

Folate and vitamin B-12 and risk of fatal cardiovascular disease: cohort study from Busselton, Western Australia

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Abstract

Objective To test the hypothesis that the incidence of fatal coronary heart disease and cardiovascular disease in a general population is related to serum and red cell folate and vitamin B-12 concentrations.

Design Cohort study with follow up of 29 years.

Setting Busselton, Western Australia.

Participants 1419 men and 1531 women aged 20 to 90 years, who were alive more than three years after their participation in the 1969 Busselton health survey. 2314 (78.4%) had no cardiovascular disease at the initial survey.

Main outcome measures Hazard ratios for fatal coronary heart disease and cardiovascular disease in men and women according to baseline concentrations of serum and red cell folate and serum vitamin B-12.

Results 213 men and 159 women died from coronary heart disease, and 342 men and 302 women died from cardiovascular disease. Serum and red cell folate concentrations showed a moderate positive correlation ($r=0.26$, $P<0.001$) but otherwise serum and red cell folate and serum B-12 concentrations were not strongly correlated with each other or with other standard risk factors. After age and standard risk factors were adjusted for, there was no independent association between folate and B-12 concentrations and death from coronary heart disease or cardiovascular disease in the full cohort or the subcohort with no cardiovascular disease at baseline. The multivariate adjusted hazard ratio for death from cardiovascular disease in the lowest versus the highest category of red cell folate concentration was 1.05 (95% confidence interval 0.77 to 1.43) in men and 1.10 (0.81 to 1.51) in women.

Conclusions These findings do not support the hypothesis that lower folate and B-12 concentrations increase the risk of fatal cardiovascular disease in a general population. The routine use of these vitamins for preventing cardiovascular disease should await evidence from clinical trials.

Introduction

Epidemiological studies have shown that moderate hyperhomocysteinaemia is an independent risk factor for coronary, cerebral, and peripheral vascular disease.^{1 2} Studies have also shown that moderately raised concentrations of homocysteine are prevalent in the general population and that an inverse relation exists between homocysteine concentration and concentrations of folate and vitamins B-6 and B-12.^{3 4} Randomised clinical trials have shown that low dose vitamin supplementation, particularly with folic acid, significantly lowers homocysteine concentrations.⁵ However, there are no clinical trial data that prove that lowering homocysteine concentrations prevents deaths from cardiovascular disease or coronary heart disease.

Prospective evidence linking circulating or dietary levels of folate and vitamins B-6 and B-12 in the general population to incident cardiovascular disease remains limited and inconsistent.⁶⁻¹¹ The purpose of this cohort study was to test the hypothesis that an increased incidence of fatal coronary heart disease and cardiovascular disease was related to lower concentrations of serum and red cell folate and serum vitamin B-12 in a community population.

Participants and methods

This study is based on 1772 men and 1904 women aged 20 to 90 years who attended the 1969 Busselton health survey, representing a 90% participation rate for the total adult population; cross sectional health surveys of adults listed on the electoral roll were undertaken in Busselton, Western Australia, every three years from 1966 to 1981.

The methods of the surveys have been described elsewhere.^{12 13} The participants were asked to complete a comprehensive health and lifestyle questionnaire and to undergo various measurements and tests (see bmj.com).

Outcome

Data on deaths were available to 31 December 1998. Deaths among survey participants were identified by linkage to the death register. Survival was confirmed by linkage to the electoral roll and Telecom White Pages and through relatives. We ascertained vital status at 31 December 1998 for 98% of the cohort. The survival times for the remaining 2% were censored at the last time known to be alive. The underlying cause of death was determined from the death certificates.

Analysis of data

There were 1486 men and 1570 women available for analysis after we excluded pregnant women and those with missing values for primary risk factors or adjustment variables. Of these, 67 men and 39 women died within three years of the 1969 survey and have also been omitted, leaving a total of 1419 men and 1531 women. A total of 1113 men and 1201 women (78.4% of cohort) had no history of coronary heart disease, leg claudication, or stroke at initial survey and were considered free from cardiovascular disease. We analysed data from this subcohort separately.

