ± 0.28	0.90 ± 0.15	1.21 ± 0.23
LDL cholesterol (mmol/L)	3.74 ± 0.93	3.82 ± 0.83#	3.64 ± 0.98	4.02 ± 0.75	3.43 ± 0.59	2.92 ± 0.72
VLDL cholesterol (mmol/L)	0.75 ± 0.44#	0.72 ± 0.41¶	0.62 ± 0.41	0.52 ± 0.36	0.49 ± 0.26	0.26 ± 0.15
VLDL triglycerides (mmol/L)	1.36 ± 0.90‡	1.16 ± 1.54#	1.01 ± 0.62	0.86 ± 0.58	1.13 ± 0.60	0.62 ± 0.33
Lp(a) (g/L)	0.22 ± 0.48	0.25 ± 0.12	0.26 ± 0.39	0.74 ± 0.43	0.34 ± 0.15	0.28 ± 0.12

NOTE. For Lp(a), the median is given.
*$P < .01$, †$P < .02$, ‡$P < .03$: *v* controls.
§$P < .01$, ‖$P < .003$, ¶$P < .0004$, #$P < .02$, **$P < .002$, *v* healthy subjects.

not differ significantly from the controls matched for age and cardiovascular risk factors. Within the female groups, the same was true for cysteine and glutathione. Homocysteine levels were not significantly different in any of the three female groups. The finding that there was not a higher prevalence of hyperhomocysteinemia among patients with premature coronary heart disease is unexpected, since most clinical studies investigating this matter suggest an underlying homocysteinemia in these cases.

The majority of retrospective studies to investigate fasting homocysteine concentrations in patients with coronary heart disease found a positive association. Kang et al[20] first demonstrated in an angiographically controlled study of 443 subjects that patients with coronary heart disease had higher mean total homocysteine concentrations (5.48 ± 1.72 *v* 4.25 ± 1.40 nmol/mL). Similar results have been presented by other groups.[24-29,39-41] This seems in contrast to the results presented in this study. However, a closer look at the study design may explain some of the discrepancies.

In the study by Kang et al,[20] the prevalence of smokers and the mean cholesterol concentrations were not reported. Reporting the cholesterol concentrations would have been particularly important, as homocysteine concentrations are reported to correlate positively with total cholesterol.[20,29,34] Likewise, other studies on this subject had a higher prevalence of hypercholesterolemia in the patient group.[23,28-30] In the study by Genest et al,[34] the patient group had a higher prevalence of hypertensives (42.2% *v* 5.5%), diabetics (12.5% *v* 3.9%), and smokers (67.0% *v* 30.4%). Patients with several cardiovascular risk factors had higher homocysteine concentrations—the higher the homocysteine level, the more risk factors the patient had. Hopkins et al[28] also observed a higher prevalence of hypertension among patients with coronary heart disease and hyperhomocysteinemia. However, mild hyperhomocysteinemia has been associated with hypertension in several studies.[23,33,34,42] Furthermore, hyperhomocysteinemia has been observed in diabetic nephropathy.[43] Further, to the study by Genest et al,[44] several groups investigating homocysteine levels in coronary heart disease reported a higher prevalence of diabetes in the patient group.[23,27,28,30] However, no tests for renal function were mentioned, and diabetic patients were not compared with nondiabetic subjects to rule out any influence of diabetic nephropathy. In the study by von Eckhardtstein et al,[41] the problem of a higher prevalence of cardiovascular risk factors was partially eliminated by multivariate analyses, but differences in the mean homocysteine concentration between patients and controls were not present after adjustment for fibrinogen levels.

Since homocysteine has been positively correlated with age,[27,33,34,40,41] age must be considered in the interpretation of clinical studies on homocysteine. In several instances, significantly younger controls have been used,[25,27-29] the maximum age difference being 14 years between the patient and control groups.[25] If we had selected the young healthy subjects as a control group, there would have been a significant prevalence of homocysteinemia in the patient group (10.5%).

These findings do not exclude the possibility that hyperhomocysteinemia may be provoked by other cardiovascular risk factors or that its prevalence is a result of a difference in age between the study groups. In the present study, we tried to avoid problems relating to group differences. Patients with diabetes or hypertension were excluded. We compared age-matched populations with virtually identical LDL cholesterol concentrations and a similar prevalence of smokers. The difference in homocysteine levels between patients and young healthy subjects are thought to be mainly due to the difference in age, menopausal status in the female subgroups, and different smoking behavior. The results do not exclude that hyperhomocysteinemia may carry an increased risk of developing coronary heart disease. However, they suggest that in a population with premature coronary heart disease and a low prevalence of primary cardiovascular risk factors, hyperhomocysteinemia is probably rare. Obviously, our observation needs to be confirmed by large population studies; however, there have been trials presenting similar results. Boers et al,[32] who excluded patients with diabetes, hypertension, or hyperlipoproteinemia, did not observe a significant difference in postmethionine homocysteine levels in patients with premature coronary heart disease compared with age-matched controls. Likewise, in a prospective clinical trial, Alfthan et al[33] found virtually equal homocysteine levels in patients with different cardiovascular endpoints and their respective controls. Our conclusion is further supported by the results of a large population study in which homocysteine was found to be related to smoking, high blood pressure, and total cholesterol levels.[34]

The cardiovascular risk for patients in the present study may be attributable to differences in lipoprotein metabolism. Male patients had higher mean VLDL triglycerides and lower HDL_3 cholesterol. Both have been associated with an increased risk to develop coronary heart disease.[45-47] Further, the male patients had a significantly higher waist to hip ratio than their respective controls, indicating a higher prevalence of central obesity in the patient group. Central obesity has been associated with low HDL cholesterol, elevated triglycerides, insulin resistance, and a high risk for cardiovascular disease,[48,49] and appears to play a role in this population. It is noteworthy that there was not a higher prevalence of obesity in the patient group. LDL cholesterol and Lp(a) concentrations, both of which were not significantly different from the control levels, do not seem to play a role in this population.

In summary, our results suggest that mild hyperhomocysteinemia is not more frequent in patients with premature coronary heart disease and a low cardiovascular risk profile. The higher prevalence found in other studies may partly relate to differences in age and to the prevalence of more primary cardiovascular risk factors. Further studies in large population samples will be needed to determine the prevalence of hyperhomocysteinemia in the normal population and the significance of the correlation of homocysteine with major cardiovascular risk factors, thus defining whether hyperhomocysteinemia is a marker of vascular disease or a primary, independent, risk factor.

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