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Serum folate and homocysteine and the incidence of acute coronary events: the Kuopio Ischaemic Heart Disease Risk Factor Study[1–3]

Sari Voutilainen, Jyrki K Virtanen, Tiina H Rissanen, Georg Alfthan, Jari Laukkanen, Kristiina Nyyssönen, Jaakko Mursu, Veli-Pekka Valkonen, Tomi-Pekka Tuomainen, George A Kaplan, and Jukka T Salonen

ABSTRACT

Background: Several, but not all, prospective studies have shown that low folate intakes, low circulating folate concentrations, or high plasma total homocysteine (tHcy) concentrations are associated with an increased risk of coronary artery disease (CAD).

Objective: We examined the relations of both serum folate and serum tHcy concentrations with acute coronary events in middle-aged men from eastern Finland who had no CAD at baseline.

Design: In a population-based prospective cohort study, 1027 men aged 46–64 y were examined in 1991–1993 as part of the Kuopio Ischaemic Heart Disease Risk Factor Study. During an average follow-up of 7.7 y (7900 person-years of follow-up), 114 acute coronary events were observed in 61 men who had no previous history of CAD ($n = 810$).

Results: In a Cox model, compared with men whose serum folate concentrations were in the lowest tertile, those whose concentrations were in the highest tertile had a risk factor–adjusted relative risk of acute coronary events of 0.35 (95% CI: 0.17, 0.73; $P = 0.005$). Serum tHcy concentrations were not significantly associated with the risk of acute coronary events (for the highest tertile compared with the lowest, adjusted relative risk = 1.03; 95% CI: 0.57, 1.87; $P = 0.932$).

Conclusions: The results of this prospective cohort study do not support the hypothesis that a high circulating tHcy concentration is a risk factor for acute coronary events in a male population free of prior heart disease. However, they do suggest that moderate-to-high serum folate concentrations are associated with a greatly reduced incidence of acute coronary events. *Am J Clin Nutr* 2004;80:317–23.

KEY WORDS Serum folate, serum homocysteine, acute coronary events, Kuopio Ischaemic Heart Disease Risk Factor Study, men, Finland

INTRODUCTION

During the past few years, elevated plasma total homocysteine (tHcy) has been one of the most controversial risk factors for heart disease. Homocysteine is a sulfur-containing amino acid that is synthesized from the essential amino acid methionine. Defects in intracellular homocysteine metabolism lead to elevated plasma tHcy concentrations. These metabolic defects can have a genetic or a nutritional background, ie, an inadequate intake of folate or vitamins B-6 or B-12 that serve as cofactors or substrates for the enzymes involved in homocysteine metabolism (1). Approximately two-thirds of cases of elevated tHcy concentration were estimated as being due to low or moderate concentrations of these vitamins (2), of which folate is considered the most important (3). Few previous epidemiologic studies addressed the link between folate and the risk of cardiovascular disease (CVD) (4–13). In some studies, subjects with lower circulating folate concentrations or lower dietary intakes of folic acid had a higher risk of coronary events than did those with higher concentrations or intakes (4–8); however, not all studies found this association (9–13).

Although an elevated plasma or serum tHcy concentration has been hypothesized as a risk factor for CVD, the risk-increasing mechanisms are still poorly understood. It has been proposed that an elevated plasma tHcy concentration may alter the anticoagulant properties of endothelial cells to a procoagulant phenotype, cause dysfunction of the vascular endothelium, or enhance lipid peroxidation (14). Although the intake of folate or folic acid could lower the risk of CVD through the reduction of plasma tHcy concentrations, elevated homocysteine may only be a marker of low folate or vitamin B-6 status or an indicator of an unhealthy lifestyle or existing atherosclerosis rather than a causal risk factor per se (14–16).

In the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD), we previously showed that high serum concentrations and dietary intakes of folate are associated with a significantly reduced risk of acute coronary events (6, 7), but in a nested case-control setting in this same cohort, elevated plasma tHcy concentrations were not associated with any elevated risk of coronary events (17). Therefore, we studied the association of serum folate and tHcy concentrations with coronary events in a subpopulation of the KIHD cohort.

[1] From the Research Institute of Public Health (SV, JKV, JL, KN, JM, V-PV, T-PT, and JTS) and the Department of Public Health and General Practice (THR), University of Kuopio, Kuopio, Finland; the Biomarker Laboratory, National Public Health Institute, Helsinki (GA); the Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor (GAK); and the Inner Savo Health Centre, Suonenjoki, Finland (JTS).