The risk factors of primary interest were serum and red cell folate concentrations and serum B-12 concentration. We also included other baseline risk factors for cardiovascular disease in the multivariate analysis: age, systolic and diastolic blood pressure, body mass index, serum cholesterol concentration, white cell count, smoking, menopause (in women), treatment for diabetes, treatment for hypertension, alcohol intake, and history of coronary heart disease, stroke, or leg claudication.^{14 15}

We used Cox proportional hazards regression analysis of survival to death or end of follow up to

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Table 1 Adjusted hazard ratios* (95% confidence intervals) for death among participants surviving three years after baseline according to quarters of serum and red cell folate and serum vitamin B-12 concentrations

	Men (n=1419)			Women (n=1531)		
	All deaths	Cardiovascular disease deaths	Coronary heart disease deaths	All deaths	Cardiovascular disease deaths	Coronary heart disease deaths
Serum folate ($\mu\text{g/l}$):						
0 to 2.99	1.07 (0.80 to 1.43)	1.06 (0.70 to 1.60)	1.10 (0.63 to 1.91)	1.19 (0.88 to 1.61)	1.04 (0.69 to 1.56)	1.14 (0.65 to 2.03)
3.00 to 4.49	1.13 (0.93 to 1.38)	1.23 (0.94 to 1.62)	1.44 (1.01 to 2.06)	1.15 (0.93 to 1.43)	0.97 (0.73 to 1.30)	1.13 (0.77 to 1.67)
4.50 to 5.99	0.95 (0.78 to 1.17)	0.91 (0.68 to 1.21)	1.34 (0.92 to 1.89)	1.07 (0.84 to 1.35)	0.88 (0.64 to 1.20)	0.95 (0.62 to 1.47)
≥ 6.00 †	1.00	1.00	1.00	1.00	1.00	1.00
Trend P value	0.93	0.81	0.58	0.23	0.79	0.80
Red cell folate ($\mu\text{g/l}$):						
0 to 199.9	1.03 (0.83 to 1.29)	1.05 (0.77 to 1.43)	1.26 (0.86 to 1.85)	1.15 (0.91 to 1.46)	1.10 (0.81 to 1.51)	1.00 (0.65 to 1.53)
200 to 274.9	1.00 (0.81 to 1.22)	1.03 (0.78 to 1.36)	1.07 (0.75 to 1.53)	0.97 (0.76 to 1.24)	0.89 (0.64 to 1.24)	0.75 (0.48 to 1.18)
275 to 349.9	1.14 (0.92 to 1.42)	0.89 (0.64 to 1.22)	0.90 (0.60 to 1.36)	1.11 (0.86 to 1.42)	1.00 (0.71 to 1.39)	0.93 (0.60 to 1.46)
≥ 350 †	1.00	1.00	1.00	1.000	1.00	1.00
Trend P value	0.72	0.80	0.35	0.92	0.93	0.63
Vitamin B-12 (ng/l):						
0 to 269.9	0.99 (0.80 to 1.22)	1.14 (0.85 to 1.53)	1.09 (0.74 to 1.60)	1.04 (0.83 to 1.31)	0.88 (0.65 to 1.20)	0.74 (0.48 to 1.14)
270 to 329.9	1.10 (0.89 to 1.35)	1.23 (0.92 to 1.65)	1.30 (0.90 to 1.89)	1.00 (0.78 to 1.27)	0.94 (0.68 to 1.29)	0.82 (0.52 to 1.27)
330 to 389.9	0.90 (0.71 to 1.14)	0.87 (0.62 to 1.23)	1.09 (0.72 to 1.65)	0.96 (0.75 to 1.24)	0.89 (0.64 to 1.25)	0.88 (0.56 to 1.37)
≥ 390 †	1.00	1.00	1.00	1.00	1.00	1.00
Trend P value	0.65	0.24	0.30	0.86	0.50	0.37

*Hazard ratios are adjusted for age, systolic and diastolic blood pressure, body mass index, cholesterol concentration, white cell count, smoking, treatment for diabetes, treatment for hypertension, alcohol intake, and history of coronary heart disease, stroke, or leg claudication.

