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Prospective Study of Plasma Vitamin B_6 and Risk of Colorectal Cancer in Men

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Abstract

Vitamin B_6 may lower risk of colorectal cancer by preventing aberrations in one-carbon metabolism or by anti-inflammatory effects. We prospectively evaluated the association between plasma levels of pyridoxal 5′-phosphate (PLP; the active form of vitamin B_6) and risk of colorectal cancer in a nested case-control study within the Physicians' Health Study. Among 14,916 men who provided blood specimens in 1982–1984, we identified 197 incident colorectal cancer cases through 2000 and individually matched them to 371 controls by age and smoking status. Plasma PLP levels were positively correlated with cold cereal intake and with plasma levels of folate and vitamin B_{12} (age- and smoking-adjusted partial correction r=0.28–0.48) and slightly inversely correlated with body mass index (BMI, r=−0.11) and plasma levels of homocysteine, C-reactive protein (CRP), tumor necrosis factor-α receptor (TNF-αR)2, and interleukin (IL)-6 (r ranged from −0.23 to −0.14). With control for these factors and known risk factors for colorectal cancer, plasma PLP levels were significantly inversely associated with risk of colorectal cancer; compared with men in the lowest quartile, those with PLP in quartiles 2–4 had relative risks (RR, 95% confidence interval, CI) of 0.92 (0.55–1.56), 0.42 (0.23–0.75), and 0.49 (0.26–0.92; P-trend = 0.01), respectively. In conclusion, vitamin B_6 may protect against colorectal cancer independent of other one-carbon metabolites and inflammatory biomarkers.

Keywords

Vitamin B_6; pyridoxal 5′-phosphate; colorectal cancer; prospective study

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Introduction

Vitamin B_6 is involved in DNA synthesis by acting as a co-enzyme in the conversion of tetrahydrofolate to 5,10-methylenetetrahydrofolate and in the transsulfuration of homocysteine to cysteine. Therefore, deficiency of vitamin B_6 may increase the risk of cancer through aberrations in DNA methylation, imbalance in DNA precursors, and disruption in DNA repair (1). Low levels of vitamin B_6 have also been associated with elevation of C-reactive protein (CRP) (2), a systemic inflammatory marker, and increases in cell proliferation, oxidative stress, nitric oxide synthesis, and angiogenesis (3).

A lower risk of colorectal cancer associated with higher intake of vitamin B_6 has been summarized in a recent review of three cohort and six case-control studies (4). Two prospective epidemiologic studies of blood vitamin B_6 levels also found an inverse association with colorectal cancer (5,6). However, it remains unclear whether this association is mediated by other one-carbon co-factors and/or inflammatory factors, which may be associated with the risk of colorectal cancer (7–10).

We therefore examined the association between plasma levels of pyridoxal 5′-phosphate (PLP; the active form of vitamin B_6) and colorectal cancer risk in a prospective, nested case-control study of U.S. male physicians. We especially examined whether the association for plasma PLP was independent of plasma levels of one-carbon metabolites and markers of inflammation, including not only CRP but also two other pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin (IL)-6. The effect of TNF-α is mediated by tumor necrosis factor-α receptor (TNF-αR)1 and TNF-αR2 (11). The circulating soluble forms of TNF-αR2 are a better marker of TNF-α because of their higher sensitivity and reliability than TNF-α itself (12).