[2] Supported by grants 201688 and 80185 from the Academy of Finland (to SV).

[3] Reprints not available. Address correspondence to S Voutilainen, Research Institute of Public Health, University of Kuopio, PO Box 1627, 70211 Kuopio, Finland. E-mail: sari.voutilainen@uku.fi.

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SUBJECTS AND METHODS

Study design and population

The KIHD is an ongoing, population-based prospective cohort study designed to investigate risk factors for CVD, atherosclerosis, and related outcomes in middle-aged men from eastern Finland (18), a population with one of the highest recorded rates of coronary artery disease (CAD). A total of 2682 participants (82.9% those eligible) aged 42, 48, 54, or 60 y were enrolled in the study between 1984 and 1989. The baseline examinations for the present prospective cohort study were carried out from 1991 to 1993. These examinations were conducted at the same time as the 4-y follow-up survey of the KIHD cohort. Of a total of 1229 men eligible for the study, 52 had died, were suffering from severe illness, or had migrated from the region, and 139 either could not be contacted or refused to participate. Of the remaining 1038 men, data on serum folate and tHcy concentrations were available from 1027. Because previous disease may affect the diet, men with prior CAD ($n = 217$) were excluded from the main analyses of this study. Prior CAD was defined as either a history of myocardial infarction or angina pectoris, the use of nitroglycerin for chest pain $\geq$1 time/wk, or chest pain as the reason for stopping the exercise stress test at baseline. All participants provided written informed consent. The study protocol was approved by the Research Ethics Committee of the University of Kuopio.

Measurements

The subjects donated a venous blood sample between 0800 and 1000. They were instructed to abstain from ingesting alcohol for 3 d and from smoking and eating for 12 h. After the subjects had rested in the supine position for 30 min, blood samples were obtained by venipuncture and collected into vacuum tubes (Venoject; Terumo, Leuven, Belgium). No tourniquet was used. Blood for folate and cholesterol determination and for lipoprotein separation and α-tocopherol, lycopene, and β-carotene measurements was drawn into serum tubes.

Serum folate concentrations were measured by using a radioimmunoassay (Quantaphase II; Bio-Rad, Hercules, CA). Folate measurements were carried out in 1998 on serum samples that were collected during 1991–1993 and kept frozen at −80 °C. The between-batch CVs for quality-control serums (Lyphochek Immunoassay Plus Control levels 1, 2, and 3; Bio-Rad Laboratories, ECS Division, Anaheim, CA) for concentrations of 5.5, 13.4, and 23.6 nmol/L were 6.4%, 6.7%, and 6.7%, respectively ($n = 16$).

Serum tHcy concentrations were analyzed in 2001 in the National Public Health Institute, Helsinki, with the use of HPLC as described by Schwab et al (19). The between-batch CVs ($n = 30$) for 2 pooled serum samples were 4.3% and 5.4%.

Serum for α-tocopherol, lycopene, and β-carotene measurements was stored at −80 °C until the compounds were extracted with ethanol and hexane and measured with the use of an HPLC method and α-tocopherol acetate as an internal standard (20). Lipoproteins were separated from fresh serum samples by combined ultracentrifugation and precipitation (21). An autoanalyzer (Kone Specific; Kone Instruments, Espoo, Finland) was used to enzymatically determine serum total, LDL-, and HDL-cholesterol concentrations (Kone Instruments) and serum triacylglycerol concentrations (Boehringer Mannheim, Mannheim, Germany).

Two trained nurses measured resting blood pressure with a random-zero mercury sphygmomanometer (Hawksley, Lancing, United Kingdom). The measuring protocol was as follows: after the subjects rested in a supine position for 5 min, 3 measurements were made while the subjects were in a supine position, one measurement was made while they were standing, and 2 measurements were made while they were sitting; the measurements were separated by 5-min intervals. For both systolic and diastolic blood pressures, the mean of all 6 measurements was used. Body mass index (BMI) was computed as the ratio of weight to the square of height. A subject was defined as a smoker if he had ever smoked on a regular basis and had smoked cigarettes, cigars, or a pipe within the past 30 d.

