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Association of plasma vitamin B6 with risk of colorectal adenoma in a multiethnic case–control study

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Abstract

Circulating level of vitamin B6 has been inversely associated with colorectal cancer (CRC) risk but, unlike for folate, few studies have examined the relationship of vitamin B6 to colorectal adenoma, the precursor lesion to most CRCs. We measured plasma levels of folate, vitamin B6, and vitamin B12 in 241 patients with pathologically confirmed first occurrence of colorectal adenoma and 280 controls among Caucasians, Japanese Americans, and Native Hawaiians undergoing flexible sigmoidoscopy screening in Hawaii. High plasma level of vitamin B6 was independently inversely associated with risk of colorectal adenoma [multivariate odds ratios (95% confidence intervals): 1.0, 0.71 (0.45–1.13) and 0.44 (0.26–0.74) from the lowest to the highest tertile, respectively, $p_{trend} = 0.002$]. Plasma folate was not associated with adenoma after adjustment for plasma vitamin B6 ($p_{trend} > 0.3$). No association was observed with plasma vitamin B12. No significant interaction was detected between the three B vitamins and alcohol intake, multivitamin use or *MTHFR* C677T. The results provide evidence for an inverse association of plasma vitamin B6 levels with risk of colorectal adenoma. This study expands previous findings

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Electronic supplementary material

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and suggests that vitamin B6 may be protective against the early stages of colorectal carcinogenesis.

Keywords

Folate; Vitamin B6; Vitamin B12; Methylenetetrahydrofolate reductase; Colorectal adenoma

Introduction

Folate, in all coenzymatic forms, and other B vitamin cofactors mediate the transfer of one-carbon groups and may play an important role in two key cancer-related pathways, namely, nucleic acid synthesis for DNA replication and repair and provision of methyl groups for DNA methylation. The role of folate in colorectal carcinogenesis has been extensively studied, and evidence is mounting that it may have a dual effect, protecting the normal colonic epithelium but enhancing the progression of existing neoplastic lesions [1]. However, data for other B vitamins are more limited, such as for vitamin B6, which is a cofactor for cystathionine-β-synthase, and for vitamin B12, which is a cofactor for methionine synthase and methionine synthase reductase, all of which catalyze reactions that affect levels of homocysteine. Vitamin B12 intake has mostly been found not to be associated with colorectal neoplasia (reviewed in [2]). In contrast, a number of studies have consistently linked dietary or plasma levels of vitamin B6 with reduced risk of colorectal cancer (CRC) (e.g., [2–8]). The evidence for a similar inverse association of vitamin B6 with colorectal adenoma (a common CRC precursor lesion) is weaker, with only a handful of studies published to date [2, 3, 9].

Specifically, plasma pyridoxal-5′-phosphate (PLP), the main circulating form of vitamin B6, was suggested in the Nurses Health Study to reduce risk of advanced (≥1 cm in size, or villous or tubulovillous) distal colorectal adenoma (p_{trend} = 0.08), but no association was observed for early-stage adenoma (small and tubulous) or with total vitamin B6 intake [3]. A borderline significant inverse association between plasma PLP and adenoma recurrence was observed in the Aspirin/Folate Polyp Prevention Study (p_{trend} = 0.08) only among subjects who did not drink alcohol ($p_{interaction}$ = 0.03) [2]. Finally, a higher intake of vitamin B6 was associated with a lower risk of adenoma recurrence in the Wheat Bran Fiber intervention trial [9].

We examined the associations of plasma levels of folate, vitamin B6, and vitamin B12 with adenoma risk among individuals undergoing flexible sigmoidoscopic screening in Hawaii.

Methods

Study design and data collection for this colorectal adenoma study have been described in detail elsewhere [10]. Briefly, two flexible sigmoidoscopy screening clinics were used to recruit participants on Oahu, Hawaii. Adenoma cases were identified either as part of the baseline screening exam at the Hawaii site of the Prostate Lung Colorectal and Ovarian (PLCO) screening trial between July 1996 and February 2000 or at the Kaiser Permanente Hawaii (KPH)’s Gastroenterology Screening Clinic between January 1995 and June 2004.

