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Plasma total homocysteine concentration and the risk of acute coronary events: the Kuopio Ischaemic Heart Disease Risk Factor Studytpdel

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Abstract. Voutilainen S, Lakka TA, Hämelahti P, Lehtimäki T, Poulsen HE, Salonen JT (University of Kuopio, Kuopio, Finland; Tampere University Medical School, Tampere, Finland; University of Copenhagen, Copenhagen, Denmark). Plasma total homocysteine concentration and the risk of acute coronary events: the Kuopio Ischaemic Heart Disease Risk Factor Study. *J Intern Med* 2000; **248:** 217–222.

Objectives. Results from prospective studies concerning the association between plasma total homocysteine (tHcy) concentration and coronary heart disease (CHD) are conflicting. The purpose of this study was to test the hypothesis that plasma tHcy is associated with an increased risk of acute coronary events in middle-aged men.

Design and subjects. We investigated this association in a prospective nested case–control study among Eastern Finnish men aged 42–60 years. Plasma tHcy measurements were carried out for 163 men who had an acute coronary event during an average 8 years and 11 months follow-up of the whole cohort and for 163 control subjects. Both the cases and the controls were from a cohort of 2005 men who had no clinical CHD at the Kuopio Ischaemic Heart Disease (KIHD) baseline.

Results. Men in the highest plasma tHcy concentration quarter had no increase in the risk of coronary events compared with men with lower tHcy concentrations (odds ratio = 0.88, 95% confidence interval 0.44–1.76). Average follow-up time before the first coronary event was 4.9 years (SD 3.2) in men in the highest plasma tHcy quarter and 5.5 years (SD 3.1) in men in the three lowest quarters ($P = 0.368$).

Conclusion. We conclude that plasma tHcy is not associated with an increased risk of coronary events in the middle-aged male population in eastern Finland.

Key words: acute coronary events, homocysteine, myocardial infarction, population studies, prospective studies.

Introduction

A number of clinical and epidemiological studies have noted that increased levels of plasma total homocysteine (tHcy) is an independent risk factor for atherosclerosis and atherothrombosis [1, 2]. Homocysteine is a sulphur-containing amino acid, which is formed only from the dietary essential amino acid methionine [3]. Defects in intracellular homocysteine metabolism lead to the elevation of plasma tHcy. These metabolic defects can have a genetic background, i.e. an inherited enzyme deficiency (cystathionine β-synthetase or 5,10-methylenetetrahydrofolate reductase), or a nutritional background, i.e. an inadequate intake of folate or vitamins B_6 or B_{12} that serve as cofactors or substrates to the enzymes involved in homocysteine metabolism [3]. Men usually have higher tHcy values than women, and homocysteine also increases with age [1, 2].

Although several case–control studies have reported higher plasma tHcy levels in coronary heart disease (CHD) patients than in the control subjects, epidemiological evidence concerning the association between plasma tHcy concentration and CHD is inconsistent [4–16] (Table 1). The purpose of this

study was to test the hypothesis that high tHcy concentration is associated with acute coronary events in middle-aged Finnish men who are free of previous atherosclerotic vascular disease.

Methods

The Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) is a population-based study of risk factors for cardiovascular diseases, atherosclerosis and related outcomes in men from Eastern Finland [17]. The baseline examinations were carried out between March 1984 and December 1989. The study protocol was approved by the Research Ethics Committee of the University of Kuopio. The study sample was composed of 3235 men aged 42, 48, 54 or 60 years at baseline examination. Of these, 2682 (82.9%) participated. Men with prevalent CHD at baseline (n = 677) were excluded from the present analyses, as these diseases could have influenced their dietary habits. Prevalent CHD was defined as either a history of acute coronary event or angina pectoris or positive angina pectoris on effort in Rose interview or the use of nitroglycerin tablets once a week or more frequently. Of the remaining 2005 men, 163 had a coronary event during the follow-up time from March 1984 to December 1996. For each case subject, one control subject was matched for age, examination year and residence.

