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Low serum folate concentrations are associated with an excess incidence of acute coronary events: the Kuopio Ischaemic Heart Disease Risk Factor Study

S Voutilainen[1], TA Lakka[1], E Porkkala-Sarataho[1], T Rissanen[1], GA Kaplan[2] and JT Salonen[1]*

[1]*Research Institute of Public Health, University of Kuopio, Kuopio, Finland; and* [2]*University of Michigan, School of Public Health, Department of Epidemiology, Michigan, USA*

Objective: To test the hypothesis that low serum folate concentrations are associated with an increased risk of acute coronary events in men free of prior coronary heart disease.
Setting: Research Institute of Public Health, University of Kuopio, Kuopio, Finland.
Design: Prospective study in a cohort of 734 men aged 46–64 y examined in 1991–1993 as part of the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) and followed for 5 y and 3 months.
Intervention: Acute coronary events during the follow-up period were obtained by national hospital discharge registry. Baseline serum folate concentrations were measured by radioimmunoassay.
Results: During the follow-up, six (2.5%) men with higher serum folate concentrations (highest third >11.3 nmol/1) and 28 (5.7%) men with lower serum folate (two lowest thirds) developed an acute coronary event ($P = 0.008$). In a Cox model adjusting for age, examination years, and plasma lycopene concentration, in men with higher serum folate concentrations the relative risk for an acute coronary event was 0.31 (95% CI 0.11–0.90, $P = 0.031$) when compared with men with lower serum folates.
Conclusion: This prospective cohort study in middle-aged men from eastern Finland indicates that moderate-to-high levels of serum folate are associated with a greatly reduced incidence of acute coronary events.
Descriptors: myocardial infarction; acute coronary event, folate; homocysteine; cohort studies; prospective studies; Finland
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Introduction

A number of cross-sectional studies have noted higher concentrations of plasma total homocysteine (tHcy) in patients with cardiovascular disease (CVD) as compared with controls, suggesting that elevated plasma tHcy concentration is a risk factor for cardiovascular disease CVD (Boushey *et al*, 1995; Refsum *et al*, 1998). This association has been confirmed in some, but not in all prospective studies (Stampfer *et al*, 1992; Verhoef *et al*, 1994; Perry *et al*, 1995; Arnesen *et al*, 1995; Folsom *et al*, 1998; Wald *et al*, 1998; Alfthan *et al*, 1994; Evans *et al*, 1997). In a cross-country study with WHO MONICA data (Alfthan *et al*, 1997) high plasma tHcy concentrations were associated with increased cardiovascular mortality, and both the mean plasma tHcy concentration and cardiovascular mortality were the highest in Finland. Plasma tHcy concentrations may be elevated due to deficiencies of enzyme activities in homocysteine metabolism or deficiencies of folic acid, vitamin B_6 or B_{12}. Approximately two-thirds of the cases of elevated tHcy have been estimated to be due to low or moderate concentrations of these vitamins (Selhub *et al*, 1993), of which folic acid is considered the most important (Ubbrink *et al*, 1994).

Few previous epidemiological studies have addressed the link between folate and the risk of CVD (Verhoef *et al*, 1998). In these studies, subjects with lower circulating folate concentrations or lower dietary intake of folic acid have had a higher risk of coronary events compared with others, although all studies have not found this association. In a recent European multi-center case–control study (Robinson *et al*, 1998) with 750 cases and 800 controls, low circulating concentrations of folate and vitamin B_6 conferred an increased risk of atherosclerosis.

Although it has been supposed that elevated plasma tHcy concentration is an independent risk factor for CVD, the risk-increasing mechanisms are still poorly understood. It has been proposed that it alters the anti-coagulant properties of endothelial cells to a procoagulant phenotype, causes dysfunction of the vascular endothelium or enhances lipid peroxidation (Jacobsen, *et al* 1998). Although folic acid could lower the risk of CVD through reducing plasma tHcy concentrations, elevated homocysteine may also be only a marker for low folate and vitamin B_6 status rather than a causal risk factor.