†Reference group for hazard ratio.

assess the influence of primary risk factors after adjustment for age only and also after adjustment for age and other risk factors.¹⁴⁻¹⁵ As the age adjusted and multivariate adjusted results were similar, only the multivariate results are reported. Vitamin concentrations were examined as continuous variables and also in categories.

Results

At baseline the average age was about 48 years and the risk factor characteristics were typical of population samples surveyed around that time. Serum folate and red cell folate concentrations showed a moderate positive correlation ($r=0.26$; $P<0.001$) but neither serum nor red cell folate and vitamin B-12 concentrations were correlated with each other or with other risk factors.

After 29 years' follow up, 665 men and 537 women had died (excluding subjects who died within three years of initial survey). Death was due to cardiovascular disease in 342 men and 302 women and to coronary heart disease in 213 men and 159 women. In the subcohort of participants without cardiovascular disease, 475 men and 362 women had died; deaths were due to cardiovascular disease in 226 men and 187 women and to coronary heart disease in 131 men and 98 women.

Tables 1 and 2 show the multivariate adjusted hazard ratios for folate and vitamin B-12 quarters in relation to deaths from all causes, cardiovascular disease, and coronary heart disease. There was no consistent or significant pattern of relative risk for serum and red cell folate and serum B-12 concentrations in men and women in the full cohort. Among women in the subcohort without cardiovascular disease, there was a generally inverse association between serum folate concentrations and risk of death, but the only significant relative risk was for all cause mortality, and the trend P value was not significant at the 5% level.

Among men in the subcohort without cardiovascular disease, the trend model suggested an

unexpected positive association between serum folate concentration and risk of death from cardiovascular disease ($P=0.05$), but the categorical model showed no association. When a subgroup of 85 men (7% of the disease-free cohort) with serum folate concentrations $>9 \mu\text{g/l}$ and about double the risk of death from cardiovascular disease were removed from the analysis, there was no longer evidence of a trend for death from cardiovascular disease ($P=0.66$). There was no evidence of any other significant relation.

Discussion

We found no evidence of an independent association between folate or vitamin B-12 concentrations and death from cardiovascular or coronary heart disease. The trends in risk factors and mortality in the Busselton population are similar to those in other parts of Australia.¹⁵ In addition, adjusted estimates of relative risk of coronary heart disease and stroke in the Busselton population seem consistent with estimates in other populations of similar age and ethnicity.¹⁴⁻¹⁶

The 1969 Busselton health survey measured serum and red cell folate and serum vitamin B-12 concentrations as a means of estimating the nutritional status of the population.¹⁷ Folate and B-12 nutrition seemed to be generally sufficient, with only 3.1% of the population having a reduced folate and 0.4% having a reduced B-12 concentration based on the normal reference intervals for red cell folate (115-600 $\mu\text{g/l}$) and serum B-12 (160-850 ng/l).¹⁷ However, the absolute vitamin concentrations were unimportant as analysis was based on estimating relative risks.

Folate

Red cell folate indicates tissue folate status and reflects folate turnover over the preceding two to three months.¹⁸ It is a more reliable indicator of long term folate intake than serum folate, which has a high intraindividual variability because it reflects intake only in the preceding few days.¹⁸

Table 2 Adjusted hazard ratios* (95% confidence intervals) for death among participants who had no cardiovascular disease at baseline and who survived three years according to quarters of serum and red cell folate and serum vitamin B-12 concentrations