Outcome measurements

Acute coronary events that occurred between March 1984 and December 1992 were registered as a part of the multinational MONICA (MONItoring of Trends and Determinants in Cardiovascular Disease) Project [1–8] (see also http://www.ktl.fi/publications/monica/manual/index.htm). Data on coronary events between 1993 and 1996 were obtained by record linkage from the national computerized hospital discharge registry. Identical diagnostic classification with that of the FINMONICA project was used. According to diagnostic classification of the events [19] there were 81 definite and 54 probable acute myocardial infarctions (AMI) and 28 typical prolonged chest pain episodes indicating CHD. According to this classification myocardial infarction is probable in patients with typical symptoms but whose ECG and enzyme values do not place them in the definite AMI category. The cases of the present study were 163 of the 2005 men at risk who had their first coronary event by the end of 1996. The average follow-up period for the whole cohort was approximately 8 years 11 months assuming no events.

Determination of plasma total homocysteine concentration

EDTA blood samples were obtained from subjects between 8.00 and 10.00 am after an overnight fast. Subjects were instructed to abstain from alcohol for at least one week. The subjects were also instructed to avoid strenuous exercise during the previous 24 h. Plasma was separated within 60 min and stored at −20 °C until analysis. The plasma tHcy concentration was analysed in 1998 at the Department of Clinical Pharmacology, Rigshospitalet, by gas chromatography mass spectrometry using isotope dilution method [19, 20].

Other measurements

The collection of blood specimens [21] and the measurement of serum lipids [22], lipoproteins [22], blood pressure [21] and urinary excretion of nicotine metabolites [22] have been described previously.

The C677T mutation of methylenetetrahydrofolate reductase (MTHFR) genotypes were determined using PCR amplification, and subsequent restriction analysis by Hinfi enzyme, as described previously [23] in Research Laboratory for Atherosclerosis Genetics, University Hospital of Tampere, Tampere, Finland. Data on MTHFR mutation was available for 56 cases and for 112 control subjects.

Statistical analysis

Differences in risk factors between the cases and the controls were tested for statistical significance with One-way Analysis of Variance (Table 2). To establish the effects of potential confounding factors, we studied Pearson's correlation coefficients (r) between plasma tHcy and cardiovascular risk factors (Table 3). The subjects were classified into quarters according to their plasma tHcy concentration. Odds ratios (ORs) for acute coronary events, adjusted for risk factors, were estimated by stepwise and forced conditional multivariate regression modelling in

Table 1 Prospective studies on plasma tHcy and CHD in subjects free of CHD in study baseline examinations

Study, publication year [Ref.]	Follow-up (Years)	Study population	Cases or Events/ Controls	Sex	Age	tHcy cases/ others	Outcome	Sample storage temperature	Main result (Adjusted Relative Risk, 95% (CI)
Physicians' Health Study 1992 [4]	5	14 916	271/271	M	40–84	11.1/10.5	MI,CHDmort	– 80 °C	3.4 (1.3–8.8)[a]
Thromsø Study*, 1995 [5]	4	21 826	123/492	M + F	12–61	12.7/11.3	CHD	– 20 °C	1.32(1.05–1.65)[b]
BUPA, 1998 [6]	8.7	21 520	229/1126	M	35–64	13.1/11.8	IHD	– 40 °C	2.9 (1.8–4.7)[c]
Framingham Study*, 1999 [7]	10	1933	244	M + F	70 Å 7	?	CVD mort	– 20 °C	1.52 (1.16–1.98)[d]
The Women's Health Study, 1999 [8]	3	28 263	122/244	F	Postmenop.	14.1/12.4	CVD		2.2 (1.2–4.0)[c]
Jerusalem Study*, 1999 [9]	9–11	1788	405	M + F	> 50	?	CVD mort	– 20 °C	1.81(1.19–2.76)[e]
Finnish Study, 1994 [10]	9	7424	149/149	M + F	40–64	10.0/9.8(M)	MI, Stroke	– 20 °C	1.06(0.64–1.77)[f]
Physicians' Health Study 1996 [11]	7.5	14 916	333/333	M	40–84	?	MI	– 80 °C	1.7(0.9–3.3)[a]
MRFIT Study, 1997 [12]	> 11	12 866	93/186	M	35–57	12.6/13.1	MI	– 50–70 °C	0.82(0.55–1.54)[c]
	> 11	12 866	147/286	M	35–57	12.8/12.7	CHDmort	– 50–70 °C	
ARIC Study, 1998 [13]	3.3	15 792	232/395	M + F	Middle-aged	8.9/8.5	CHD	– 70 °C	1.28(0.5–3.2)[e]
Caerphilly cohort, 1998 [14]	5	2290	154	M	50–64	12.4/11.7	IHD	– 20–70 °C	1.4(0.8–2.3)[e]
Rotterdam Study*, 1999 [15]	2.7	7983	120/533	M + F	> 60	17.3/15.2	MI	– 20 °C	2.10(0.88–5.03)[e]
British Regional Heart Study, 1999 [16]	12.8	5661	386/454	M	40–59	14.2/13.5	MI	– 20 °C	1.45(0.88–2.38)[e]