Ascertainment of follow-up events

The province of Kuopio participated in the multinational MONICA (MONItoring of Trends and Determinants in CArdiovascular Disease) project (22), in which detailed diagnostic information on all heart attacks that occurred by December 1992 was collected prospectively. The diagnostic classification was made by the FINMONICA coronary registry group (18). Data on acute coronary events between January 1993 and December 1999 were obtained via computer linkage to the national hospital discharge register and were classified by using diagnostic criteria, including symptoms, cardiac enzymes, and electrocardiographic findings, that were identical to those used in the MONICA project, as explained previously (12). The average follow-up time was 7.7 y. If multiple nonfatal events occurred during the follow-up, the first event for each subject was considered as the endpoint for the analyses. According to the diagnostic classification of the events, there were 37 definite and 17 possible acute myocardial infarctions and 7 typical prolonged chest pain episodes in the men who were free of prior CAD at baseline.

Analysis of data

Data are expressed as means $\pm$ SDs. Means were compared by using analysis of variance. The subjects were classified into tertiles according to their serum folate and tHcy concentrations. The relations of serum folate and tHcy with the risk of acute coronary events were analyzed by using Cox proportional hazards models and SPSS version 10.0 (SPSS Inc, Chicago). We used 4 sets of covariates. Model 1 was adjusted for age and examination year; model 2, for the covariates in model 1 plus smoking, BMI, and systolic blood pressure; model 3, for the covariates in model 2 plus serum LDL and HDL cholesterol; and model 4, for the covariates in model 3 plus the following dietary factors: serum lycopene, α-tocopherol, and β-carotene. Relative hazards (risks) adjusted for risk factors were estimated as the antilogarithms of coefficients from multivariate models. The CIs were estimated on the basis of the assumption of asymptotic normality of the estimates. All tests of significance were two-sided.

RESULTS

At the beginning of the follow-up, the mean age of the men in the study was 55.3 y. During the average follow-up time of 7 y and 8 mo, 61 men with no previous heart disease at baseline experienced acute coronary events. Compared with the subjects who did not experience an acute coronary event, those who did were significantly older and had significantly higher systolic blood pressure and serum total and LDL-cholesterol concentrations and significantly lower serum lycopene concentrations (**Table 1**). The mean serum folate and tHcy concentrations in the study cohort were 10.4 nmol/L (range: 2.3–38.7 nmol/L) and

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TABLE 1
Baseline characteristics of subjects who participated in the Kuopio Ischaemic Heart Disease Risk Factor Study and had no previous coronary artery disease at baseline[1]

Characteristic	Subjects who experienced an acute coronary event ($n = 61$)	Other subjects ($n = 749$)
Serum folate (nmol/L)	9.4 ± 4.1[2,3]	10.5 ± 3.9
Serum tHcy (μmol/L)	10.74 ± 3.04	10.79 ± 3.37
Age (y)	57.1 ± 6.3[4]	55.2 ± 6.6
BMI (kg/m^2)	28.0 ± 3.3	27.4 ± 3.6
Systolic blood pressure (mm Hg)	140 ± 14[4]	134 ± 16
Serum cholesterol (mmol/L)		
Total	5.74 ± 0.83[5]	5.49 ± 0.91
LDL	4.16 ± 0.73[6]	3.90 ± 0.80
HDL	1.08 ± 0.32	1.12 ± 0.28
Serum triacylglycerols (mmol/L)	1.79 ± 1.04	1.56 ± 1.03
Serum lycopene (μmol/L)	0.11 ± 0.11[7]	0.17 ± 0.14
Serum α-tocopherol (μmol/L)	29.55 ± 6.96	28.45 ± 7.38
Smoking (%)	32.8	26.0

[1] tHcy, total homocysteine.
[2] $\bar{x}$ ± SD (all such values).
[3] Nearly significantly different from other subjects, $P = 0.052$ (one-way ANOVA).
[4–7] Significantly different from other subjects (one-way ANOVA): [4]$P = 0.029$, [5]$P = 0.045$, [6]$P = 0.019$, [7]$P = 0.003$.

10.8 μmol/L (range: 3.3–51.2 μmol/L), respectively. The mean serum folate concentration was 10% lower ($P = 0.052$) in the men who experienced an acute coronary event than in those who did not. The 2 groups did not differ significantly in mean serum tHcy concentration. Eleven subjects (1 who experienced an acute coronary event and 10 who did not) had a serum tHcy concentration > 20 μmol/L.