Materials and Methods

The Physicians' Health Study was a randomized, double-blind, placebo-controlled trial of aspirin and β-carotene among 22,071 U.S. male physicians aged 40 to 84 in 1982, without a history of heart disease, cancer, or major chronic diseases (13). Participants were excluded if they had a diagnosis of myocardial infarction, stroke, or transient ischemic attack, cancer (except non-melanoma skin cancer), renal or liver disease, peptic ulcer, or gout or used vitamin A or β-carotene supplements. The aspirin component of the trial was terminated on January 25, 1988, because of a substantial reduction in the risk of myocardial infarction (13). After the end of the aspirin trial, participants were offered a choice of either aspirin or placebo along with their randomly assigned β-carotene supplement or placebo. Blood samples were obtained from 14,916 of the participants during 1982 and 1984 (more than 70% of participants provided blood between September and November 1982) before randomization. Blood collection kits were sent to all participants with instructions to have their blood drawn into the EDTA tubes. These samples were centrifuged to collect both plasma and whole blood, sent to investigators, divided into aliquots, and stored at −82°C. The median interval (10^{th}–90^{th} percentile) between blood collection and freezing in this analysis was 1.00 (1.0–2.0) days. Samples used in this analysis were thawed only once. Information on height, weight, physical activity, alcohol intake, multivitamin use, and smoking habits was collected by self-administered questionnaires at baseline. The frequency of intake of specified food groups was obtained on the 18-week or 12-month questionnaires. Follow-up is >99% complete for mortality and morbidity. This study was approved by the Institutional Review Board of Brigham and Women's Hospital.

Cases were identified through annual follow-up questionnaires and then confirmed by subsequent review of medical records by the end-point committee. Among those who provided baseline blood samples, 197 cases were ascertained through July 31, 2000. The majority of the

cases were matched to two controls on age (±1 year for younger participants, ±5 years for older participants) and smoking status (never, past, current). However, for 23 cases, only one appropriate control was available. Thus a total of 371 controls were included in our study here; 407 of these blood samples were collected less than 8 h after their last meal (non-fasting).

Pyridoxal 5′-phosphate (PLP), an active form of vitamin B_6, was measured by an enzymatic procedure using radioactive tyrosine and the apo-enzyme tyrosine decarboxylase (14). The mean intraassay coefficient of variation (CV) was 5%. Plasma vitamin B_{12} was measured with a commercially available radioassay kit (Ciba-Corning, MA). Plasma homocysteine and cysteine were measured by high-performance liquid chromatography with fluorescence detection (15). Plasma folate was measured by the microbiological method (16). All assays for folate, vitamins B_6 and B_{12}, and homocysteine were conducted at the Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University. Plasma TNF-αR2 and IL-6 were measured with enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN), and plasma high-sensitive CRP was determined by a highly sensitive immunoturbidimetric assay (Denka Seiken, Niigata, Japan) at the Boston Children's Hospital laboratory. The mean intraassay CVs were all <10%. Cases and their controls were analyzed in the same batch, and laboratory personnel were blinded to case, control, and quality-control status.

DNA was extracted from whole-blood samples, with a commercially available process based on the absorption of DNA to a silica membrane after lysis with a proprietary agent (Qiagen, Chatsworth, CA). The TaqMan assay was used to genotype methylenetetrahydrofolate reductase (MTHFR) C677T (17).

We compared medians and proportions of the baseline risk factors between cases and controls. To test for differences between cases and controls, the paired t-test was used for $\log_e$-transformed plasma biomarker levels and the Mantel-Haenszel test for categorical variables. We examined the associations between plasma PLP and other biomarkers as well as intakes of food groups potentially related to colorectal cancer risk using partial Spearman correlations among the controls adjusted for age, smoking status, and lab assay batches.