M = male, F = female, MI = myocardial infarction, CHD = coronary heart disease, IHD = ischaemic heart disease, CVD = cardiovascular disease, mort = mortality, CeVD = cerebrovascular disease; *nonfasting samples. [a]For highest 95th vs. lowest 90th percentile, [b]Per 4 μmol L^{-1} increment, [c]for highest vs. lowest quarter, [d]For highest vs. three lowest quarters, [e]for highest vs. lowest fifth, [f]for highest 90th vs. lowest 90th percentile.

Table 2 Characteristics of the study subjects

	Cases Mean ± SD	Controls Mean ± SD	*P* for difference
Plasma tHcy, μmol L^{-1} (n = 326)	11.24 ± 2.86	11.20 ± 3.39	0.804
Age, years (n = 326)	54.2 ± 3.9	54.2 ± 3.9	0.999
Body mass index, kg m^{-2} (n = 326)	27.4 ± 3.9	26.8 ± 3.3	0.112
Systolic blood pressure, mmHg (n = 326)	140.1 ± 17.0	133.8 ± 17.6	0.001
Diastolic blood pressure, mmHg (n = 320)	92.2 ± 10.9	88.6 ± 10.2	0.003
Serum total cholesterol, mmol L^{-1} (n = 325)	6.27 ± 1.23	6.10 ± 1.10	0.192
Serum HDL cholesterol, mmol L^{-1} (n = 326)	1.21 ± 0.27	1.31 ± 0.30	0.002
Serum LDL cholesterol, mmol L^{-1} (n = 326)	4.39 ± 1.04	4.19 ± 1.05	0.081
Urinary excretion of nicotine metabolites, mg day^{-1} (n = 247)	8.04 ± 10.20	4.34 ± 7.97	0.002

paired data (Egret for Windows software, version 1.0). Covariates considered for the logistic regression model were examination years and urinary excretion of nicotine metabolites. We also split plasma tHcy into fifths and tenths and adjusted the model for other cardiovascular risk factors. Confidence intervals (CI) were estimated based on the assumption of asymptotic normality of estimates. All statistical tests were two-tailed.

Results

In this study population mean plasma tHcy concentration was 11.2 (SD 3.1) μmol L^{-1}, ranging from 5.3 to 30.3 μmol L^{-1}. There was no statistically significant difference in the mean tHcy concentration between the case and control subjects (Table 2). Seven (two case and five control) subjects had plasma tHcy concentration more than 20 μmol L^{-1}. The cases had significantly higher systolic and diastolic blood pressure, lower HDL cholesterol and higher urinary excretion of nicotine metabolites than the control subjects (Table 2).

To establish the effects of potential confounding factors, associations between tHcy and established cardiovascular risk factors were explored (Table 3). There was a significant positive correlation between tHcy and urinary excretion of nicotine metabolites. All other correlations were weak and statistically nonsignificant.

In a multiple logistic regression analysis including examination years and urinary excretion of nicotine metabolites, men with a high plasma tHcy concentration (highest quarter, above 12.6 μmol L^{-1}) did not have an increased risk of a coronary event compared to other men (OR = 0.88, 95% CI 0.44–1.76). Splitting plasma tHcy concentration into fifths or tenths or adjustment for other risk factors did not reveal an association between plasma tHcy and coronary event.

Average follow-up time before first coronary event was 4.9 years (SD 3.2) in men in the highest plasma tHcy quarter and 5.5 years (SD 3.1) in men in three lowest quarters (P = 0.368).

