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Serum Total Homocysteine and Coronary Heart Disease

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Background. Several studies have observed high plasma levels of homocysteine among patients with coronary heart disease (CHD). The only prospective study was based on US physicians, and concluded that homocysteine was associated with subsequent myocardial infarction (MI). However, the association was limited to those above a threshold level of homocysteine.

Methods. We conducted a nested case-control study among the 21 826 subjects, aged 12–61 years, who were surveyed in the municipality of Tromsø, Norway. Among those free from MI at the screening, 123 later developed CHD. Four controls were selected for each case.

Results. Level of homocysteine was higher in cases than in controls (12.7 ± 4.7 versus 11.3 ± 3.7 μmol/l (mean ± SD); $P = 0.002$). The relative risk for a 4 μmol/l increase in serum homocysteine was 1.41 (95% confidence interval (CI) : 1.16–1.71). Adjusting for possible confounders reduced the relative risk to 1.32 (95% CI : 1.05–1.65). There was no threshold level above which serum homocysteine is associated with CHD events.

Conclusions. In the general population serum total homocysteine is an independent risk factor for CHD with no threshold level.

Keywords: Coronary heart disease, serum total homocysteine, nested case-control study, cardiovascular risk factors

Cardiovascular diseases are a dominant clinical feature among patients with inborn errors of homocysteine metabolism which causes high plasma homocysteine levels and homocystinuria.[1] Notably, these patients are at increased risk of cardiovascular diseases irrespective of the site of the enzymic defect, suggesting that the vascular lesions are caused by homocysteine itself.[2] This hypothesis is supported by a number of clinical studies showing that symptomatic patients with occlusive atherosclerotic disease tend to have elevated plasma total homocysteine levels.[3,4] Also, carotid artery intima-media wall thickening, which probably reflects atherosclerosis,[5] has been associated with high homocysteine levels in a cross-sectional study of asymptomatic subjects.[6]

The only prospective study of homocysteinaemia and cardiovascular disease was based on a selected population of US male physicians participating in a randomized trial.[7] The study indicated that only high levels of homocysteine were independently associated with increased risk of myocardial infarction, suggesting that there may be a threshold level below which homocysteine is not a cardiovascular disease risk factor. The purpose of the present study was to examine the association between homocysteine and coronary heart disease (CHD) by using a population-based prospective study design among the participants in the third Tromsø health study.

MATERIALS AND METHODS

Population Characteristics and Study Design

We conducted a prospective nested case-control study, in which both cases and controls were selected from the same population-based cohort. The Tromsø health study in 1986–1987 examined 10 963 males (mean age = 37.6, range 12–61 years) and 10 863 females (mean age = 35.5, range 12–56 years), representing 81.4% of the eligible population in the municipality of Tromsø.[8] The survey was conducted by teams from the National Health Screening Service in co-operation with the University of Tromsø and local health services and followed almost the same protocol as former screenings in Tromsø[9,10] and the cardiovascular disease study in

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Norwegian counties.[11] Among other items, age, sex, state of health, number of cigarettes smoked per day, and time since last meal were recorded, and serum total cholesterol, high density lipoprotein (HDL) cholesterol and triglycerides, systolic and diastolic blood pressure, body weight and height were measured.

Since the completion of the survey, records have been kept of all patients with a diagnosis of CHD discharged from the University Hospital (the only hospital in the municipality) and all deaths in the screened population have been registered by computer linkage to the National Death Register in the Central Bureau of Statistics. The coding principles follow international rules with minor exceptions.[12] The cases were identified after a follow-up of death until 31 December 1989 and an examination of all discharged from the hospital until 31 December 1990. Among those free from myocardial infarction at the survey (more than 99% of the screened population), 123 had a diagnosis of CHD (ICD-9 410–412) or died suddenly after onset of chest pain (n = 5) during follow-up. Twenty-seven died on the first day of the attack, of whom 20 had an autopsy. When the matching was done, cause of death for the year 1990 was not available for those who had permanently left Tromsø. If cause of death for 1990 had been available, the number of cases would have increased by five.