The men with higher serum folate concentrations (highest tertile) differed significantly from those with lower concentrations in serum tHcy concentration, age, BMI, systolic blood pressure, serum triacylglycerol concentration, and the concentrations of certain nutritional factors (eg, serum α-tocopherol and lycopene). The men with higher serum tHcy concentrations (highest tertile) differed significantly from those with lower concentrations in serum folate concentration, age, serum total and LDL-cholesterol concentrations, serum triacylglycerol concentration, and serum lycopene concentration (**Table 2**).

Serum folate and acute coronary events

During the follow-up, 10 (3.8%) men with higher serum folate concentrations (highest tertile, serum folate concentration > 11.3 nmol/L) and 28 (10.0%) men with lower serum folate concentrations (lowest tertile, serum folate concentration < 8.4 nmol/L) experienced an acute coronary event ($P = 0.011$) (**Figure 1**). In a Cox proportional hazards model with adjustment for age and examination year, the men with serum folate concentrations in the highest tertile had a relative risk (RR) of acute coronary events of 0.38 (95% CI: 0.19, 0.79) relative to that of the men with concentrations in the lowest tertile. In a Cox model including examination year, age, and traditional CVD risk factors (BMI, smoking, systolic blood pressure, and serum LDL and HDL cholesterol), the RR for those with higher serum folate concentrations remained almost unchanged (0.35; 95% CI: 0.17, 0.73). Furthermore, adjustment for nutritional factors did not attenuate this association (**Table 3**).

To check whether this effect was independent of tHcy concentrations, we also adjusted models 1 and 3 for serum tHcy. This resulted in RRs of coronary events of 0.35 (95% CI: 0.17, 0.72) and 0.33 (95% CI: 0.16, 0.69) in models 1 and 3, respectively, when the men with serum folate concentrations in the highest tertile were compared with those with concentrations in the lowest tertile.

TABLE 2
Characteristics of the study subjects according to baseline serum folate and total homocysteine (tHcy) concentrations[1]

	Serum folate (nmol/L)[2]			Serum tHcy (μmol/L)[2]		
Characteristic	≤11.3 ($n = 545$)	>11.3 ($n = 265$)	P[3]	≤11.26 ($n = 559$)	>11.26 ($n = 251$)	P[3]
Serum folate (nmol/L)	8.3 ± 1.8[4]	14.8 ± 3.5	<0.001	10.9 ± 4.1	9.2 ± 3.4	<0.001
Serum tHcy (μmol/L)	11.05 ± 3.62	10.26 ± 2.63	0.002	9.40 ± 1.29	13.87 ± 4.32	<0.001
Age (y)	55.8 ± 6.5	54.4 ± 6.6	0.007	54.8 ± 6.5	56.5 ± 6.6	0.001
BMI (kg/m^2)	27.2 ± 3.5	28.1 ± 3.6	0.001	27.4 ± 3.6	27.5 ± 3.5	0.759
Systolic blood pressure (mm Hg)	134 ± 17	137 ± 16	0.037	134 ± 17	137 ± 17	0.050
Serum cholesterol (mmol/L)						
Total	5.47 ± 0.82	5.59 ± 0.92	0.077	5.56 ± 0.92	5.39 ± 0.88	0.014
LDL	3.92 ± 0.82	3.92 ± 0.80	0.979	3.95 ± 0.82	3.83 ± 0.78	0.047
HDL	1.10 ± 0.27	1.11 ± 0.30	0.146	1.13 ± 0.28	1.03 ± 0.29	0.121
Serum triacylglycerols (mmol/L)	1.51 ± 1.01	1.70 ± 1.08	0.014	1.56 ± 1.07	1.58 ± 1.01	0.022
Serum α-tocopherol (μmol/L)	27.7 ± 7.5	30.3 ± 7.6	<0.001	28.7 ± 7.2	28.0 ± 8.5	0.221
Serum lycopene (μmol/L)	0.15 ± 0.13	0.20 ± 0.16	<0.001	0.17 ± 0.15	0.14 ± 0.12	0.003
Smoking (%)	27.9	23.7	0.214	28.2	22.7	0.098
Subjects who experienced an acute coronary event (%)	9.4	3.8	0.005	6.6	9.6	0.143

[1] The subjects were participants in the Kuopio Ischaemic Heart Disease Risk Factor Study who had no previous coronary artery disease at baseline.
[2] Highest tertile compared with the 2 lower tertiles.
[3] One-way ANOVA.
[4] $\bar{x}$ ± SD (all such values).