The C677T mutation of the MTHFR gene was present as heterozygous in 33.9% (n = 57) and as homozygous in 6.5% (n = 11) of the subjects whose MTHFR genotype was available (n = 168). Mean plasma tHcy concentration was 11.9 (SD 3.04) μmol L^{-1} in men with the homozygous MTHFR

Table 3 Pearsons correlation coefficients (r) and statistical significance between plasma tHcy concentration and cardiovascular risk factors in middle-aged eastern Finnish men

	r	*P*
Body-mass index, kg m^{-2} (n = 326)	0.02	0.777
Systolic blood pressure, mmHg (n = 326)	0.08	0.147
Diastolic blood pressure, mmHg (n = 320)	0.05	0.379
Serum total cholesterol, mmol L^{-1}(n = 326)	− 0.06	0.266
Serum HDL-cholesterol, mmol L^{-1}(n = 326)	− 0.04	0.465
Serum LDL-cholesterol, mmol L^{-1}(n = 325)	− 0.01	0.930
Urinary excretion of nicotine metabolites, mg day^{-1} (n = 247)	0.16	0.014

C677T genotype, 9.9 (SD 1.95) μmol L^{-1} in heterozygotes and 10.9 (SD 2.15) μmol L^{-1} in noncarriers ($P = 0.074$).

Discussion

In this prospective population-based study no association was found between plasma tHcy concentration and either the incidence of acute coronary events or the length of follow-up time before first coronary event amongst cases.

Earlier, the association between plasma tHcy and CHD or CHD mortality was investigated in several prospective studies [4–16] (Table 1). These studies consist of more than 140 000 study subjects with more than 900 000 person-years of follow-up. The reason for the conflicting results is not known, as study populations, age of subjects or follow-up time do not appear to explain this discrepancy (Table 1).

When evaluating our results, some limitations have to be taken into account. First, we can not rule out the possibility that plasma tHcy samples have deteriorated during the storage time of approximately 8.8 years at − 20 °C. In the Physicians' Health Study [4, 11], in the MRFIT Study [12] and in the ARIC Study [13], samples were stored at − 50 to − 80 °C for 3–11 years and these studies also showed no association between tHcy and CHD. However, in most of the prospective studies with positive association, samples have been stored at − 20 °C (Table 1) for as long as for 12 years. Homocysteine is known to be stable for at least one year at − 20 °C [25], and the distribution of values from stored samples is generally similar to those from assays on freshly drawn blood. In our study the samples of the cases and the control subjects had been stored for a similar period of time and were analysed in random order. Thus the long storage time at − 20 °C is not likely to explain the lack of association between plasma tHcy and acute coronary event.

In our study, average follow-up time for the cohort was 8 years 11 months. When the follow-up interval after baseline becomes prolonged, the relationship between tHcy and events may become attenuated. However, there are few studies with almost equal follow-up interval, in which a positive association between tHcy and CHD has been observed [6, 7, 9]. We also analysed the association between plasma tHcy levels and the acute coronary events in shorter follow-up periods. Men with a high plasma tHcy concentration did not have any increased risk of coronary event after either 3 or 5 years of follow-up (data not shown). Thus the length of follow-up is an unlikely explanation for the lack of association. Secondly, we had plasma tHcy concentrations available only from 163 acute coronary event cases and 163 controls. Because we found an association between plasma tHcy levels and coronary events in the prospective nested case–control design, it is very unlikely that this association would be different in the whole cohort.

Although in Finnish studies the mean tHcy concentrations have been similar to that in other studies [10, 26, 27], severe and moderate hyperhomocysteinaemia is relatively uncommon in Finland. In our study there were only seven subjects (2%) who had tHcy values of more than 20 μmol L^{-1}. In 1994 Alfthan and coworkers [10] reported no association between serum tHcy and the risk of stroke or myocardial infarction in a nested case–control study based on 7424 Finnish men and women aged 40–64 years. There was no significantly increased risk for subjects with serum tHcy levels in the upper 5th or 10th percentiles, as compared with the lower percentiles. Serum tHcy samples were collected in 1977, the follow-up period lasted on average nine years until the year 1986, and the measurements were carried out in the 1990s. Alfthan and coworkers suggest that their null findings may have been due to low prevalence of mutations predisposing to hyperhomocysteinaemia in the Finnish population. In our study the prevalence of homozygotes for the MTHFR C677T mutation was 6.5%, which is somewhat lower than what has been reported in other studies (10–48%) [28, 29]. Our results are consistent with the earlier Finnish study [10], and most likely the explanation for our negative results is that highly elevated tHcy values are not common in Finland, which may be due to low frequency of mutations predisposing to high tHcy values.

In conclusion, our results are consistent with the other longitudinal studies that have observed no increased risk of cardiovascular diseases in men with elevated plasma tHcy concentrations.

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