To estimate the relative risks (RRs) and 95% confidence intervals (95% CIs), we categorized participants in quartiles or by the median of plasma PLP level among the controls and used a conditional logistic regression model to account for the matched case-control study design. To test for trend across quartiles, we assigned participants the median value of their quartile level. This variable was entered as a continuous term in the model, the coefficient for which was evaluated by the Wald test. In multivariate analyses, we adjusted for BMI (<23, 23–<25, 25–<27, ≥27 kg/m^2), vigorous exercise (<2, ≥2 times/wk), multivitamin use (never, past, current use), fasting status (<8 hours, ≥ 8 hours), red meat consumption (quartiles), cold cereal intake (<2/wk, ≥2/wk), alcohol intake (<1/wk, 1/wk-<1/d, ≥1/d), and aspirin assignment (yes, no). In additional analyses, we adjusted for plasma levels of folate, homocysteine, vitamin B_{12}, TNF-αR2, IL-6, and CRP to examine whether the association for vitamin B_6 was independent of other one-carbon metabolites and inflammatory biomarkers. We examined whether the associations between plasma PLP level and colorectal cancer risk differed by alcohol intake (<1 or ≥1 drinks/d), cold cereal intake (<2 or ≥2 servings/wk), multivitamin use (never, ever), BMI (<25, ≥25 kg/m^2), MTHFR C677T genotype (CC/CT, TT), aspirin assignment (yes, no), β-carotene assignment (yes, no), and plasma levels of folate, TNF-αR2, CRP, and IL-6 (two categories based on the median) using conditional logistic regression. To test the null hypothesis that there was no interaction by the potential effect modifiers, we used a Wald test of the cross-product term of PLP as a dichotomous variable, with the specific modifier variable modeled as a dichotomous variable. The study was analyzed with the SAS 9.1 statistical package (SAS Institute, Cary, NC). $P<0.05$ was considered statistically significant.

Results

Table 1 shows the baseline characteristics of the colorectal cancer cases and controls. We examined whether the distribution of known risk factors for colorectal cancer differed between cases and controls. Multivitamin use, aspirin assignment, BMI, intakes of alcohol, red meat, fish, and dairy products, and vigorous exercise were not statistically significantly different between cases and controls, but cold cereal intake was higher among cases than among controls (P value = 0.09).

After controlling for baseline age, smoking status, and assay batch, plasma PLP levels among controls were significantly and positively correlated with plasma levels of folate and vitamin B_{12}, but slightly inversely, though statistically significantly, correlated with BMI and plasma levels of homocysteine. We observed weak but significant inverse correlations of PLP levels with plasma levels of CRP, IL-6, and TNF-αR2 (Table 2). PLP levels were not correlated with intakes of fish, dairy products, red meat, and alcohol, but were positively correlated with cold cereal consumption. Current multivitamin users had more than 1.7 times higher median plasma PLP levels than nonusers and past users; the median levels of plasma PLP in controls were 118 pmol/ml among current users (n=75) versus 66 pmol/ml among those who never used multivitamins (n=238) and 61 pmol/ml among past users (n=57).

Plasma PLP levels were inversely associated with colorectal cancer risk; a lower risk of colorectal cancer was observed mainly among participants in the two higher quartiles. Compared with the lowest quartile, the age- and smoking-adjusted RRs (95% CIs) were 1.02 (0.63–1.64), 0.55 (0.32–0.92), and 0.68 (0.42–1.10) for each subsequent quartile (Model 1; Table 3). When we categorized men into two groups based on the median levels of PLP, the age- and smoking-adjusted RR (95% CI)$_{\text{high vs. low}}$ was 0.61 (0.42–0.87; Model 1). The results did not appreciably change after controlling for BMI, vigorous exercise, fasting status, red meat consumption, alcohol intake, and aspirin assignment ($RR_{\text{high vs. low}}$ = 0.58; 0.39–0.85). However, adjustment for cold cereal intake and multivitamin use strengthened the association (Model 2). To evaluate whether the effect of plasma PLP was independent of plasma levels of other one-carbon metabolites or biomarkers related to inflammation, we further included these plasma levels of folate, vitamin B_{12}, homocysteine, CRP, TNF-αR2, and IL-6 in the multivariate models and found no appreciable change of the association. The multivariate RR (95% CI)$_{\text{high vs. low}}$ was 0.47 (0.30–0.73) after further adjustment for plasma folate, vitamin B_{12}, and homocysteine (Model 3). Further adjustment for plasma CRP, TNF-αR2, and IL-6 did not change the results (Model 4).