Cases were patients with histologically confirmed first-time adenoma(s) of the colorectum and were of Japanese, Caucasian, or Native Hawaiian ancestry. Controls were selected among individuals without a history of adenoma and found to have a normal colon and rectum at sigmoidoscopy and were individually matched to the cases (with a one-to-one ratio) on age, sex, race/ethnicity, screening date (±3 months), and screening clinic. The overall study participation rate was 68% for cases and 69% for controls. Blood was provided by 87.5% of cases and 86.3% of controls who were interviewed. Subjects who were interviewed between June 1998 and December 2002 and who provided a blood sample (241 cases, 280 controls) were included in the present analyses.

Exposure information was collected via an interview-administered questionnaire designed to obtain demographic and lifestyle information, including lifetime histories of tobacco smoking and alcohol drinking, weight at time of examination, usual physical activity, personal medical history, family history of colorectal cancer, and for women, reproductive and hormone use histories. The interview also included a validated food frequency questionnaire with >200 food items [11]. The reference period was the year before the examination. If a change had occurred in types and amounts of foods consumed in the three years preceding the sigmoidoscopy, the reference period was the year before the change. Daily intake in grams for each food item was computed for each subject. A food composition nutrient database primarily based on the US Department of Agriculture nutrient database was applied to the items to assess nutrient intake. The computation of folate intake took into account folic acid fortification of grains in the United States and was expressed in dietary folate equivalents (DFE). Detailed information on vitamin (including multivitamins and B vitamins) and mineral supplement use was also collected. Finally, each subject was asked to give a blood sample that was drawn in the morning after a 10-h fast. Specimens were processed within 2 h of collection and stored at −80°C until laboratory analysis. All participants with an available plasma sample were included in the study regardless of whether both members of a matched case–control set had given blood.

Plasma folate, vitamin B6, and vitamin B12 were analyzed at Tufts University blinded to the subject's case–control status. Samples of cases and controls were assayed together in the same analytical batch giving priority to complete matched sets and, in situations where plasma was not available for all members of a matched set (due to refusal to give blood), to cases and controls of the same sex, ethnicity, and similar age. Plasma folate and vitamin B12 concentrations were determined by a radioimmunoassay method using a commercially available kit from Biorad (Richmond, CA). The intra-batch coefficient of variation from 35 blind duplicate pairs was 5.3% for vitamin B12 and 9.6% for folate. Plasma pyridoxal-5′-phosphate (PLP), the active form of vitamin B6, was determined enzymatically using tyrosine decarboxylase based on the principles described by Shin-Buehring et al. [12]. In this method, PLP activity in the plasma sample is determined on the basis of release of tritiated tyramine following the incubation of tyrosine decarboxylase apo-enzyme with the supernatant fraction of TCA-precipitated serum sample and tritium-labeled tyrosine. The intra-batch coefficient of variation for this assay was 5.2%.

DNA was purified from blood buffy coat using QIAamp DNA Blood Kits (Qiagen, Valencia, CA). Genotyping of the missense single-nucleotide polymorphism (SNP)

rs1801133 (*MTHFR* C677T) was performed as previously described [7]. Genotype frequencies for *MTHFR* C677T in the controls conformed to Hardy–Weinberg equilibrium in all ethnic groups at significance level 0.05. The genotyping call rate was 98.9%.

Characteristics of cases and controls were compared with Pearson's χ^2 test for categorical variables or Wilcoxon's rank sum test for continuous traits. Among unaffected subjects, we tested for differences in plasma biomarker levels (folate, vitamin B6, vitamin B12) across ethnic groups with a general linear model (GLM) using log-transformed variables, with or without adjustment for age at blood draw and sex. Partial Spearman's correlation coefficients were also computed to examine the relationships among the plasma levels and the dietary and total intakes of folate, vitamin B6, and vitamin B12, adjusting for age at blood draw, sex, and ethnicity.

To estimate the risk of colorectal adenoma conferred by the blood biomarkers, we used unconditional logistic regression to obtain odds ratios (ORs) and 95% confidence intervals (CIs). Because all subjects who gave blood were used in this analysis regardless of the matched-set completion status, we adjusted for age at blood draw, race/ethnicity, sex, and recruitment site to account for potential confounding effects. The regression models were further adjusted for variables previously found to be associated with adenoma risk, namely, BMI, lifetime hours of leisure time physical activity, pack-years of smoking, daily intake of alcohol (quartiles), and daily caloric intake, where alcohol intake was expressed as nutrient density. To account for the differences in time between screening exam and blood draw and for the effect of the folic acid fortification initiated in the United States in 1996, we adjusted for year of exam and year of blood draw in the regression models. However, the risk estimates did not change substantially after the adjustment so these two variables were dropped from the final models. Other potential confounders, such as multivitamin use, aspirin use, previous endoscopy (Yes/No), years of schooling, dietary fiber intake, waist and hip circumferences, waist-to-hip ratio, total calcium, and processed meat, did not materially change the OR estimates or increase model-fit, and they were also not included in the final model. Plasma B vitamin levels were categorized into 3 levels according to tertiles based on their distributions among all study subjects. Linear trends in ORs were tested using the median values for each tertile.