	Men (n=1113)			Women (n=1201)		
	All deaths	Cardiovascular disease deaths	Coronary heart disease deaths	All deaths	Cardiovascular disease deaths	Coronary heart disease deaths
Serum folate (µg/l):						
0 to 2.99	0.88 (0.61 to 1.26)	0.77 (0.45 to 1.33)	0.84 (0.40 to 1.77)	1.51 (1.03 to 2.21)	1.39 (0.79 to 2.46)	1.35 (0.61 to 3.10)
3.00 to 4.49	1.00 (0.79 to 1.26)	0.98 (0.70 to 1.38)	1.15 (0.72 to 1.84)	1.23 (0.95 to 1.59)	1.05 (0.73 to 1.51)	1.11 (0.68 to 1.81)
4.50 to 5.99	1.00 (0.79 to 1.28)	0.98 (0.69 to 1.39)	1.67 (1.07 to 2.60)	1.11 (0.83 to 1.49)	1.04 (0.70 to 1.55)	1.10 (0.64 to 1.89)
≥6.00†	1.00	1.00	1.00	1.00	1.00	1.00
Trend P value	0.23	0.05	0.47	0.08	0.64	0.69
Red cell folate (µg/l):						
0 to 199.9	0.96 (0.74 to 1.25)	1.06 (0.73 to 1.53)	1.17 (0.71 to 1.91)	1.08 (0.81 to 1.45)	1.07 (0.72 to 1.59)	0.91 (0.53 to 1.54)
200 to 274.9	0.98 (0.77 to 1.25)	1.04 (0.73 to 1.46)	1.17 (0.75 to 1.84)	1.01 (0.75 to 1.36)	0.96 (0.63 to 1.47)	0.73 (0.41 to 1.31)
275 to 349.9	1.13 (0.87 to 1.47)	0.92 (0.61 to 1.39)	0.96 (0.55 to 1.67)	0.98 (0.73 to 1.33)	0.76 (0.50 to 1.17)	0.71 (0.40 to 1.24)
≥350†	1.00	1.00	1.00	1.00	1.00	1.00
Trend P value	0.83	0.79	0.42	0.72	0.75	0.82
Vitamin B-12 (ng/l):						
0 to 269.9	1.05 (0.82 to 1.35)	1.16 (0.81 to 1.66)	1.17 (0.72 to 1.91)	0.94 (0.71 to 1.25)	0.76 (0.51 to 1.15)	0.68 (0.39 to 1.21)
270 to 329.9	1.25 (0.98 to 1.60)	1.44 (1.01 to 2.05)	1.65 (1.04 to 2.62)	1.03 (0.78 to 1.36)	1.02 (0.69 to 1.49)	0.92 (0.55 to 1.54)
330 to 389.9	1.02 (0.77 to 1.34)	0.83 (0.53 to 1.28)	1.09 (0.63 to 1.88)	0.77 (0.56 to 1.06)	0.68 (0.44 to 1.07)	0.68 (0.37 to 1.24)
≥390†	1.00	1.00	1.00	1.00	1.00	1.00
Trend P value	0.85	0.35	0.46	0.62	0.21	0.42

*Adjusted for age, systolic and diastolic blood pressure, body mass index, cholesterol concentration, white cell count, smoking, treatment for diabetes, treatment for hypertension, and alcohol intake.

†Reference group for hazard ratio.

Studies examining the association between serum or dietary folate concentrations and coronary heart disease have produced inconsistent results. Morrison et al found a significant inverse association between serum folate concentration and 15 year mortality from coronary heart disease, especially in women.⁶ However, they found no association between dietary folate consumption and risk of fatal coronary heart disease. Other cohort studies with follow up periods ranging from 3.3 to 20 years have found no significant increase in risk of non-fatal or fatal coronary heart disease for participants in the lowest versus highest group of serum folate concentration.⁷⁻⁹ The high intraindividual variability of serum folate measurements may have diluted any observed association with risk of coronary heart disease in these studies as well as in our study.