In all the subsequent analyses, we compared men with above the median level (>66.1 and >73.5 pmol/ml in each batch) and men with PLP below the median and controlled for matching factors, BMI, vigorous exercise, fasting status, red meat consumption, cold cereal intake, multivitamin use, alcohol intake, and aspirin assignment. In separate analyses of colon cancer and rectal cancer, we found similar associations for both colon and rectal cancer; the RRs (95% CI)$_{\text{high vs. low}}$ were 0.51 (0.31–0.84) for colon cancer and 0.58 (0.20–1.66) for rectal cancer.

When we examined the associations between plasma PLP level and colorectal cancer risk in two follow-up periods, we found a stronger inverse association in the earlier follow-up period (1982–1992; 100 cases and 194 controls) than in the later follow-up period (1993–2000; 97 cases and 177 controls) or in the entire follow-up period (1982–2000); multivariate RRs (95% CI) $_{\text{high vs. low}}$ were 0.37 (0.19–0.71) in the earlier follow-up period and 0.63 (0.35–1.14) in the later follow-up period. Because plasma PLP level among cases diagnosed near the time when blood was drawn could be altered by preclinical disease, we conducted analyses from which 25 cases diagnosed during the first 4 years of follow-up and their matched controls were

excluded. The results were similar to those when all cases were included; the multivariate RR (95% CI) $_{\text{high vs. low}}$ was 0.53 (0.33–0.84).

The associations between plasma PLP levels and colorectal cancer risk did not vary by alcohol intake (<1 or ≥1 drinks/d), BMI (<25, ≥25 kg/m^2), MTHFR C677T genotype (CC/CT, TT), aspirin assignment (yes, no), β-carotene assignment (yes, no), or each of plasma levels of folate, CRP, TNF-αR2 and IL-6 (two categories based on the median)(P values for interactions ranged between 0.17–0.67; data not shown). Also, multivitamin use (never, ever) did not modify the association between plasma PLP level and colorectal cancer risk. Compared with never multivitamin users with plasma PLP below the median, the multivariate RRs (95% CIs) were 0.50 (0.30–0.84) for never multivitamin users with PLP above the median, 1.35 (0.78–2.34) for ever multivitamin users with PLP below the median, and 0.64 (0.39–1.06) for ever multivitamin users with PLP above the median (P-interaction = 0.78). However, we found a suggestive interaction between plasma PLP and cold cereal intake (<2, ≥2/wk). Compared with <2 servings/wk of cold cereal intake and PLP below the median, the multivariate RRs (95% CI) were 0.67 (0.38–1.16) for <2 servings/wk of cold cereal intake and PLP above the median, 2.14 (1.28–3.57) for ≥2 servings/wk of cold cereal intake and PLP below the median, and 0.72 (0.44–1.20) for ≥2 servings/wk of cold cereal intake and PLP above the median (P- interaction = 0.07).

Discussion

In this prospective, nested case-control study, we found that men with higher plasma PLP levels (above the median level) at baseline had a significantly lower risk of developing future colorectal cancer. The association was independent of conventional risk factors and plasma levels of biomarkers involved in one-carbon metabolism or inflammatory processes. The inverse association remained statistically significant after exclusion of cancer cases diagnosed during the first 4 years of follow-up, suggesting that the protective effect of PLP was unlikely to be influenced by preclinical disease or by change of diet.

We found a significant 51% lower risk of colorectal cancer among men in the highest quartiles than in the lowest quartiles of plasma PLP levels. Our findings in men are consistent with the previous findings in women (5), which observed a significant 52% reduction in colorectal cancer risk comparing the highest with the lowest quartiles (P-trend = 0.07). A recent nested case-control study in Finnish male smokers found a RR of 0.30 (95% CI = 0.11–0.82) for colon cancer, comparing the highest with the lowest quintile of serum PLP levels (6). Findings of these biomarker studies of PLP concur with the evidence from prospective and case-control studies showing that individuals with high dietary vitamin B_6 intake from food and/or supplements had a lower risk of colorectal cancer (4). A recent prospective study in the Netherlands reported a positive association between dietary vitamin B_6 intake and rectal cancer among women (18). However, we observed inverse associations of plasma PLP with both colon and rectal cancers. Heterogeneity by tumor site (proximal, distal, and rectal cancers) warrants further studies.