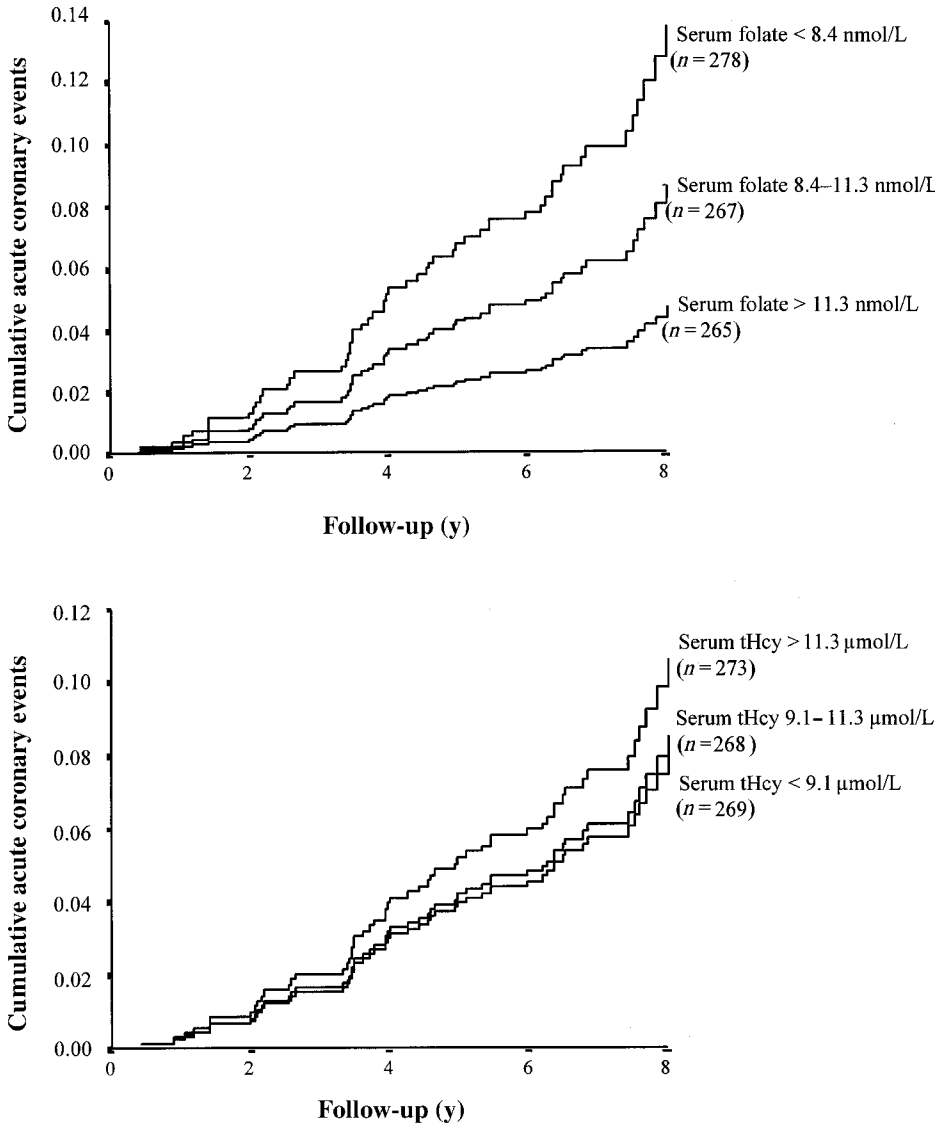

FIGURE 1. Cumulative incidence of acute coronary events in men according to tertiles of serum folate and total homocysteine (tHcy) concentrations after adjustment for age and examination year.