For each of the cases, four controls were selected after matching for sex, age (whole year at 31 December 1986), and the number of hours since last meal. We chose those subjects with the survey date nearest to the case and who were without disease at the time of diagnosis of the case. For non-fatal cases the controls were selected among subjects living in Tromsø at the time of diagnosis. Serum samples were available for 122 cases and 478 of their controls. The mean duration of follow-up was 4 years.

Collection of Blood Samples and Biochemical Analysis

A non-fasting blood sample was drawn from an antecubital vein. After about 30 minutes the coagulated sample was centrifuged at 1000 g for 10 minutes and the serum fraction aspirated and transferred to plastic tubes. It was kept between 1°C and 10°C, and analysed within 48 hours. The concentrations of total cholesterol, HDL cholesterol, and triglycerides were measured at the Department of Clinical Chemistry, University Hospital of Tromsø by standard methods as previously described.[8] Thereafter the serum samples were stored at −20°C. Most of the samples have been thawed once.

Serum total homocysteine concentration was determined blindly in the stored serum samples using a modification[13] of an automated assay, based on precolumn derivatization with monobromobimane, followed by HPLC.[14] The assay was performed at the Department of Pharmacology and Toxicology, University of Bergen, Norway. The coefficient of variation of the method is 3%.[13]

Statistical Methods

In the analysis we used two-way analysis of variance with match-number of quintuples (1–122) and myocardial infarction (yes/no) as factors[15] and conditional logistic regression.[16] All *P*-values are two-tailed.

RESULTS

Table 1 shows the characteristics of the cases and their matched controls. The cases smoked more cigarettes and had higher mean serum cholesterol and triglycerides and lower HDL cholesterol levels than the controls (all $P < 0.0001$). They also had higher prevalence of angina pectoris and diabetes mellitus (all $P < 0.0001$). The mean blood pressures did not differ significantly between cases and controls.

The cases had 1.4 μmol/l higher mean level of homocysteine than the controls (12.4%, $P = 0.0002$) (Table 1). Figure 1 shows that the whole frequency distribution curve of serum total homocysteine level was shifted to the right for those who later had a myocardial infarction compared to those who remained free of the disease.

In a logistic regression analysis (Table 2), taking into account the individual matching, the coefficient of homocysteine was 0.0860 ± 0.0245 ($\beta \pm$ SE). This corresponds to a relative risk of 1.41 (95% confidence interval (CI) : 1.16–1.71) for each 4 μmol/l (about 1 SD) increase in serum homocysteine level, i.e. an average 41% increase for each 1 SD increase in homocysteine. To assess the independent predictive value of homocysteine, we included serum total cholesterol, HDL cholesterol, triglycerides, systolic blood pressure, the number of cigarettes smoked per day, and history of diabetes and angina pectoris simultaneously in the logistic regression model. In this model, the estimated relative risk decreased to 1.32 (95% CI : 1.05–1.65). The other variables in the analysis came out as expected, although blood pressure and serum triglycerides were not statistically significant independent variables. Serum homocysteine level correlated with total cholesterol ($r = 0.12$; $P = 0.0031$), systolic blood pressure ($r = 0.13$; $P = 0.019$) and the number of cigarettes smoked per day ($r = 0.17$; $P = 0.0001$), but not with HDL cholesterol ($r = 0.01$) or triglycerides ($r = 0.05$) ($n = 600$).

We also assessed the risk for each 4 μmol/l increase in homocysteine among those below or above the

TABLE 1 *Cardiovascular risk factors in 122 cases of coronary heart disease and 478 matched controls*

	Cases $\bar{x} \pm SD$	Controls $\bar{x} \pm SD$	
Age (years)	51.3 ± 7.3	51.2 ± 7.3	Matching factor
Hours since last meal	2.9 ± 2.6	3.0 ± 2.5	Matching factor
Male sex (%)	90.2	90.0	Matching factor
Serum lipids (mmol/l)			
Total cholesterol	7.16 ± 1.18	6.42 ± 1.22	< 0.0001
HDL cholesterol[a]	1.26 ± 0.35	1.46 ± 0.39	< 0.0001
Triglycerides	1.92 ± 0.93	1.49 ± 0.77	< 0.0001
No. of cigarettes per day	11.8 ± 9.3	7.0 ± 9.1	< 0.0001
Blood pressure (mmHg)			
Systolic	135.9 ± 18.2	133.5 ± 17.8	0.2606
Diastolic	83.2 ± 11.3	81.5 ± 11.2	0.1820
History of (%)			
Angina pectoris	11.5	1.0	< 0.0001
Diabetes mellitus	6.6	0.2	< 0.0001
Homocysteine (μmol/l)	12.7 ± 4.7	11.3 ± 3.7	0.0002

[a] High density lipoprotein cholesterol.

