Plasma Vitamin B₆ and the Risk of Colorectal Cancer and Adenoma in Women

Esther K. Wei, Edward Giovannucci, Jacob Selhub, Charles S. Fuchs, Susan E. Hankinson, Jing Ma

Background: Vitamin B₆, whose main circulating form is pyridoxal 5′-phosphate (PLP), is important in one-carbon metabolism, which is critical for DNA synthesis and DNA methylation, both of which are potentially involved in colorectal carcinogenesis. However, no previous epidemiologic studies have directly evaluated the association of plasma PLP with risk for colorectal neoplasia. Methods: We conducted a prospective nested case–control study of 32826 female participants of the Nurses’ Health Study who provided blood specimens in 1989–1990. From 1989–1990 to 2000 (1998 for adenoma), a total of 194 incident colorectal cancer cases and 410 incident colorectal adenoma cases were identified from medical records. Multivariable-adjusted relative risks (RRs) and 95% confidence intervals (CIs) were calculated using logistic regression. All statistical tests were two-sided. Results: A suggestive inverse association was observed between plasma PLP concentration and risk for colorectal cancer when comparing the highest quartile versus the lowest (RR = 0.56, 95% CI = 0.31 to 1.01; \( P_{\text{trend}} = .07 \)); the association of PLP concentration with colon cancer was statistically significant (RR = 0.42, 95% CI = 0.21 to 0.85; \( P_{\text{trend}} = .02 \)). Both associations were statistically significant and stronger after controlling for intakes of folate, of multivitamins, and of methionine (for colorectal cancer, RR = 0.48, 95% CI = 0.25 to 0.92; \( P_{\text{trend}} = .03 \); for colon cancer, RR = 0.38, 95% CI = 0.18 to 0.80; \( P_{\text{trend}} = .01 \)). Total vitamin B₆ intake was also statistically significantly inversely associated with colon cancer risk (RR = 0.51, 95% CI = 0.27 to 0.97; \( P_{\text{trend}} = .007 \)). There was a suggestive inverse association between plasma PLP concentration and advanced distal colorectal adenoma (RR = 0.65, 95% CI = 0.37 to 1.11; \( P_{\text{trend}} = .08 \)), but the association with early-stage adenoma was weaker (RR = 0.85, 95% CI = 0.52 to 1.38; \( P_{\text{trend}} = .52 \)). Conclusions: Our results suggest that vitamin B₆ may be inversely associated with risk of colorectal neoplasia. [J Natl Cancer Inst 2005;97:684–92]

Folate, methionine, vitamin B₆, and vitamin B₁₂ play important roles in one-carbon metabolism, which is critical for nucleotide synthesis and DNA methylation. Aberrations in either nucleotide synthesis or DNA methylation can contribute to carcinogenesis in general and colorectal cancer in particular (1,2). Of the nutrients involved in one-carbon metabolism, folate has been the most widely studied in relation to risk for colorectal neoplasia, with most epidemiologic studies reporting an inverse association, particularly with dietary folate (3). In addition, both high alcohol intake, which has potent antagonistic effects on folate status and methylation, and low methionine intake have been associated with an increased risk for colorectal neoplasia (4–8). Despite its importance in one-carbon metabolism and several animal studies that have shown a protective role for vitamin B₆ against colorectal carcinogenesis (9), few epidemiologic studies have evaluated the association between vitamin B₆ and risk of colorectal neoplasia (10–15).

Vitamin B₆ is needed to regenerate 5,10-methylene tetrahydrofolate, which is involved in the conversion of uracil to thymidine. Inadequate levels of 5,10-methylene tetrahydrofolate can lead to misincorporation of uracil instead of thymidine in DNA synthesis and, consequently, to chromosomal instability (16–18). Methionine is converted to S-adenosyl-methionine, the main methyl donor in the body, which is converted in turn to S-adenosyl-homocysteine and then to homocysteine. Vitamin B₆ is involved in converting homocysteine to cysteine, via cystathionine. In addition, vitamin B₆ is a cofactor for at least 100 other physiological processes (19). Major contributors of vitamin B₆ intake are vitamin B supplements and multivitamins. Other dietary sources include fortified breakfast cereals, chicken, beef, potatoes, broccoli, and bananas. Heating may cause foods to lose vitamin B₆, and some evidence suggests that bioavailability from plant sources is lower than from animal sources (20).