Serum tHcy and acute coronary events

Serum tHcy concentrations were not associated with acute coronary events (**Table 4**). With adjustment for age and examination year, the RR of acute coronary events in the men with higher serum tHcy concentrations (highest tertile) was 1.02 (95% CI: 0.56, 1.85) relative to that of the men with the lowest serum

TABLE 3
Relative risks of acute coronary events by tertile of serum folate concentration in subjects who participated in the Kuopio Ischaemic Heart Disease Risk Factor Study and had no previous coronary artery disease at baseline[1]

	<8.4 nmol/L (*n* = 278)	8.4–11.3 nmol/L (*n* = 267)	>11.3 nmol/L (*n* = 265)	*P* for trend[2]
Model 1[3]	1	0.77 (0.44, 1.35)	0.38 (0.19, 0.79)	0.009
Model 2[4]	1	0.76 (0.44, 1.33)	0.35 (0.17, 0.73)	0.005
Model 3[5]	1	0.76 (0.43, 1.33)	0.35 (0.17, 0.73)	0.005
Model 4[6]	1	0.78 (0.44, 1.38)	0.39 (0.18, 0.83)	0.016

[1] 95% CIs in parentheses.
[2] Cox proportional hazards model.
[3] Adjusted for age and examination year.
[4] Adjusted for the covariates in model 1 plus smoking, BMI, and systolic blood pressure.
[5] Adjusted for the covariates in model 2 plus serum LDL and HDL cholesterol.
[6] Adjusted for the covariates in model 3 plus the following dietary factors: serum lycopene, α-tocopherol, and β-carotene.

TABLE 4
Relative risks of acute coronary events by tertile of serum total homocysteine concentration in subjects who participated in the Kuopio Ischaemic Heart Disease Risk Factor Study and had no previous coronary artery disease at baseline[1]

	<9.55 μmol/L (n = 273)	9.55–11.26 μmol/L (n = 268)	>11.26 μmol/L (n = 269)	P for trend[2]
Model 1[3]	1	0.78 (0.56, 1.85)	1.02 (0.56, 1.85)	0.926
Model 2[4]	1	0.78 (0.39, 1.48)	1.00 (0.54, 1.84)	0.958
Model 3[5]	1	0.73 (0.38, 1.43)	1.04 (0.57, 1.93)	0.854
Model 4[6]	1	0.74 (0.38, 1.46)	1.01 (0.55, 1.87)	0.925

[1] 95% CIs in parentheses.
[2] Cox proportional hazards model.
[3] Adjusted for age and examination year.
[4] Adjusted for the covariates in model 1 plus smoking, BMI, and systolic blood pressure.
[5] Adjusted for the covariates in model 2 plus serum LDL and HDL cholesterol.
[6] Adjusted for the covariates in model 3 plus the following dietary factors: serum lycopene, α-tocopherol, and β-carotene.

tHcy concentrations (lowest tertile). Subdividing the tHcy concentrations into quarters or adjustment for other dietary or risk factors did not uncover any association between tHcy and coronary events.

We also studied the role of tHcy in the primary and secondary prevention of CAD. The mean serum tHcy concentration among 217 men with previous CAD at baseline (P = 0.010 for the difference in tHcy concentration between the men with or without previous CAD at baseline) was 11.5 μmol/L (range: 2.7–32.6 μmol/L). Twenty-four percent (n = 53) of these men had a coronary event during follow-up. Compared with the men with serum tHcy concentrations in the lowest tertile, those with concentrations in the highest tertile had RRs of 1.09 (95% CI: 0.56, 2.14) and 0.96 (95% CI: 0.48, 1.92) in models 1 and 3, respectively. Evaluation of the follow-up time suggested that there was no association for a short follow-up (3 or 5 y) in the men either with or without heart disease at baseline.

Interaction analyses

We repeated the analyses in smokers and nonsmokers. The mean serum folate concentrations in the nonsmokers (n = 595) and smokers (n = 215) were 10.5 ± 4.0 and 10.1 ± 3.9 nmol/L, respectively (P = 0.175). The mean tHcy concentrations in the smokers and nonsmokers were 10.9 ± 3.5 and 10.5 ± 3.0 μmol/L (P = 0.194). The association between serum tHcy and the risk of acute coronary events appeared to be stronger among the smokers than among the nonsmokers. In the nonsmokers, with adjustment for examination year, age, BMI, systolic blood pressure, and serum LDL and HDL cholesterol, the RR of acute coronary events in the men with serum tHcy concentrations in the highest tertile was 0.82 (95% CI: 0.40, 1.69) relative to that of the men with concentrations in the lowest tertile. In the smokers, the respective RR was 2.34 (95% CI: 0.78, 7.01). In a similar analysis of the relation between serum folate and the risk of acute coronary events, the respective RRs were 0.37 (95% CI: 0.15, 0.90) and 0.38 (95% CI: 0.10, 1.42) in the nonsmokers and smokers, respectively.