We explored the overall shape of the functional relationships between the three B vitamins and adenoma risk by fitting a generalized additive model (GAM) with all the matching and risk factors (as in logistic regression modeling) as linear terms and the plasma levels of the B vitamins as non-linear smoothing effects. Each non-linear effect was estimated by a smoothing spline function as described in [13].

Interactions between plasma biomarkers and sex, race, age at blood draw (≤65 and >65 years), alcohol intake (≤1.8 and >1.8 g/d), multivitamin supplement use, and *MTHFR* C677T genotype (TT vs. CC or CT) were assessed with the likelihood ratio test, comparing the likelihood of a main effect model with a model including both main effect and interaction terms.

All statistical tests were performed with a significance level of 0.05 (2-sided) using SAS (version 9.1).

Results

Table 1 displays the main characteristics of the participants by case–control status. Compared to cases, controls smoked less, were more likely to have had a previous colorectal endoscopy and to use multivitamins and consumed less alcohol and more folate. The distribution of other variables, including aspirin use and lifetime recreational physical activity, was similar between cases and controls. In this study, only two subjects (one case, one control) had plasma folate deficiency (<3 ng/mL). No statistically significant differences across ethnic groups were observed in mean plasma folate, vitamin B6, and B12, before and after adjustment for age at blood draw and sex (p's > 0.08).

The partial Spearman's correlation coefficients are shown in Table 2 among plasma levels and dietary and total intakes of the B vitamins, after adjustment for age at blood draw, sex, and race/ethnicity. Highly statistically significant correlations (p's ≤ 0.0001) were observed: the correlations ranged from 0.35 to 0.44 between plasma values, from 0.36 to 0.72 between dietary intakes, and from 0.73 to 0.88 between total intakes (from foods and supplements). The correlations between total intakes (from foods and supplements) and plasma levels for folate, vitamin B6, and vitamin B12 were 0.40, 0.42, and 0.30 (p's ≤ 0.0001), respectively, compared to 0.26 ($p \leq 0.0001$), 0.17 ($p = 0.005$), and 0.04 ($p = 0.5$) for the correlations between dietary intakes and plasma levels, respectively.

The ORs for adenoma associated with tertiles of plasma B vitamins are presented in Table 3. With adjustment for the matching variables only, increasing level of plasma folate was associated with a decrease in adenoma risk ($p_{trend} = 0.02$); however, after adjusting for additional riskfactors and plasma vitamin B6 and B12, this association was not statistically significant ($p_{trend} = 0.35$). This change was mainly due to the inclusion of plasma B6 as a covariate in the model. Higher plasma vitamin B6 levels were associated with a decreased adenoma risk, independently of the risk factors and plasma folate and vitamin B12($p_{trend} = 0.002$). The OR for the third tertile (≥106 pmol/mL) compared to the first tertile (≤51 pmol/mL) of plasma vitamin B6 was 0.44 (95% CI: 0.26–0.74). Plasma vitamin B12 did not show any significant association with adenoma. These effects did not differ by sex, race, or age at blood draw (p values for interactions > 0.13). In addition, GAM modeling did not detect a significant non-linear component in the relationships between the three vitamins and adenoma risk (Supplementary Figure).

We also examined the interactions of the three plasma B vitamins with, successively, alcohol intake, multivitamin use, and *MTHFR* C677T on the risk of adenoma. No statistically significant interaction was observed (p's > 0.2). However, there was the suggestion, though not statistically significant ($p_{interaction} = 0.26$), that the inverse association with plasma vitamin B6 was stronger among non-users compared to users of multivitamins (Table 4). Among non-users ($n = 223$), the ORs of the middle and upper tertile compared to the lower tertile were 0.59 (95% CI: 0.32–1.10) and 0.30 (95% CI: 0.13–0.73), respectively ($p_{trend} = 0.005$). The corresponding ORs among users were 1.02 (95%

CI: 0.52, 1.99) and 0.68 (95% CI: 0.36, 1.29) (p_{trend} = 0.17). However, our statistical power was limited for such subgroup analyses and these results should not be over-interpreted.