We found a slightly stronger inverse association in the earlier follow-up period than in the later follow-up period or in the entire follow-up period. This may be because vitamin B_6 delays progression of colorectal cancer (19), which is also supported by the findings from the Nurses' Health Study that the association between plasma PLP levels and early-stage adenoma was weaker than that with advanced adenoma (5). Also, it is possible that a single vitamin B_6 measurement would not reflect long-term vitamin B_6 levels, especially if the participants changed their vitamin B_6 consumption in the later follow-up period. Further studies are warranted to examine whether benefit of vitamin B_6 is limited to late progression.

Several possible mechanisms could explain the observed association. First, vitamin B_6 may reduce the risk of colorectal cancer by preventing disturbance in the one-carbon metabolism pathway, in which three key enzymes require vitamin B_6: serine hydroxymethyltransferase, cystathionine-β-synthase, and cystathionine γ–lyase. Serine hydroxymethyltransferase catalyzes the methylation of tetrahydrofolate to 5,10-methylenetetrahydrofolate, the crucial intermediate of the three pathways involved in thymidylate, purine, and methionine synthesis. A recent study found that pyridoxal kinase, a key enzyme in the metabolism of PLP, improved genome stability, likely through its influence on dTMP synthesis (20). Cystathionine-β-synthase catalyzes the condensation of homocysteine with serine to form cystathionine. Cystathionine γ–lyase breaks cystathionine into cysteine and alpha-ketobutyrate. These two enzymes, the cofactor of which is vitamin B_6, in the transsulfuration pathway may partially explain an increase in homocysteine levels among individuals with inadequate vitamin B_6, indicating disturbance in one-carbon metabolism (21).

Second, vitamin B_6 may be associated with inflammation, a potential risk factor for colorectal cancer (7). Evidence of the link between vitamin B_6 and inflammation includes lower PLP levels in patients with rheumatoid arthritis (22), a typical chronic inflammatory disease, and inverse associations of plasma PLP with cardiovascular disease (23,24) and CRP (2). In our study, we found that plasma PLP levels were inversely associated with not only plasma levels of CRP, but also IL-6 and TNF-αR2. However, adjusting for inflammatory biomarkers had little influence on the association for plasma PLP, suggesting that the protective role of vitamin B_6 against colorectal cancer may be independent of these inflammatory markers.

Finally, vitamin B_6 may protect against cancer by reducing cell proliferation (25,26). Studies of a mouse model demonstrated that supplemental vitamin B_6 reduced cell proliferation and the number of tumors in the colon (27), possibly with modification by fat intake (26). In addition, several studies found that PLP was an effective inhibitor of many enzymes, including RNA polymerase (28), reverse transcriptase (29), and DNA polymerase (30), overexpression of which drives cell proliferation and oncogenic transformation. Vitamin B_6 has also been shown to reduce oxidative stress (31), suppressing nitric oxide (27) and inhibiting angiogenesis (19).