*Correspondence: JT Salonen, Research Institute of Public Health, University of Kuopio, PO Box 1627, 70211 Kuopio, Finland.
E-mail: Jukka.Salonen@uku.fi
Guarantor: Professor JT Salonen.
Contributors: Sari Voatilainen undertook most of the data analyses and drafted the paper, TA Lakka classified coronary events, E Porkkala-Sarataho supervised serum folate assays, T Tissanen contributed to the data analyses and checked food recording data, GA. Kaplan and JT. Salonen designed the project and initiated this study.
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Table 1 Characteristics of the study population[a]

	Subjects who developed an acute coronary event (n = 34)	*Other subjects (n = 700)*	*P for difference in means*	*All subjects (n = 734)*
Serum folate (nmol/l)	9.37 ± 4.07	10.47 ± 3.92	0.101	10.42 ± 3.93
Age (y)	57.7 ± 6.3	55.0 ± 6.6	0.018	55.1 ± 6.6
Body mass index (kg/m^2)	27.7 ± 3.2	27.4 ± 3.6	0.699	27.4 ± 3.6
Systolic blood pressure (mmHg)	139.6 ± 14.9	134.7 ± 16.5	0.091	135.0 ± 16.5
Serum total cholesterol (mmol/l)	5.70 ± 0.83	5.49 ± 0.92	0.192	5.50 ± 0.91
Serum LDL cholesterol (mmol/l)	4.13 ± 0.76	3.90 ± 0.81	0.105	3.91 ± 0.81
Serum HDL cholesterol (mmol/l)	1.07 ± 0.31	1.12 ± 0.28	0.343	1.12 ± 0.29
Serum triglycerides (mmol/l)	1.72 ± 0.99	1.55 ± 0.97	0.308	1.56 ± 0.97
Serum lycopene (μmol/l)	0.11 ± 0.12	0.17 ± 0.14	0.019	0.17 ± 0.14
Serum retinol (μmol/l)	2.02 ± 0.41	2.13 ± 0.44	0.166	2.12 ± 0.44
Serum α-tocopherol (μmol/l)	29.66 ± 7.55	28.49 ± 7.65	0.384	28.54 ± 7.64
Smoking (%)	35.3 ± 48.5	26.7 ± 44.2	0.268	27.0 ± 44.4

[a] $\bar{x}$ ± s.d.; statistical analyses were based on one-way ANOVA.

The purpose of this prospective study was to test the hypothesis that low serum folate concentrations are associated with an increased risk of acute coronary events in men free of prior coronary heart disease (CHD).

Methods

Subjects

The Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) is an ongoing prospective population-based cohort study designed to investigate risk factors for CVD, atherosclerosis and related outcomes in middle-aged men from eastern Finland (Salonen, 1988), the population with one of the highest recorded rates of CHD. 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 examination for the present prospective cohort study was carried out during 1991–1993. These examinations were at the same time the 4 y follow-up survey of the KIHD cohort. Out of a total of 1229 men eligible for the study, 52 had died, suffered severe illness, or had migrated from the region, and 139 could not be contacted or refused to participate. Thus, 1038 men were examined in 1991–1993. As previous disease affects the diet, men with prevalent CHD ($n = 298$) were excluded from the present analyses. Of the remaining 740 men, data on serum folate concentration were available for 734 men.

Measurements

The subjects came to gave venous blood samples between 8 and 10 a.m. in the morning. They were instructed to abstain from ingesting alcohol for 3 days and from smoking and eating for 12 h. After the subject had rested in 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 was drawn into serum tubes and blood for α-tocopherol, lycopene, β-carotene and retinol measurements was collected in tubes containing lithium and heparin.

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

Heparin plasma for lycopene, α-tocopherol, β-carotene and retinol determinations was stored at −80°C until extracted with ethanol and hexane and measured by HPLC using alpha-tocopherol acetate as an internal standard (Porkkala-Sarataho *et al*, 1998). Lipoproteins were

Table 2 Characteristics of the study subjects in the two lowest thirds and in the highest third of baseline serum folate concentration[a]

	Serum folate concentration (nmol/l)		
	≤ 11.3 (n = 493)	*>11.3 (n = 241)*	*P for difference*
Serum folate (nmol/l)	8.3	14.7	
Age (y)	55.6	54.1	0.004
Body mass index (kg/m^2)	27.2	28.0	0.005
Systolic blood pressure (mmHg)	134.3	136.3	0.116
Serum triglycerides (mmol/l)	1.49	1.70	0.006
Serum LDL cholesterol (mmol/l)	3.91	3.90	0.845
Serum HDL cholesterol (mmol/l)	1.11	1.14	0.173
Smoking (%)	28.3	24.6	0.294
Serum retinol (μmol/l)	2.05	2.26	< 0.001
Serum α-tocopherol (μmol/l)	27.7	30.3	< 0.001
Serum lycopene (μmol/l)	0.15	0.20	< 0.001
Serum beta-carotene (μmol/l)	0.41	0.40	0.881
Number of subjects who developed an acute coronary event (%)	28 [5.7]	6 [2.5]	0.008

[a] $\bar{x}$ percentage in brackets. Statistical analyses were based on one-way ANOVA.

separated from fresh serum samples by combined ultrasentrifugation and precipitation (Salonen *et al*, 1995). Serum total, LDL and HDL cholesterol (Kone Instruments, Espoo, Finland) and triglyceride (Boehringer Mannheim, Mannheim, Germany) concentrations were determined enzymatically with an autoanalyser (Kone Specific, Kone Instruments, Finland).