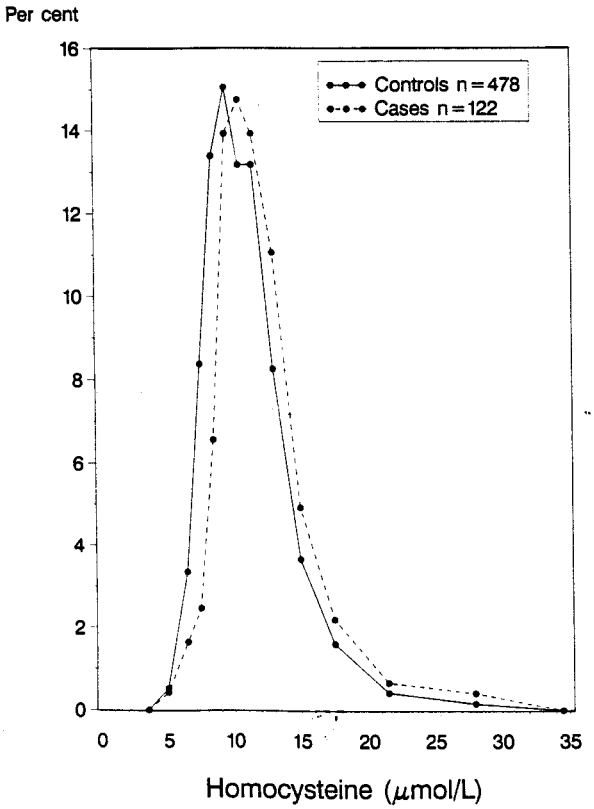

FIGURE 1 *Serum total homocysteine distribution in cases and controls*

median age (53 years), retaining the match in a conditional logistic regression. Among the youngest subjects, the adjusted relative risk was 1.42 (95% CI : 1.05–1.92). This relative risk was higher than that observed in older subjects, in whom the relative risk was 1.18 (95% CI : 0.84–1.65). For women the adjusted relative risk was 1.66 (95% CI : 0.67–4.12).

DISCUSSION

The principal finding of this prospective study is that serum total homocysteine level is an independent predictor of the risk of myocardial infarction in the general population. The association was not materially altered after adjustment for conventional coronary risk factors. The predictive value of homocysteine was lower than for total cholesterol, smoking and HDL cholesterol, but higher than for diabetes mellitus, systolic blood pressure and triglycerides. Because non-response at the baseline survey was low and follow-up almost 100% complete, the generalizability of the results appears sound.

The present study was based on serum samples from non-fasting subjects. This is not optimal for homocysteine determination since the level increases 4–8 hours after food intake (Guttormsen *et al.* unpublished results). The bias was minimized by using time since last meal as matching criteria.

There is an increase in homocysteine level when whole blood is left at room temperature due to the continuous production and release of homocysteine

TABLE 2 *Predictors for coronary heart disease in 122 cases of coronary heart disease and 478 matched controls, using conditional logistic regression analysis*

	Adjusted for the other variables			
	No		Yes	
	β ± SE	*P*	β ± SE	*P*
Homocysteine (μmol/l)	0.086 ± 0.024	0.0004	0.069 ± 0.029	0.0178
Serum lipids (mmol/l)				
Total cholesterol	0.487 ± 0.086	< 0.0001	0.411 ± 0.107	< 0.0001
HDL cholesterol[a]	−1.782 ± 0.355	< 0.0001	−1.258 ± 0.432	0.0036
Triglycerides	0.593 ± 0.123	< 0.0001	0.088 ± 0.166	0.5955
No. of cigarettes per day	0.056 ± 0.011	< 0.0001	0.058 ± 0.013	< 0.0001
Systolic blood pressure (mmHg)	0.006 ± 0.006	0.2649	0.005 ± 0.007	0.4993
History of (yes = 1, no = 0)				
Angina pectoris	3.828 ± 1.039	0.0002	3.362 ± 1.109	0.0024
Diabetes mellitus	3.466 ± 1.061	0.0011	2.378 ± 1.128	0.0351