By contrast, the US Nurses' health study¹⁰ and the Kuopio ischaemic heart disease risk factor study¹¹ of Finnish men found a significant inverse association between dietary folate intake and coronary heart disease events over 14 and 10 years respectively. However, these results need to be interpreted with caution as a high folate intake from food and vitamin supplements may also be related to other dietary factors or unmeasured risk behaviours that are independently associated with coronary heart disease.

Vitamin B-12

We found no association between vitamin B-12 concentration and death from cardiovascular or coronary heart disease. This may not be surprising given that vitamin B-12 supplementation has a relatively small effect on homocysteine concentration^{4,5} and B-12 deficiency is uncommon in the general population, being usually related to a problem of absorption rather than nutrition.

Implications

Our negative findings together with the inconsistent results of previous cohort studies leave many questions unanswered about the effect of folate and vitamin B-12

on homocysteine concentrations and risk of cardiovascular disease in the general population. The epidemiological data for homocysteine as a risk factor for cardiovascular disease is strong, and clinical trials have shown that homocysteine concentrations can be lowered by safe and inexpensive doses of folic acid and vitamin B-12.

Our results do not argue against public health efforts to raise folate consumption in the general population by increased intake of fruits, vegetables, and fortified grains and cereals.¹⁹ However, use of vitamin supplements to lower homocysteine concentrations should not be routinely recommended in the general

What is already known on this topic

Moderate hyperhomocysteinaemia is thought to be an independent risk factor for cardiovascular disease

High homocysteine concentrations in the general population are mainly due to insufficient folate and B vitamin concentrations

Evidence linking serum or dietary folate and B vitamin levels to incident cardiovascular disease is inconclusive

What this study adds

A large community cohort followed for 29 years showed no independent association of baseline serum and red cell folate and serum B-12 concentrations with mortality from cardiovascular disease

Vitamin therapy to lower homocysteine concentrations should not be routinely recommended in the general population until the benefit is proved by controlled clinical trials

population for prevention of cardiovascular disease until their benefit is proved by controlled clinical trials.

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- 1 Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *JAMA* 1995;274:1049-57.
- 2 Eikelboom JW, Lonn E, Genest J Jr, Hankey G, Yusuf S. Homocyst(e)ine and cardiovascular disease: a critical review of the epidemiologic evidence. *Ann Intern Med* 1999;131:363-75.
- 3 Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *JAMA* 1993;270:2693-8.
- 4 Ubbink JB. Vitamin nutrition status and homocysteine: an atherogenic risk factor. *Nutr Rev* 1994;52:383-7.
- 5 Homocysteine Lowering Trialists' Collaboration. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. *BMJ* 1998;316:894-8.
- 6 Morrison HI, Schaubel D, Desmeules M, Wigle DT. Serum folate and risk of fatal coronary heart disease. *JAMA* 1996;275:1893-6.
- 7 Chasan-Taber L, Selhub J, Rosenberg IH, Malinow MR, Terry P, Tishler PV, et al. A prospective study of folate and vitamin B6 and risk of infarction in US physicians. *J Am Coll Nutr* 1996;15:136-42.
- 8 Folsom AR, Nieto FJ, McGovern PG, Tsai MY, Malinow MR, Eckfeldt JH, et al. Prospective study of coronary heart disease incidence in relation to fasting total homocysteine, related genetic polymorphisms, and B vitamins: the atherosclerosis risk in communities (ARIC) study. *Circulation* 1998;98:204-10.
- 9 Ford ES, Byers TE, Giles WH. Serum folate and chronic disease risk: findings from a cohort of United States adults. *Int J Epidemiol* 1998;27:592-8.
- 10 Rimm EB, Willett WC, Hu FB, Sampson L, Colditz GA, Manson JE, et al. Folate and vitamin B6 from diet and supplements in relation to risk of coronary heart disease among women. *JAMA* 1998;279:359-64.
- 11 Vuolteenaho S, Rissanen TH, Virtanen J, Lakka TA, Salonen JT, Kuopio Ischemic Heart Disease Risk Factor Study Group. Low dietary folate intake is associated with an excess incidence of acute coronary events. *Circulation* 2001;103:2674-80.
- 12 Cullen KJ. Mass health examinations in the Busselton population, 1966 to 1970. *Med J Aust* 1972;2:714-8.
- 13 Knuiman MW, Jamrozik K, Welborn TA, Bulsara MK, Divitini ML, Whittall DE. Age and secular trends in risk factors for cardiovascular disease in Busselton. *Aust J Public Health* 1995;19:375-82.
- 14 Knuiman MW, Vu HT, Bartholomew HC. Multivariate risk estimation for coronary heart disease: the Busselton health study. *Aust NZ J Public Health* 1998;22:747-53.
- 15 Knuiman MW, Vu HT. Risk factors for stroke mortality in men and women: The Busselton study. *J Cardiovasc Risk* 1996;3:447-52.
- 16 Knuiman MW, Vu HT. Prediction of coronary heart disease mortality in Busselton, Western Australia: an evaluation of the Framingham, national health epidemiologic follow up study, and WHO ERICA risk scores. *J Epidemiol Commun Health* 1997;51:515-9.
- 17 Davis RE. Serum vitamin levels and human nutrition. *Proc Nutr Soc Aust* 1979;4:45-52.
- 18 Babior BM. The megaloblastic anemias. In: Beutler E, Lichtman M, Coller B, Kipps T, Seligsohn U, eds. *Williams hematology*. 6th ed. Chicago: McGraw-Hill, 2001:425-45.
- 19 Malinow MR, Bostom AG, Krauss RM. Homocyst(e)ine, diet, and cardiovascular diseases: a statement for healthcare professionals from the nutrition committee, American Heart Association. *Circulation* 1999;99:178-82.