Resting blood pressure was measured by two trained nurses with random-zero mercury sphygmomanometer (Hawksley, UK). The measuring protocol included, after supine rest of 5 min, three measurements in supine, one on standing and two in sitting position with 5 min intervals. The mean of all six measurements was used as the systolic and diastolic blood pressure.

Dietary intake of folic acid was assessed with a 4 day food recording at the KIHD baseline examinations. Intake of nutrients was estimated using NUTRICA® software. The data bank of NURTICA® is compiled using mainly Finnish values of nutrient composition of foods.

Ascertainment of follow-up events

The province of Kuopio participated in the multinational MONICA (Monitoring of Trends and Determinants of Cardiovascular Disease) project (Tuomilehto *et al*, 1992), in which detailed diagnostic information of all heart attacks that occurred by December 1992 was collected prospectively. The diagnostic classification was made by the FINMONICA coronary registry group (Salonen, 1988). Data on acute coronary events between January 1993 and December 1997 were obtained by computer linkage to the national hospital discharge register and classified by one internist (TAL) using identical diagnostic criteria, including symptoms, cardiac enzymes and electrocardiographic findings, as explained previously (Tuomilehto *et al*, 1992). The average follow-up time was 5 y and 3 months. If multiple non-fatal events occurred during the follow-up, the first event for each subject was considered as end point for the analyses. According to the diagnostic classification of the events there were 20 definite and 10 possible acute myocardial infarctions (AMI), and 4 typical prolonged chest pain episodes.

Statistical methods

The data are expressed as means ± s.d. Means were compared by analysis of variance (ANOVA). The subjects were classified into thirds according to their serum folate concentration. The relationship of serum folate to the risk of acute coronary events was analysed using Cox proportional hazard models. Risk factor adjusted relative hazards (risks), adjusted for other risk factors, were estimated as the antilogarithms of coefficients from multivariate models. The confidence intervals were estimated on the basis of the assumption of asymptotic normality of the estimates. All tests of significance were two-sided.

Results

The baseline characteristics of the study cohort, separately for cohort members who developed an acute coronary event and subjects who did not are shown in Table 1. The mean age of the study men was 55.1 (± 6.6) y. Subjects who developed an acute coronary event were older ($P = 0.018$) and had lower serum lycopene concentration ($P = 0.019$) than other subjects. The mean serum folate concentration was 10.4 nmol/l (2.3 − 38.7 nmol/l) in the study cohort. Serum folate concentration was 10.5% lower in subjects who developed an acute coronary event than in those who did not.

We compared men in the highest third of serum folate concentration (>11.3 nmol/l) to those with lower serum folate concentrations. Men with higher serum folate concentration differed statistically significantly from men with lower folate concentrations with regard to age, serum triglycerides, body mass index, and with regard to nutritional factors including serum lycopene, serum retinol, and serum alpha-tocopherol (Table 2). During the follow-up time of 5 years and 3 months, six (2.5%) men with higher serum folate concentrations (highest third >11.3 nmol/l) and 28 (5.7%) men with lower serum folate (two lowest thirds) developed an acute coronary event ($P = 0.008$) (Table 2). In a Cox proportional hazard model adjusting for age, examination years, and plasma lycopene concentration, in men with higher serum folate concentrations the relative risk for acute coronary event was 0.31 (95% CI 0.11 − 0.90, $P = 0.031$) when compared with men with lower serum folates. In a Cox model including examination years, age, and traditional CVD risk factors (smoking, systolic blood pressure, serum LDL and HDL cholesterol), the relative risk for those with higher serum folates remained almost identical (0.30, 95% CI 0.10 − 0.84, $P = 0.023$).

Discussion

This prospective cohort study in middle-aged men from eastern Finland indicates that moderate-to-high concentrations of serum folate are associated with greatly reduced incidence of acute coronary events. The association is strong and adjustment for other dietary factors or traditional CVD risk factors did not attenuate the observed association.

There are a few limitations in this study. First, we have no plasma tHcy data available. In a prospective Physician's Health Study (Chasan-Taber *et al*, 1996), after adjusting for six other risk factors men in the lowest plasma folate fifths had an increased risk of myocardial infarction (MI) compared with those with higher concentrations. After adding plasma tHcy in the model, the relative risk remained unchanged. The authors suggested that the increased risk for MI may be partly independent of homocysteine elevation. Because plasma tHcy data were not available, we could not examine whether plasma tHcy levels could explain any of the association between serum folate concentration and acute coronary events. Secondly, we cannot fully exclude the possibility that part of the association may reflect confounding by other dietary and lifestyle factors associated with a reduced risk of CHD. In the Finnish diet, folic acid is found mostly in foodstuffs of plant origin (National Public Health Institute: Nutrition Report, 1998), and other nutrients such as carotenoids or flavonoids may contribute to the apparent benefit. However, scrum concentrations of lycopene, vitamin A and E, also markers of healthy diet, did not confound this association in our study cohort. Finally, our follow-up period is quite short (5.25 y) and we have a very limited number of events, so we could not study the association in possibly relevant subgroups.

