The Use of B Vitamin Supplements and Peripheral Arterial Disease Risk in Men Are Inversely Related[1]

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ABSTRACT Peripheral arterial disease (PAD) causes morbidity and is associated with mortality. B vitamin intake has been inversely associated with coronary heart disease, but their effects on PAD are not known. We examined prospectively the relationships between dietary folate, vitamin B-6 and B-12 and PAD risk in 51,529 male U.S. health professionals, aged 40 to 75 y, who answered a detailed 131-item questionnaire to assess diet and vitamin supplement use. The study population consisted of 46,036 men free of PAD, cardiovascular disease and diabetes at baseline followed for 12 y during which we documented 308 incident PAD cases. For every 400 μg/d increment of folate intake, the multivariate adjusted PAD risk decreased by 21% [relative risk (RR) = 0.79, 95% CI 0.64–0.96]. Men in the top category of folate intake (median = 840 μg) were at 33% lower risk of PAD than men in the bottom category (median = 244 μg) (RR = 0.67, 95% CI 0.45–0.96, *P*-value, test for trend = 0.03) after multivariate adjustment. There were weak inverse associations between intake of vitamin B-6 and PAD risk (RR = 0.70, 95% CI 0.48–1.02, *P*-value, test for trend = 0.06) and B-12 (RR = 0.77, 95% CI 0.54–1.11, *P*-value, test for trend = 0.12). These results suggest that higher consumption of folate may contribute to the prevention of PAD. J. Nutr. 133: 2863–2867, 2003.

KEY WORDS: • *folate* • *peripheral arterial disease* • *vitamin B-6* • *vitamin B-12* • *homocysteine*

High plasma homocysteine increases platelet aggregation (1), oxidative stress (2) and vascular smooth muscle proliferation, decreases nitric oxide production (3) and impairs endothelial function (4). Consistent with these adverse cardiovascular effects, elevated concentrations of homocysteine have been positively associated with the risk of coronary heart disease (CHD)[3] (5) and peripheral arterial disease (PAD) (6). A low intake of folate limits the remethylation of homocysteine to methionine (7) and increases the concentration of plasma homocysteine (8). Vitamins B-6 and B-12 are cofactors that contribute to the conversion of homocysteine to cysteine or methionine, respectively (9); low intakes of these vitamins can potentially increase homocysteine. A meta-analysis with serum homocysteine as the outcome has estimated a 25% reduction in homocysteine after folate supplementation, which increased by 7% when vitamin B-12 was added (8). In a prospective study, serum concentrations of folate were inversely associated with carotid artery thickening (10). An inverse association between folate intake and risk of CHD (11) has also been reported, but there are no studies on PAD. We therefore studied prospectively intakes of folate, vitamin B-6 and vitamin B-12 in relation to PAD in a large group of men.

SUBJECTS AND METHODS

Study population. The Health Professionals Follow-up Study began in 1986 when 51,529 male U.S. health professionals, aged 40–75 y, volunteered to participate after Institutional Review Board approval (12). The participants received questionnaires at baseline and biennially to determine lifestyle and medical conditions, and validated food-frequency questionnaires (FFQ) every 4 y to determine diet.

We excluded men with a history of PAD (n = 41), CHD (n = 2288), stroke (n = 342) and diabetes (n = 1381) because they may have changed their diets as a result of disease, and 1437 men with inadequate dietary data (reported energy intake <3352 or >17598 kJ, or >70 blanks out of 131 items in the FFQ) leaving 46,036 men in this analysis. We censored men at time of death, development of diabetes or date of return of last questionnaire, whichever came first. Deaths were reported by family members, the Postal Service or ascertained through state registries or the National Death Index. Ascertainment of death was >98% complete (13).

Case ascertainment. If a participant reported intermittent claudication or surgery for PAD during follow-up, we requested permission to review his medical record to confirm the diagnosis and the

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[3] Abbreviations used: ABPI, ankle brachial blood pressure index; CHD, coronary heart disease; FFQ, food-frequency questionnaire; MET, metabolic equivalents; MTHFR, methylene tetrahydofolate reductase; PAD, peripheral arterial disease; RDA, Recommended Dietary Allowance; RR, relative risk.