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Impact of changing diagnostic criteria on incidence, management, and outcome of acute myocardial infarction: retrospective cohort study

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Acute myocardial infarction used to be defined by criteria based on symptoms, changes in electrocardiograms and the concentrations of cardiac enzymes, as recommended by the World Health Organization.¹ Specific markers of myocardial damage, including troponin T, are more sensitive indicators than total creatine kinase concentration for ischaemic myocardial necrosis and prognosis.²

In 2000, the European Society of Cardiology and the American College of Cardiology recommended changing the diagnostic criteria for acute myocardial infarction to include raised troponin T concentrations in addition to changes in electrocardiograms or coronary intervention.³ Some patients with acute coronary syndrome who had been diagnosed as having unstable angina are now classified as having myocardial infarction. We investigated the impact of using the new criteria on the incidence, management, and outcome of myocardial infarction.

Participants, methods, and results

Since 1997, all patients admitted with chest pain to Monklands Hospital, Airdrie, had their troponin T concentrations measured. We identified patients admitted between April 1997 and December 2000 with a principal diagnosis of acute myocardial infarction,

according to the old criteria, from routine discharge data, the databases of the coronary care unit and laboratory, and case notes. We used the databases to identify patients admitted for chest pain who had raised troponin T concentrations (≥ 0.1 ng/ml) in the absence of non-myocardial causes such as renal failure, thromboembolic disease, or myocarditis. The new criteria increased admissions for myocardial infarction by 58%, from 1671 to 2637; this equated to approximately 160 000 additional myocardial infarctions per year in the United Kingdom.

Compared with patients who met the old criteria, the additional 966 patients identified were older (median age 74 v 68 years; $P < 0.001$; Mann Whitney U test) and a higher proportion were women (47% v 38%; $P < 0.0001$, χ^2 test). Thrombolysis was given to only 13 of the additional patients compared with 672 patients who met the old criteria (1% v 40%; $P < 0.0001$; χ^2 test). As a result, thrombolysis rates fell from 40% (95% confidence interval 38% to 42%) to 26% (24% to 28%).

Linkage to national admission (Scottish morbidity record) and death data (General Registrar's Office) provided information on survival, readmission for ischaemic heart disease, coronary angiography, and coronary revascularisation. We calculated cumulative