An association between dietary intake of folic acid or circulating folate concentration and CVD has been studied earlier in several studies (Chasan-Taber *et al*, 1996; Morrison *et al*, 1996; Rimm *et al* 1988; Folsom *et al*, 1998; Giles *et al*, 1995, 1998). In the prospective Physician's Health

Study (Chasan-Taber *et al*, 1996), men with the lowest 20% of plasma folate concentrations had a 1.4 fold (95% CI 0.9–2.3) relative risk compared with those in the top 80%, adjusting for common cardiovascular risk factors. Although the results were not statistically significant, the authors concluded that low dietary intake of folic acid contributes to the risk of MI. In the Nutrition Canada Survey cohort study (Morrison *et al*, 1996), low serum folate concentrations were associated with an increased 15-y CHD mortality among both men and women. Increased risks were not restricted to individuals with extremely low serum folate concentrations, but were observed for individuals with normal concentrations as well. In the Nurses' Health Study (Rimm *et al*, 1988) after controlling for cardiovascular risk factors, the relative risk of CHD comparing the extreme folic acid intake fifths was 0.69 (95% CI 0.55–0.87). The authors also found the strongest apparent benefit of a high-folate diet among women who consumed alcohol. In the Atherosclerosis Risk in Communities Study, neither plasma folate concentration nor folic acid dietary intake had an association with CHD (Folsom *et al*, 1998). In the prospective First National Health and Nutrition Examination Survey Epidemiologic Follow-Up Study (Giles *et al*, 1998), the relative risk of CHD was the greatest for persons in the lowest serum folate quarter among persons aged 35–55 y, whereas among persons over 55 y the relative risk for CHD was the greatest in persons in the highest serum folate quarter. In a recent European multi-center case-control study (Robinson *et al*, 1998) with 750 cases and 800 control subjects, low circulating concentrations of folate and vitamin B_6 conferred an increased risk of atherosclerosis. The reason for the conflicting results is not known, neither as study populations, nor differences in age of subjects or follow-up time appear to explain this discrepancy.

Although it is supposed that even moderately increased plasma tHcy concentrations are associated with an elevated risk of vascular disease in the coronary, cerebral and peripheral arteries, the risk-increasing mechanisms of plasma tHcy are still poorly understood. Theoretically folate is an important substrate in the remethylation of homocysteine back to methionine. According to a recent meta-analysis (Homocysteine Lowering Trialists' Collaboration, 1998), after standardization to pretreatment blood concentrations of homocysteine of 12 μmol/l and folate of 12 nmol/l (approximate average concentrations for Western populations), supplemented folic acid reduced blood homocysteine concentrations by 25%, with similar effects in the range of 0.5–5 mg folic acid daily. Although folic acid could lower the risk of CHD through reducing plasma homocysteine concentrations, homocysteine may also be only a marker for folate and vitamin B_6 status rather than a causal risk factor. Brattström and co-workers examined recently in their meta-analysis an association between the common methylenetetrahydrofolate reductase gene mutation, which causes hyperhomocysteinaemia, with homocysteine concentrations, and the risk of vascular diseases (Brattström *et al*, 1998). They concluded that, although this gene mutation is a major cause of mild hyperhomocysteinaemia, it does not increase cardiovascular risk. They concluded that mild hyperhomocysteinaemia found frequently in vascular disease patients is not causally related to the pathogenesis of the vascular disease.

The mean serum concentrations of the folate in our study were similar to those reported previously in other studies (Giles *et al*, 1995 1998). The mean daily dietary intake of folic acid measured at the KIHD baseline was 227 μg/day, and mean daily intake more than the currently recommended daily allowance (RDA) 300 μg/day was observed for only 13% of the study subjects. Before 1986, the RDA for dietary folic acid was 400 μg/day, and it was then changed to 180 μg/day for women and 200 μg/day for men and again in 1997 to 300 μg/day for both men and women. We believe that the recently recommended daily allowance of 400 μg/day could be beneficial for cardiovascular health.

Together with the previous findings, our results support the theory that folate has a role in the prevention of CHD. Intervention studies are required to test the effect of folic acid supplementation in cardiovascular health.

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