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date of occurrence of the disease. Cases of PAD were considered definite if the medical record contained either a report of surgery for peripheral arterial disease, ankle systolic blood pressure index (ABPI) <0.80, a physician diagnosis, or an angiogram or Doppler ultrasound reporting ≥50% obstruction of at least one artery plus symptoms in the ipsilateral limb. Participants who confirmed the diagnosis of PAD by letter or by telephone contact but without available medical records were considered probable PAD cases.

Diet and exposure information. We assessed diet in 1986 and updated it every 4 y via validated semiquantitative FFQ as described elsewhere (12). The deattenuated correlation between FFQ and diet records for vitamin B-6 was 0.64; the correlation between FFQ and serum folate was 0.63, and FFQ and serum vitamins B-6 and B-12, 0.37 (14).

The enrollment and follow-up questionnaires included information on age, smoking, hypertension, diabetes mellitus, hypercholesterolemia, angina, supplement use, weight and physical activity. Metabolic equivalents (MET), a measure of energy expenditure defined for each activity as multiples of time spent sitting quietly in a chair, were used to assess physical activity. BMI was computed by dividing weight in kilograms by the squared height in meters. All time-varying covariates were updated every 2 y.

Statistical analysis. We derived nutrients from the FFQ and adjusted them for total energy intake using the residual method (15). We then calculated the energy-adjusted nutritional exposures in three ways. In the first approach, we assessed nutrient intakes at baseline in 1986, and used these estimates for the entire period of follow-up. In the second or simple updating approach, for each 2-y period, we used the most recent (within the last 4 y) available measure of nutrient intakes. In the third approach, we used cumulatively averaged intake to estimate the long-term dietary pattern, and calculated the means of all available dietary measurements (16). PAD incidence between each questionnaire cycle was related to the mean dietary intake before that period (17). For example, disease incidence from 1986 to 1988 was related to intake measured in 1986, and disease incidence from 1990 to 1992 was related to the mean intake from 1986 to 1990. We stopped updating diet in the event of myocardial infarction, stroke or coronary artery graft by-pass surgery and assigned it the value of the preceding time period (16). If dietary data were missing for one questionnaire, we used the value derived from the immediately preceding questionnaire. In addition to analyses based on total nutrient intake, we analyzed nutrients from foods in the following two ways: *1*) excluding men who took supplements or nutrients from multivitamins; and *2*) adjusting for nutrients from supplements.

We measured incidence rates of PAD by quintile categories of nutrient intakes stratifying by age and smoking using the Mantel-Haenszel method (18). For the multivariate analyses, we used the Cox proportional hazard model with failure time measured in age in months. In these analyses, we adjusted for variables shown to be confounders or effect modifiers in previous studies (19). We assessed smoking (never smokers, past smokers who had smoked for <20 y, 20–30 y and >30 y, and current smokers who smoked 1–14, 15–24 or ≥25 cigarettes/d), BMI (<21 to 21–22.9, 23–24.9, 25–29.9 and 30+ kg/m^2), alcohol use (never, 0.1–4.9, 5.0–14.9, 15.0–29.9 and 30+ g/d), and physical activity (quintile categories of MET); hypertension, hypercholesterolemia, family history of heart disease and use of supplements (vitamin C, E and folate) were treated as dichotomous variables; total energy was continuous. We adjusted associations for measurement error of folate intake using data from a validation study in a subsample of men who completed detailed, weighed diet records using the regression calibration method (20). All tests were two-sided and the significance level of the *P*-values was 0.05.

RESULTS

In 12 y of follow-up, there were 308 incident cases of PAD. In the 223 definite cases 113 (51%) had a history of surgery, 58 (26%) ABPI <0.8, 47 (21%) had a physician diagnosis on the chart and 7 (3%) had abnormal Doppler ultrasound results. In 73 of the 85 probable cases, we received a note confirming the diagnosis, whereas the remaining 12 were confirmed over the telephone. Because the results were consistent after excluding the probable cases, we included them in all analyses.