Our analysis has several limitations. We had only a single measure of plasma PLP level, which did not allow us to examine changes in levels over time. Because other risk factors for colorectal cancer were assessed at baseline, we were not able to take into account the changes in risk factors or their status at earlier ages. We also cannot rule out the possibility of influence of residual confounding or unmeasured factors, such as microflora in the large intestine (32). Although multivitamin use and cold cereal intake were strong predictors of PLP level, the inverse association became stronger after controlling for these two factors. Therefore, our findings are unlikely to be explained by other vitamins, minerals, or fiber in cold cereals. Because we had only a limited number of food items on the questionnaires, we were unable to assess total energy intake. However, body mass index and physical activity, which we adjusted for, may reflect prolonged energy intake and energy expenditure. We did not ask whether participants underwent a colonoscopy or sigmoidoscopy, but it is unlikely that screening explains the inverse association we observed, given that adjustment for variables related to healthy behaviors did not markedly change the results. We do not have information on the stability of plasma PLP with the blood collection/storage methods used, but we have generally found no indication of deterioration for biomarkers examined in this study under the conditions used. The prospective design of our study allowed us to examine interactions by prospectively measured variables, but we had limited statistical power to examine these interactions. Although participants are not a random sample of U.S. men, it is unlikely that the basic biological relations among these physicians will differ from well-nourished men in general.

The important strengths of our study include a prospective design in which blood samples were collected before colorectal cancer diagnosis and high follow-up rates. Because we measured plasma levels of biomarkers involved in one-carbon metabolism or inflammatory processes, we were able to examine whether plasma PLP level was associated with a lower risk of colorectal cancer risk independent of these biomarkers. In addition, we were able to eliminate the potential influence of preclinical disease by excluding cases diagnosed during the first few years after blood draw, further demonstrating a temporal relationship.

In summary, our prospective data in men are consistent with previous findings that higher plasma PLP levels and vitamin B_6 intake are associated with a lower risk of colorectal cancer (4–6). Further, our findings provide additional evidence that vitamin B_6 is associated with a lower risk of colorectal cancer independent of other one-carbon metabolites and inflammatory biomarkers.

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Abbreviations

PLP
: pyridoxal 5′-phosphate

CRP
: C-reactive protein

TNF-αR2
: tumor necrosis factor-α receptor-2

IL-6
: interleukin-6

RR
: relative risk

CI
: confidence interval

CV
: coefficient of variation

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Table 1

Baseline characteristics of men with colorectal cancer and male controls*

	Cases	Controls	P value
N	197	371	
Age at randomization, y†	56 (51–61)	56 (50–59)	Matched
Smoking status, N (%)			Matched
Past	94 (48)	178 (48)	
Current	19 (10)	33 (9)	
Follow-up years, y†	10 (6–13)	10 (6–13)	Matched
Age at diagnosis, y†	64 (59–70)	-	
Tumor site, colon, N (%)	147 (75)	-	
Fasting time < 8 hours, N (%)	145 (78)	262 (76)	0.53
Current multivitamin use, N (%)	38 (19)	75 (20)	0.75
Aspirin assignment, N (%)	96 (49)	194 (52)	0.47
Body mass index ≥ 25 kg/m^2, N (%)	94 (48)	154 (42)	0.24
Alcohol intake ≥ 1 drinks/day, N (%)	59 (30)	102 (28)	0.62
Red meat intake ≥ 1 servings/day, N (%)	47 (24)	101 (28)	0.42
Dairy food intake ≥ 1 servings/day, N (%)	102 (52)	201 (54)	0.54
Fish intake ≥ 2 servings/wk, N (%)	86 (44)	168 (46)	0.59
Cold cereal intake ≥ 2 servings/wk, N (%)	100 (51%)	160 (43%)	0.09
Vigorous exercise ≥2 times/wk, N (%)	106 (54)	216 (59)	0.26
Plasma biomarkers†			
Pyridoxal 5′-phosphate (PLP), pmol/ml	65 (50–90)	70 (52–103)	0.14
Folate, ng/ml	5 (3–7)	5 (3–7)	0.79
Vitamin B_{12}, pg/ml	434 (374–575)	467 (364–572)	0.93
Homocysteine, nmol/ml	11 (9–13)	10 (8–13)	0.13
C-reactive protein, mg/l	1 (0.5–2)	1 (0.5–2)	0.54
Tumor necrosis factor receptor-2, pg/ml	2,368 (1,986–2,790)	2,244 (1,917–2,629)	0.10
Interleukin-6, pg/ml	1 (0.8–2)	1 (0.7–2)	0.19

*All P values > 0.05. P values were calculated using the Mantel-Haenszel test for categorical variables and the paired t-test performed on $\log_e$-transformed plasma biomarker levels.