As in our earlier studies, we divided the cohort into 2 groups on the basis of their alcohol consumption [no or light alcohol consumption and heavy alcohol consumption (>30 g/wk)]. We found no effect of interaction between alcohol consumption and serum folate or tHcy on the risk of acute coronary events.

DISCUSSION

The results of this prospective cohort study in middle-aged men from eastern Finland do not support the hypothesis that a high circulating tHcy concentration is a risk factor for acute coronary events in a male population free of prior CAD. However, they do suggest that moderate-to-high serum folate concentrations are associated with a greatly reduced incidence of acute coronary events. This association was strong, and even adjustment for other dietary factors or traditional CVD risk factors did not attenuate the observed association.

When evaluating our results, some limitations have to be taken into account. First, we cannot rule out the possibility that the serum tHcy samples had deteriorated during storage at −20 °C for ≈7 y. tHcy is known to be stable for ≥1 y at −20 °C (23), and the distribution of values from assays of stored samples is generally similar to that from assays of freshly drawn blood. Alfthan et al (24) evaluated the effect of storage on serum tHcy concentrations in 1994 and observed that samples that were stored for 7 y at −20 °C and thawed twice were stable; the mean serum tHcy concentrations in 1985 and 1990 were 9.1 (range: 6.9–13.2) and 9.3 (range: 7.5–13.2) μmol/L, respectively. In our study, all samples had been stored for a similar period of time and were analyzed in random order. Thus, the storage of serum samples is not likely to explain the lack of association between tHcy and acute coronary events.

Second, our follow-up period was quite short (7.7. y), and we had only a limited number of outcome events. During a longer follow-up period, diet could have changed and attenuated the associations between dietary biomarkers and diseases. However, the association was similar when using a follow-up time of either 3 or 5 y. There have also been other prospective studies in which the follow-up times were equal to or shorter than the follow-up time in our study (25–27). Thus, the length of the follow-up is an unlikely explanation for the lack of association between tHcy and coronary events.

Third, we cannot fully exclude the possibility that part of the association between serum folate and coronary events may reflect confounding by other dietary and lifestyle factors associated with the risk of CAD. In the Finnish diet, folate is found mostly in foodstuffs of plant origin (28); thus, other plant-derived nutrients, such as carotenoids or flavonoids, or even a healthier diet may have contributed to the apparent benefit. However, other

markers of a healthy diet, such as dietary intakes of lycopene, β-carotene, and vitamin E, did not attenuate the association between folate and coronary events in our study. Subjects who exercise more may eat a healthier diet and consume more energy, and they are likely to be the same subjects whose dietary folate intake is the highest. Nonetheless, it is possible that the influence of a healthy lifestyle cannot be fully controlled for in any multivariate model. An increase in the consumption of vegetables, fruit, and whole cereal will also displace other foods considered less healthy, such as meat, sweets, and pastries.

In the same KIHD cohort, we showed previously that high circulating folate concentrations (6) and high dietary folate intakes (7) are associated with a significantly reduced risk of acute coronary events, but in a nested case-control setting in this same cohort, elevated plasma tHcy concentrations were not associated with an elevated risk of coronary events (17). In this earlier report on tHcy from the KIHD, plasma tHcy concentrations at baseline were available from only 164 cases with acute coronary events and only 164 controls (17). Compared with the present study, our earlier study on serum folate had a shorter follow-up time (5 y), only 34 events, and no available serum tHcy measurements (6). In the present study, we had serum tHcy and folate measurements from KIHD 4-y examinations for 1200 cohort members. Because we found no association between circulating tHcy concentrations and coronary events in either of these studies, it is very unlikely that this association would be substantially different in the whole cohort (ie, all subjects for whom measurements were taken during the study's baseline examinations). There are a few previous studies concerning the association between dietary folate intakes or circulating folate concentrations and CAD (4–13). In these studies, both dietary folate intakes (5) and plasma or serum folate concentrations (4, 8) were inversely associated with the risk of CAD, although this association was not found in all prospective cohort studies (9–13). In the Physician's Health Study (9), after adjustment for common CAD risk factors, men in the lowest quintile of plasma folate concentration had a nonsignificantly increased risk of myocardial infarction relative to the risk of men with higher concentrations, and further adjustment for plasma tHcy did not change the observed associations. Therefore, the authors suggested that the increased risk of myocardial infarction might be partly independent of tHcy concentration.