The median baseline intakes in the lowest and highest quintile categories ranged from 244 to 840 μg/d for folate, 1.7 to 9.9 mg/d for vitamin B-6 and 5 to 22 μg/d for vitamin B-12. One 177-mL (6-oz) glass of orange juice for example, contains 330 μg of folate, one slice of dark bread, 0.24 mg vitamin B-6, and one egg, 1.2 μg of vitamin B-12 (21). Men in the highest quintile category of folate intake were less likely to smoke and more likely to take vitamin supplements than those in the lowest quintile category of intake. They also consumed more carotenoids and fiber and less saturated fat. Intake of vitamin B-6, but not vitamin B-12, was similarly associated with lifestyle and dietary variables (**Table 1**).

There was a significant inverse linear association between folate intake and PAD risk after multivariate adjustment. For every 400 μg/d increase in folate intake (cumulatively averaged) there was a 21% decline in PAD risk [relative risk (RR) = 0.79, 95% CI 0.64–0.96]. This association was further strengthened after measurement error correction (46% reduction in PAD risk; RR = 0.54, 95% CI 0.30–1.00). After categorizing folate intake into quintile categories, men in the top quintile category were at 32% lower risk of PAD than men in the bottom quintile category (RR = 0.67, 95% CI 0.47–0.96, *P*-value, test for trend = 0.03) (**Table 2**). This association was significant after additional adjustment for fruits and vegetables intake (RR comparing extreme quintile categories = 0.69, 95% CI 0.48–0.99, *P*-value, test for trend = 0.02), almost unchanged after adjustment for saturated fat (RR = 0.67, 95% CI 0.47–0.97, *P*-value, test for trend = 0.03), fiber (RR = 0.68, 95% CI 0.46–0.98, *P*-value, test for trend = 0.04), fish (RR = 0.65, 95% CI 0.45–0.93, *P*-value, test for trend = 0.02), nuts (RR = 0.68, 95% CI 0.47–0.97, *P*-value, test for trend = 0.03), but attenuated after adjustment for vitamin E (RR = 0.74, 95% CI 0.51–1.08, *P*-value, test for trend = 0.11), vitamin B-6 (RR = 0.72, 95% CI 0.44–1.17, *P*-value, test for trend = 0.27), and vitamin B-12 (RR = 0.73 95% CI 0.49–1.11 *P*-value, test for trend = 0.14). After excluding men taking supplemental folate, folate from food was not associated with PAD risk (RR comparing the top to bottom quintile categories of intake = 0.82, 95% CI 0.52–1.30, *P*-value, test for trend = 0.44). Also, folate from foods was not associated with PAD risk after adjusting for folate from supplements. Men taking supplemental folate (32% of total) had a 32% lower PAD risk than those who did not in the multivariate model (RR = 0.68, 95% CI 0.52–0.89).

There was an inverse association between cumulatively averaged vitamin B-6 intake and PAD risk, but this was not significant after adjustment for smoking, and other covariates (Table 2). It was further attenuated after additional adjustment for folate (RR = 0.92, 95% CI 0.58–1.46, *P*-value, test for trend = 0.73), vitamin B-12 (RR = 0.81 95% CI 0.54–1.23, *P*-value, test for trend = 0.28), or supplemental vitamin E (RR = 0.85, 95% CI 0.57–1.26, *P*-value, test for trend = 0.37). There was an inverse relationship between recent intake of vitamin B-6 and PAD risk (Table 2) (RR comparing extreme categories of intake = 0.61, 95% CI 0.42–0.88, *P*-value, test for trend = 0.01).