†Median (25^{th} – 75^{th} percentile)

Table 2

Partial Spearman correlations between plasma pyridoxal 5′-phosphate (PLP) and other biomarkers and dietary intakes among controls, adjusted for age, smoking status, and laboratory batches

	Plasma PLP (unit)	P value
Age at randomization (yr)*	−0.01	0.80
Body mass index (BMI, kg/m^2)	−0.11	0.04
Plasma levels		
Folate (ng/ml)	0.48	<0.001
Vitamin B_{12} (pg/ml)	0.34	<0.001
Homocysteine (nmol/ml)	−0.23	<0.001
Cysteine (mg/l)	0.08	0.15
Tumor necrosis factor receptor-2 (pg/ml)	−0.14	0.007
C-reactive protein (mg/l)	−0.14	0.01
Interleukin-6 (pg/ml)	−0.16	0.002
Dietary intake		
Fish (servings/d)	0.07	0.19
Dairy food (servings/d)	0.07	0.19
Red meat (servings/d)	−0.08	0.13
Alcohol (drinks/d)	0.06	0.29
Cold cereal (servings/d)	0.28	<0.001

* Not adjusted for age.

Table 3

Relative risk (RR) and 95% confidence intervals (CIs) of colorectal cancer according to plasma pyridoxal 5′-phosphate (PLP)

PLP Categories*	PLP Median, pmol/ml	RR (05% CI)				
		No. of cases/controls	Simple matched model 1†	Multivariate model 2‡	Multivariate model 3§	Multivariate model 4‖
Quartile 1	43	60/92	1.00	1.00	1.00	1.00
Quartile 2	61	62/93	1.02 (0.63–1.64)	0.99 (0.60–1.63)	0.95 (0.57–1.59)	0.92 (0.55–1.56)
Quartile 3	81	34/94	0.55 (0.32–0.92)	0.45 (0.26–0.77)	0.44 (0.25–0.76)	0.42 (0.23–0.75)
Quartile 4	144	41/92	0.68 (0.42–1.10)	0.52 (0.29–0.93)	0.50 (0.27–0.92)	0.49 (0.26–0.92)
P for Trend			0.07	0.01	0.01	0.01
<Median	52	122/185	1.00	1.00	1.00	1.00
≥Median	104	75/186	0.61 (0.42–0.87)	0.48 (0.31–0.73)	0.47 (0.30–0.73)	0.47 (0.30–0.73)

*The cut-off points in quartiles were selected among controls.

†Simple matched model 1: matched on age (±1 year for younger participants, ±5 years for older participants) and smoking status (never, past, current).

‡Multivariate model 2: adjusted for body mass index (<23, 23–<25, 25–<27, ≥27 kg/m^2), vigorous exercise (<2, ≥2 times/wk), fasting status (<8 hours, ≥ 8 hours), red meat consumption (quartiles), cold cereal intake (<2, ≥2/wk), multivitamin use (never, past, current), alcohol intake (<1/wk, 1/wk-<1/d, ≥1/d), and aspirin assignment (yes, no) in addition to matching factors.

§Multivariate model 3: adjusted for plasma levels (<median, ≥median) of folate, vitamin B_{12}, and homosyteine in addition to matching factors and covariates in model 2.

‖Multivariate model 4: adjusted for plasma levels (<median, ≥median) of folate, vitamin B_{12}, and homosyteine, tumor necrosis factor-α receptor 2, C-reactive protein, and interleukin-6 in addition to matching factors and covariates in model 2.