Discussion

In this case–control study, we observed an inverse association of plasma levels of PLP (the biologically active form of vitamin B6) with risk of colorectal adenoma, independent from known risk factors, as well as plasma folate and vitamin B12. Plasma folate and vitamin B12 were not associated with adenoma risk, after adjustment for plasma vitamin B6.

Our findings for vitamin B6 are consistent with those reported from past studies, although the evidence to date has been stronger and more abundant for CRC than for adenoma. With regard to plasma vitamin B6 and colorectal adenoma, two previous studies presented borderline significant associations. Wei et al. [3] reported no association overall and only a suggestive association with advanced adenoma [OR = 0.65 (95% CI: 0.37–1.11), p_{trend} = 0.08]. Compared to the study by Wei et al. [3], the sample size in our study was smaller, so we could not perform subgroup analysis limited to advanced adenoma. However, we note that levels of plasma vitamin B6 were higher in our study: the median plasma vitamin B6 level was 71 (pmol/mL) in our study (Table 3), compared to <63.3 (pmol/mL) in theirs (estimated from Table 5 in [3]); similarly, the 83.3 percentile point in our study was 200 (pmol/mL) (Table 3), compared to a 87.5 percentile of 129 (pmol/mL) in the study by Wei et al. This difference in data range may have contributed to the more pronounced overall association in our study. Another analysis in the Aspirin/Folate Polyp Prevention Study found a borderline significant inverse association with baseline plasma PLP and adenoma recurrence ($p$trend = 0.08); however, this association was limited to subjects who did not drink alcohol ($p_{interaction}$ = 0.03) [2]. The outcome in [2], recurrence of adenoma, is somewhat different from first-time occurrence studied here, so the comparison between the two studies is not straightforward.

The data on plasma vitamin B6 and CRC have been more consistent. In a nested case–control study in the Multiethnic Cohort study, we observed a 51% reduction in CRC risk (95% CI: 0.29–0.83, p_{trend} = 0.009) among individuals in the highest compared to the lowest quartile of plasma PLP [7]. Inverse associations were also reported between serum PLP and colon (but not rectal) cancer in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study [5] and between plasma PLP and CRC in the Physician's Health Study trial [6]. A recent meta-analysis showed a 49% CRC risk reduction for every 100 pmol/mL increase in blood PLP [8].

Several possible mechanisms by which vitamin B6 could reduce CRC risk have been proposed. Sub-optimal levels of vitamin B6 may disrupt the one-carbon metabolism since it acts as a cofactor for serine hydroxymethyltransferase, which catalyzes the formation of 5,10-methylenetetrahy-drofolate, an important carrier of one-carbon groups for DNA synthesis and repair [14]. Vitamin B6 has also been shown to reduce oxidative stress and angiogenesis [14] and may have a beneficial effect against inflammation [15]. Dietary supplementation with this vitamin has been shown to reduce cell proliferation and the number of colon tumors induced by a carcinogen administered to mice [16]. Although

vitamin B6 is found in a variety of foods, there is evidence that many older adults in the United States do not obtain an adequate intake of this nutrient [17].

Animal studies have suggested that folate may have a favorable effect at the early stage of colorectal carcinogenesis, by protecting the normal intestinal mucosa against early lesions [1]. However, once pre-neoplastic lesions are established, folate may enhance the progression of intestinal tumors [1]. Whether a similar dual role exists in humans is still being debated, and the data are difficult to reconcile. The Aspirin/Folate Polyp Prevention Study found no evidence that folic acid prevented adenoma recurrence. Instead, this trial suggested that folic acid increases risk of multiple adenomas and advanced adenoma after longer-term treatment and follow-up [18]. In this high-risk group of patients, early lesions may have been undetected at baseline colonoscopy, and it has been suggested that the treatment may have accelerated their progression [18]. Two other recent trials have reported no significant effect of folic acid on adenoma recurrence but their follow-up was shorter [19, 20]. Overall, data from cohort and case–control studies have tended to find folate intake to be inversely associated with risk of CRC and adenomas [21–26]. Studies that measured blood folate levels also suggested a protective effect against colorectal cancer and adenomas [9, 24, 27], although some studies did not [28, 29]. This inconsistency in the data may be due to differences in characteristics of study populations (such as ethnicity, dietary folate level, time and duration of study relative to the initiation of folate fortification policy, etc.) or genetic variants that modulate the effect of folate. For example, the *MTHFR* C677T variant appears to interact with folate intake and plasma levels in past studies [7, 30–32]. Our study was conducted after the initiation of folate fortification of grains in the United States.