There was a nonsignificant inverse association between vitamin B-12 and PAD after adjusting for confounders (Table 2). Vitamin B-6 and B-12 intakes measured as continuous covariates were not associated with PAD risk in multivariate models. Intakes of vitamin B-6 and B-12 were correlated with

TABLE 1

Baseline characteristics of men by highest and lowest quintile categories of energy-adjusted folate, vitamin B-6 and B-12 intakes[1,2]

	Folate		Vitamin B-6		Vitamin B-12	
	Quintile 1 244 μg/d, *n* = 9339	Quintile 5 840 μg/d, *n* = 9127	Quintile 1 1.7 mg/d, *n* = 9482	Quintile 5 10.0 mg/d, *n* = 9118	Quintile 1 5 μg/d, *n* = 10,722	Quintile 5 22 μg/d, *n* = 8825
Current smoker, %	15.8	8.3	14.8	9.3	9.0	11.5
Family history of MI, %	11.5	12.1	11.8	12.1	13.0	11.9
Hypertension, %	19.9	22.0	18.2	22.1	20.5	21.1
Hypercholesterolemia, %	9.7	13.1	8.4	13.6	12.5	10.8
Supplemental vitamin E, %	16.3	93.4	7.2	89.7	15.7	70.0
Supplemental vitamin C, %	27.6	95.2	18.4	96.5	28.9	76.4
Supplemental folate, %	3.5	94.2	3.6	69.2	8.3	62.3
Nondrinker, %	24.6	23.8	24.2	23.3	24.5	19.5
BMI, *kg/m²*	25.3	24.6	25.1	24.7	24.9	25.1
Physical activity, *MET*	7.8	15.1	8.3	14.6	12.3	12.5
Vitamin C, *mg*	117	663	130	801	183	438
Vitamin B-6, *mg*	1.8	5.9	1.7	10.0	2.0	5.7
Vitamin B-12, *μg*	7	16	7	16	5	22
Folate, *μg*	244	840	270	678	325	645
Vitamin C from food, *mg*	100	179	121	166	158	159
Vitamin E, *mg*	8.8	28.4	8.6	96.1	9.8	23.5
Vitamin E from food, *mg*	9.2	11.2	9.5	11.0	10.4	10.6
α-Carotene, *μg*	500	720	516	705	623	653
β-Carotene, *μg*	2938	5030	3108	5000	4129	4464
Lycopene, *μg*	6745	9376	7342	9154	9195	8752
Lutein and zeaxanthin, *μg*	2077	3715	2434	3592	3193	3348
Total carotene, *μg*	1620	2818	1727	2798	2297	2458
Total fiber, *g*	15.6	22.5	16.1	21.9	20.4	20.1
Cereal fiber, *g*	3.9	5.7	4.1	5.5	5.0	4.9
Fiber from fruit, *g*	2.2	4.6	2.3	4.4	3.9	3.8
Fiber from vegetables, *g*	4.7	7.0	5.1	6.8	6.4	6.4
Total saturated fat, *g*	27.4	22.5	26.9	22.9	22.8	24.6
Total monounsaturated fat, *g*	30.3	25.7	29.8	26.0	26.2	27.4
Total polyunsaturated fat, *g*	12.9	12.7	13.1	12.8	13.0	12.7
Trans-fat, *g*	3.2	2.4	3.2	2.4	2.6	2.6
Cholesterol, *mg*	300	279	284	288	247	340
Total energy, *kJ*	7605	7701	7245	7768	7739	7630
Fruit, *servings/d*	1.1	2.4	1.3	3.4	2.1	2.1
Vegetables, *servings/d*	1.9	3.0	2.0	2.0	2.7	2.7

[1] Values are percentages or median intakes.
[2] Abbreviations: MI, myocardial infarction; MET, metabolic equivalents.

intake of total folate ($r = 0.36$, and $r = 0.33$, respectively), but not with folate from food sources only ($r = 0.07$ and $r = 0.09$, respectively).

To remove confounding by multivitamin or folate supplement use we examined the associations of vitamin B-6 and B-12 with the risk of PAD among men who did not use those supplements. The relative risk of PAD comparing the extreme quintile categories of intake for vitamin B-6 was 1.29 (95% CI 0.55–3.01, *P*-value, test for trend = 0.66), and that for vitamin B-12 was 0.59 (95% CI 0.59–1.47, *P*-value, test for trend = 0.40).