[a] High density lipoprotein cholesterol.

from the formed elements of blood.[13,17,18] This may have influenced the results, but only marginally since the serum fraction was separated from whole blood within 40 minutes after collection of blood, and since blood samples from the cases and controls were processed according to the same procedures. The artificial increase in serum homocysteine gives the relative highest increase at lowest levels.[13] The sampling conditions may therefore cause an underestimation of the difference in serum homocysteine between cases and controls in the present study.

An important question is whether there is a graded risk of myocardial infarction with increasing levels of homocysteine or whether the excess risk is limited to subjects with clearly abnormal levels. Data obtained among US physicians[7] suggest that the risk does not increase until about the 95th percentile is reached (about 16 μmol/l in that study). The authors estimated that only about 7% of the cases were attributable to elevated levels of homocysteine. In contrast, we observed that the whole distribution curve for homocysteine was shifted to the right for cases compared to controls. This indicates that, within the normal range of the serum homocysteine, there is no threshold level below which homocysteine is not associated with risk of myocardial infarction. A considerable proportion of the population may therefore be at risk due to elevated serum homocysteine level.

The reasons for the discrepancy between the study by Stampfer *et al.*[7] and the present data are not known. We studied an unselected population whereas they investigated a highly selected and healthy sample of physicians.[19] The difference between homocysteine levels among cases and controls in our study was greater than in the US study (12.4% versus 5.7%).[7] The physician study was matched on smoking habits. Our data suggested that a corresponding matching might reduce the difference in homocysteine level between cases and controls to 8–9%. The use of fortified foods and vitamin supplements is common in the US; a recent study found that more than 50% of college graduates used vitamin and mineral supplements.[20] High intakes of vitamin B6, B12 and folate correlate with lower levels of homocysteine,[7] possibly because these vitamins are cofactors or substrates in the conversion of homocysteine to methionine and cysteine.[4] The age range of the US physicians was 40–84 years, while we studied a population aged 34–61 years. In both populations homocysteine was a stronger risk factor among younger than among older subjects. Age differences may therefore contribute to the somewhat conflicting results. We also included women in the study. However, this could not explain the different results, since women showed almost the same relative risk estimate for homocysteine as men, although the estimate, probably due to small numbers (n = 12), was not statistically significant.

The mechanism whereby homocysteine is associated with CHD is not known. Importantly, patients afflicted with various defects involving homocysteine catabolism or remethylation frequently suffer from premature vascular disease. Based on this it has been suggested that the vascular changes are induced by homocysteine itself, and not by a particular metabolic deletion or

some remote metabolic, epigenetic, or phenotypic defect.[2] High levels of homocysteine have been found to produce atherosclerosis in some animal models.[21–23] Experimental studies suggest diverse mechanisms, such as endothelial cell damage, intimal injury, platelet aggregation, coagulation abnormalities,[4] enhanced affinity of lipoprotein(a) for fibrin,[24] inactivation of thrombomodulin and protein C,[25] and oxidative modification of low density lipoprotein.[26,27] The latter possibility has recently been refuted by clinical studies,[28,29] and the processes which are relevant for the vascular injuries in hyperhomocysteinaemic subjects remain to be identified.

The present results support the hypothesis that homocysteine induces vascular disease not only in subjects with rare inborn diseases, but may be important in the general population. Pharmacological doses of folic acid may reduce elevated homocysteine levels both in patients with cystathionine β-synthase deficiency[1] and in healthy subjects without overt folate deficiency defects.[30–32] Although effective means are available for reduction of high homocysteine levels, studies of population determinants of homocysteine levels, with special emphasis on dietary factors, should be done before intervention studies are carried out.

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