A potential limitation of the present study is that bio-markers levels were measured after diagnosis of adenoma and only once. So it is not known whether the levels measured reflect the participants' past habitual levels. Although we think it is unlikely that colorectal adenomas would affect circulating levels of B vitamins, this may be a possibility. Past studies of variation in plasma levels of B vitamins over time have been conducted in healthy individuals. A study conducted in Australia reported a coefficient of variation of only 8.3%, and one conducted in Ireland reported a reliability coefficient of 0.94 over a 1-year period for plasma folate [33, 34], suggesting that a single measurement could reflect reasonably well long-term B vitamin status. In addition, adenoma patients are not typically asked to change their diet after diagnosis. However, it is possible that a subset of newly diagnosed patients may take up a "healthful" lifestyle, including taking vitamin supplements. If that is true, our risk estimates for plasma vitamins would have been conservative since the plasma vitamin levels in cases before diagnosis should then have been lower than observed. That would not affect the direction of the estimated effect for plasma vitamin B6 but may have contributed to the null association between plasma folate and disease. Another limitation was that flexible sigmoidoscopy was used to diagnose adenoma, and thus, any adenoma located in the proximal colon may have been missed. A direct result would be that some controls were misclassified, which would have led to diminished differences in plasma vitamin levels between cases and controls, or in other words, risk estimates that are biased

toward the null. Finally, our study was not adequately powered to allow for conclusive interaction or sub-group analyses.

In summary, this study provides evidence for an inverse association of circulating vitamin B6 with colorectal adenoma risk and suggests that this effect is independent from that of plasma folate and vitamin B12.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Main characteristics of study participants

	Cases (*n* = 241)	Controls (*n* = 280)	*p*[b]
Men (%)	68.5	66.4	0.62
Race (%)			0.92
Japanese	40.2	38.6	
Caucasian	40.7	42.1	
Hawaiians	19.1	19.3	
Site (%)			0.004
Kaiser-Hawaii	63.9	51.4	
PLCO	36.1	48.6	
Age at blood draw (years)	66 (60, 70)	65 (60, 70)	0.95
Education (years)	14 (12, 17)	14 (12, 17)	0.81
Previous endoscopy (%)	27.4	39.8	0.003
Ever used aspirin regularly (%)	27.4	26.8	0.88
Multivitamin supplement use (%)	51.5	61.9	0.02
Smoking status (%)			0.33
Non-smoker	36.3	41.4	
Past smoker	50.8	48.9	
Current smokers	12.9	9.7	
Pack-years[a]	27 (10, 50)	22 (5, 38)	0.05
BMI (kg/m^2)	25.9 (23.9, 29.2)	25.7 (23.6, 28.7)	0.42
Lifetime recreational physical activity (h)	7,584 (2,448, 17,880)	6,456 (2,316, 14,568)	0.23
Total Calories (kcal/day)	2,123 (1,630, 2,896)	2,046 (1,589, 2,657)	0.13
Alcohol consumption[c] (g/day)	3.3 (0.3, 18.5)	1.3 (0.2, 11.9)	0.03
Total folate intake[c] (dietary folate equiv.)	682 (471, 1,105)	839 (510, 1,219)	0.03
Total vitamin B6 intake[c] (mg/day)	2.8 (2.0, 4.9)	3.7 (2.1, 5.5)	0.09
Total vitamin B12 intake[c] (μg/day)	8.9 (4.7, 22.2)	10.2 (4.8, 25.9)	0.51
Total calcium intake[c] (mg/day)	812 (603, 1,190)	853 (608, 1,216)	0.74

Medians (interquartile range) or percentages

[a] Pack-years = pack-years of cigarette/cigar/pipe smoking among ever smokers

[b] From Pearson's χ^2 test for percentages and Wilcoxon's rank sum test for continuous traits comparing cases and controls