Cumulatively averaged folate intake had a consistent inverse association with PAD in subgroups, and there was no evidence of interaction after stratification by men who were <70 y old (*P*-value, test of interaction = 0.86), overweight (BMI ≥25 kg/m^2) (*P*-value, test of interaction = 0.93), took supplemental vitamin E (*P*-value, test of interaction = 0.18), or current smokers (*P*-value, test of interaction = 0.24). The results for vitamin B-6 and B-12 did not change significantly in the subgroups. We also assessed the effect of folate among men who abstained from alcohol, and those who consumed 0.1–14.9 g/d or >15 g/d. There was no significant interaction between folate intake and alcohol consumption on the risk of PAD. The inverse association between cumulatively averaged folate intake and risk of PAD seemed stronger among current smokers (RR = 0.63, 95% CI 0.39–0.99, *P*-value test for trend = 0.02) than among nonsmokers (RR = 0.89, 95% CI 0.43–1.83, *P*-value test for trend = 0.90), but the interaction was not significant.

DISCUSSION

In this large cohort of men followed for 12 y, we found an inverse association between folate intake and risk of PAD that was independent of other PAD risk factors. The prospective nature of the study minimized differential recall of folate intake by those men who developed PAD. Nondifferential recall would most likely attenuate the true association with PAD. Indeed, correction of measurement error strengthened the inverse association of folate intake and PAD. The inverse association of folate and PAD was seen mainly among men who took supplemental folate; because the primary source of supplemental folate was a multivitamin supplement, the possibility that the effect observed was due to some other constituent of supplements, some other dietary or lifestyle behaviors of supplement users cannot be excluded. Because only

TABLE 2

Association of folate, vitamin B-6 and B-12 intakes and the incidence of PAD in men[1,2]

	Quintile categories of intake					*P*-value, test for trend
	1	2	3	4	5	
Total folate						
Median intake, *μg/d*	244	317	388	517	840	
Age and smoking adjusted cumulatively averaged	1.00	0.86 (0.59–1.26)	0.80 (0.54–1.18)	0.67 (0.45–1.01)	0.76 (0.51–1.12)	0.05
Multivariate adjusted cumulatively averaged	1.00	0.82 (0.58–1.17)	0.80 (0.56–1.14)	0.66 (0.45–0.96)	0.67 (0.45–0.96)	0.03
Multivariate adjusted simple updating	1.00	0.96 (0.68–1.36)	0.81 (0.57–1.17)	0.56 (0.38–0.83)	0.74 (0.52–1.05)	0.03
Vitamin B-6						
Median intake, *mg/d*	1.7	2.1	2.6	4.1	9.9	
Age and smoking adjusted cumulatively averaged	1.00	1.02 (0.67–1.53)	1.13 (0.75–1.70)	0.96 (0.63–1.44)	0.79 (0.51–1.23)	0.24
Multivariate adjusted cumulatively averaged	1.00	0.89 (0.62–1.29)	0.94 (0.65–1.36)	0.76 (0.52–1.11)	0.70 (0.48–1.02)	0.06
Multivariate adjusted simple updating	1.00	0.77 (0.53–1.11)	0.99 (0.69–1.41)	0.63 (0.44–0.92)	0.61 (0.42–0.88)	0.01
Vitamin B-12						
Median intake, *μg/d*	5	7	10	14	22	
Age and smoking adjusted cumulatively averaged	1.00	0.96 (0.65–1.41)	0.77 (0.52–1.14)	0.80 (0.55–1.17)	0.75 (0.51–1.09)	0.07
Multivariate adjusted cumulatively averaged	1.00	1.01 (0.70–1.46)	0.84 (0.58–1.22)	0.81 (0.56–1.17)	0.77 (0.54–1.11)	0.12
Multivariate adjusted simple updating	1.00	1.04 (0.72–1.50)	0.76 (0.52–1.11)	0.75 (0.52–1.08)	0.74 (0.52–1.06)	0.06

[1] Multivariate models adjusted for smoking (never smokers, past smokers who had smoked for <20 y, 20–30 y and >30 y, and current smokers who smoked 1–14, 15–24 or ≥25 cigarettes/d); BMI (<21 to 21–22.9, 23–24.9, 25–29.9 and 30+ kg/m^2); alcohol (never users to those consuming 0.1–4.9, 5.0–14.9, 15.0–29.9 and 30+ g/d); hypertension; hypercholesterolemia; family history of early heart disease; physical activity in quintile categories of MET; and energy (continuous).