The problems with studies based on plasma or serum folate measurements are that folate may be unstable in frozen plasma and serum samples, and plasma or serum folate concentrations may not be a good indicator of long-term dietary intakes. The consequent increased intraindividual variability in measurements tends to dilute any observed association with disease risks. However, the fact that Finnish study subjects have very low dietary folate intakes increases the range of serum folate concentrations and thus increases the statistical power to detect an association between folate concentrations and the risk of coronary events. In a population with a higher dietary intake of folate only, it is impossible to find an association between nutrients and disease. Our results agree with those of previous prospective studies showing that low intakes of fruit and vegetables and low folate intakes are associated with an increased risk of CAD.

The association between elevated plasma or serum tHcy and CAD incidence or mortality was studied previously in several meta-analyses (29–33). In 2000 Cleophas et al (29) analyzed data from important case-control and cohort studies. They identified 33 studies (22 case-control and 11 cohort studies) and concluded that tHcy may be an indicator of an unhealthy lifestyle rather than an independent risk factor per se. They also pointed out the importance of sufficient adjustment for factors indicating lifestyle, such as the intakes of fat and fiber and physical activity. Ford et al (30) published their meta-analysis on plasma tHcy and CVD in 2002. They calculated the log odds ratio for a 5-μmol/L increase in tHcy concentration and found marked heterogeneity between the results of different study designs. The odds ratio of CAD for a 5-μmol/L increase in tHcy concentration was 1.06 (95% CI: 0.99, 1.13) in 2 cohort studies, 1.23 (95% CI: 1.07, 1.41) in 10 nested case-control studies, and 1.70 (95% CI: 1.50, 1.93) in 26 case-control studies. Ford et al concluded that prospective studies are weaker than case-control studies in detecting any association between tHcy and CAD. A recent meta-analysis assessed the association of serum tHcy concentrations with ischemic heart disease, deep vein thrombosis, and pulmonary embolism (31). The authors concluded that the association between tHcy and CVD may be causal. On the basis of their analysis, a 3-μmol/L reduction in current tHcy concentrations would reduce the risk of ischemic heart disease by 11–20%. In another meta-analysis, Bautista et al (32) collected 14 cohort studies and evaluated an average RR and the likelihood of publication bias. They found no evidence of publication bias, and the average RR of cardiac events was 1.49 (95% CI: 1.31, 1.70). They concluded that hyperhomocysteinemia could moderately increase the risk of a first cardiovascular event, regardless of age or duration of follow-up period. The meta-analysis published by the Homocysteine Studies Collaboration included data from 30 prospective or retrospective studies involving a total of 5073 ischemic heart disease events (33). The authors of this study found stronger associations in retrospective studies, in which blood was collected after the onset of disease, than in prospective studies among subjects who had no history of CVD when blood samples were collected. After adjustment for known CVD risk factors and regression dilution bias, tHcy concentrations that were 25% lower than usual were associated with an 11% lower risk of ischemic heart disease. The authors concluded that elevated tHcy is at most a modest independent predictor of ischemic heart disease in the healthy population. Our negative results from the KIHD published in June 2000 (17) were included in only one of these meta-analyses (31).

Although the reasons for the conflicting results are unknown, and differences in study population or in the age of subjects do not appear to explain the discrepancy, short follow-up periods do tend to result in positive associations. In addition, in some studies with positive results, a fraction of the populations had prevalent heart disease.

The findings of the present study confirm those of previous studies that a diet dominated by plant-derived foods promotes good cardiovascular health. Although a high tHcy concentration may be associated with accelerated atherosclerosis in smokers, it does not appear to predict acute coronary events in healthy men from eastern Finland. High folate intakes efficiently decrease circulating tHcy concentrations, but other CAD-lowering mechanisms may exist (34). Intervention studies may be required to provide more information about the effect of folic acid supplementation on cardiovascular health. Ongoing intervention trials should indicate whether the use of vitamin supplementation to reduce homocysteine concentrations prevents heart disease or whether high circulating tHcy concentrations and low circulating

folate concentrations are simply markers of an unhealthy lifestyle or existing atherosclerosis.

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