[c] From foods and supplements (adjusted for total calories)

Table 2

Partial Spearman rank correlation coefficients (*p* value) for B vitamin plasma levels and intakes among controls (*n* = 280)

	Plasma folate	Plasma vitamin B6	Plasma vitamin B12	Total folate intake (DFE)[a]	Total vitamin B6 intake[a]	Total vitamin B12 intake[a]	Dietary folate intake	Dietary vitamin B6 intake
Plasma vitamin B6	0.35							
Plasma vitamin B12	0.35	0.44						
Total folate intake (DFE)[a]	0.40	0.31	0.30					
Total vitamin B6 intake[a]	0.31	0.42	0.32	0.77				
Total vitamin B12 intake[a]	0.32	0.32	0.30	0.73	0.88			
Dietary folate intake	0.26	0.14 (0.02)	0.06 (0.33)	0.49	0.23	0.12 (0.04)		
Dietary vitamin B6 intake	0.23	0.17 (0.005)	0.16 (0.007)	0.43	0.40	0.24	0.72	
Dietary vitamin B12 intake	0.06 (0.32)	0.05 (0.40)	0.04 (0.51)	0.24	0.17 (0.005)	0.32	0.36	0.42

Adjusted for age at blood draw, sex, and race/ethnicity. *p* values not shown were all highly significant (≤ 0.0001)

[a]From foods and supplements (adjusted for total calories)

Table 3

Associations between plasma B vitamin levels and adenoma

Plasma B vitamin	Tertile			p_{trend}
	1	2	3	
Folate (ng/mL)				
Median	6.7	11.2	17.5	
Ca/Co	90/84	84/90	67/106	
OR[a] (95% CI)	1.00	0.90 (0.59, 1.37)	0.59 (0.38, 0.91)	0.02
OR[b] (95% (CI)	1.00	1.10 (0.70, 1.74)	0.81 (0.49, 1.34)	0.35
Vitamin B6 (pmol/mL)				
Median	34	71	200	
Ca/Co	97/77	79/95	65/108	
OR[a] (95% CI)	1.00	0.68 (0.45, 1.05)	0.45 (0.29, 0.70)	0.0007
OR[b] (95% CI)	1.00	0.71 (0.45, 1.13)	0.44 (0.26, 0.74)	0.002
Vitamin B12 (pg/mL)				
Median	299	442	652	
Ca/Co	85/89	83/91	73/100	
OR[a] (95% CI)	1.00	0.91 (0.59, 1.40)	0.73 (0.47, 1.13)	0.15
OR[b] (95% CI)	1.00	1.11 (0.70, 1.75)	1.20 (0.72, 1.98)	0.49

[a]Adjusted for age at blood draw, race/ethnicity, sex and screening center

[b]Further adjusted for BMI, pack-years of smoking, daily energy intake, daily intake of alcohol, lifetime hours of recreational physical activity, and plasma folate, B6 and B12 as appropriate

Table 4

Interaction between multivitamin use and plasma folate and vitamin B6 on the risk of adenoma

	Tertiles (medians) for plasma levels	Multivitamin use				$p_{interaction}$
		Non-user		User		
		Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	
Folate (ng/mL)	≤ 8.6 (6.7)	65/49	1.00	25/34	1.00	0.77
	8.6–13.9 (11.2)	36/33	1.07 (0.56, 2.03)	48/56	1.28 (0.65, 2.52)	
	> 13.9 (17.5)	16/24	0.70 (0.31, 1.56)	51/82	1.02 (0.52, 1.99)	
			p_{trend} = 0.49		p_{trend} = 0.88	
Vitamin B6 (pmol/mL)	≤ 51 (34)	68/41	1.00	29/36	1.00	0.26
	51–106 (71)	37/43	0.59 (0.32, 1.1)	42/51	1.02 (0.52, 1.99)	
	> 106 (200)	12/22	0.30 (0.13, 0.73)	53/85	0.68 (0.36, 1.29)	
			p_{trend} = 0.005		p_{trend} = 0.17	

ORs were after adjustment for sex, race, age at blood draw, recruitment clinic, BMI, pack-years of smoking, lifetime physical activity, alcohol intake, log of energy intake, and plasma folate and vitamin B6 levels, as appropriate