[2] Abbreviations: PAD, peripheral arterial disease; MET, metabolic equivalents.

modest attenuations of the relative risks between folate and PAD were observed with additional adjustment for physical activity, dietary saturated, polyunsaturated, monounsaturated and *trans*-fats, dietary carotenoids, fiber and fruit and vegetable consumption, it is unlikely that dietary or lifestyle factors explained this relationship. Because adjustment for vitamins E, B-6 and B-12 (all constituents of multivitamin supplements) attenuated the association between folate intake and PAD risk, we could not preclude the possibility that some constituent of supplements, other than the B vitamins, explained this association. Folate intake including supplement sources was inversely related to PAD risk in a linear dose manner; the association was strengthened after measurement error correction, and there are plausible biological mechanisms that could explain its action on PAD risk. Even though we could not separate folate intake per se from multivitamin supplement use and PAD risk in these analyses, it is clear that multivitamin supplements reduced PAD risk and possibly the effect was due to folate.

The lack of association with folate from foods could be due to its lower biological activity than folate from supplements. It is also possible that there was more measurement error in folate from foods than from supplements, but we did not have the data to evaluate this. Because diet in this analysis was last updated in 1994, we did not observe the effects of the food fortification that was introduced in the United States in 1996. Interactions between folate and alcohol consumption and cigarette smoking were not significant, but the statistical power with which to evaluate them was modest.

We did not observe clear inverse associations between long-term (cumulatively averaged) vitamin B-6 and B-12 intake and risk of PAD. This may be because there were few men with low intakes of these vitamins. The median intake in the lowest quintile category of B-6 was 1.7 mg/d [Recommended Dietary Allowance (RDA) of 2 mg/d)]. The median intake of B-12 in the lowest quintile category exceeded the RDA. Alternatively, these vitamins may have modest beneficial effects that would become apparent only in larger investigations. Because recent intake of vitamin B-6 was associated with reduced PAD risk, it is possible that current intake was more important in this relationship.

The positive association between plasma homocysteine and cardiovascular disease could be explained by damage to the endothelium caused by homocysteine (22, 23). Nevertheless, this interpretation has been challenged because individuals with methylene tetrahydrofolate reductase (MTHFR) 175 allele polymorphism (677 C→T) generally have high homocysteine levels, but not increased risk of CHD (24) or peripheral vascular disease (25). MTHFR has been associated with CHD in the presence of low folate intake (26). It has been suggested that homocysteine may not be an independent risk factor of vascular disease (5) but rather a marker of folate, vitamin B-6 and vitamin B-12 intake, which could reduce risk of CHD by different mechanisms. Folate deficiency has been shown to induce oxidative stress (27). Thus, folate may prevent atherosclerosis by absorbing free radicals and improving endothelial dysfunction rather than by reducing serum homocysteine. However, Schnyder et al. (21) found that a combination of folic acid and vitamins B-6 and B-12 decreased the rate of coronary restenosis only among participants whose plasma homocysteine was lowered.

Taylor et al. (28) found a positive association of plasma homocysteine and PAD in a prospective study. Morrison et al. (29) and Chasan-Taber et al. (30) found a higher risk of CHD among individuals with low serum folate, and Rimm et al. (31) reported a 30% lower risk of CHD among men in the top quintile category of folate intake compared with those in the bottom in this cohort. These reports are consistent with our findings.

We observed an inverse association between folate intake from supplements and PAD risk. We could not rule out a modest inverse association of recent vitamin B-6 and B-12 intake (within the past 4 y) and PAD risk.

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