No previous study, to our knowledge, has examined plasma concentrations of vitamin B₆ in relationship to colorectal neoplasia. We thus conducted a prospective nested case–control study in the Nurses’ Health Study to evaluate the association between plasma concentrations of pyridoxal 5′-phosphate (PLP), the main active form of vitamin B₆ (21), and risk for colorectal cancer and colorectal adenoma. We also evaluated the association between total vitamin B₆ intake and risk of colorectal cancer.

Subjects and Methods

Study Population

The Nurses’ Health Study began in 1976, when 121 700 female nurses between 30 and 55 years of age were enrolled and completed a baseline questionnaire about their lifestyles and medical histories. Subsequently, these women have completed a self-administered, mailed questionnaire biennially to update information on their lifestyle, medical history, and diet (every 2 to 4 years). In 1989, all participants were invited to provide a blood sample. A total of 32 826 women between 43 and 69 years of age...
agreed to participate and returned a mailed blood collection kit by overnight courier. Written informed consent was obtained from all participants, and the study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women’s Hospital, Boston, Massachusetts. Ninety-seven percent of the samples were received within 26 hours of the blood sample being collected. On arrival, the blood samples were centrifuged and separated into plasma, white blood cells, and red blood cells. All samples were stored in carefully monitored liquid nitrogen freezers. Further details on the blood collection methods have been published previously (22).

Identification of Case Patients and Control Subjects

We requested medical records from all women in the cohort who reported an incident diagnosis of colorectal cancer or colorectal adenoma on their biennial questionnaire. Medical records were reviewed, and detailed information on the diagnosis was extracted. For analysis of colorectal cancer associations, we included all incident case patients diagnosed with colon or rectal cancer between the date of blood collection and May 31, 2000. Control subjects were selected from the cohort of women who provided blood and who had no history of cancer diagnosed up until the time of their matched case patient’s diagnosis of colorectal cancer. Each case patient was individually matched to two control subjects on year of birth, month and year of blood collection, and fasting status at time of blood collection (fewer than 8 hours or 8 or more hours since last meal). A total of 194 case patients and 350 control subjects were included in this analysis. At the beginning of 2000, the follow-up rate in the sub-cohort who provided a blood sample was 99%.

For analysis of colorectal adenoma, we ensured comparability of the adenoma case patients and control subjects by requiring that control subjects had to have undergone an endoscopy in the same 2-year time period as the case patient to whom they were matched. Because a substantial proportion of endoscopic procedures were sigmoidoscopies, we restricted our analysis to all adenoma case patients with adenomas arising in the distal colorectum (sigmoid colon and rectum) that occurred between the date of blood collection and May 31, 1998 (unlike the colorectal cancer group, plasma assays were performed on the adenoma case patients and their corresponding control subjects only up to 1998). Information on histologic subtype (villous, tubular, or tubulovillous) and size of adenomas was available from medical records. One control subject from the cohort of women who provided blood was individually matched to each case patient on age, endoscopy within the same 2-year time period (and before baseline), date of blood collection, fasting status, and routine screening or gastrointestinal symptoms as indication(s) for endoscopy. A total of 410 case patients and 410 control subjects were included in this analysis.

Semi quantitative Food Frequency Questionnaire

To assess dietary intake of various nutrients, including folate, vitamin B₆, vitamin B₁₂, vitamin D, methionine and calcium, we used self-administered semi quantitative food frequency questionnaires completed in 1980, 1984, 1986, and 1990. Nutrient intakes were calculated by multiplying the reported frequency of consumption of each specified food item by the nutrient content of the specified portion size and then summing these products for all food items. Information on use of multivitamins and other supplements (including details on which brand and type was used) was collected, and an extensive database of supplement formulations was then used to calculate specific nutrient contributions from these supplemental sources. These nutrient contributions were then added to the specific nutrient intake from foods to calculate a total daily intake for each woman. This method of dietary assessment has been extensively validated and its reliability evaluated (23–25). Vitamin B₆ intake as assessed by the 1980, 1984, and 1986 food frequency questionnaires has been shown to correlate with four 1-week diet records, with correlation coefficients ranging from 0.54 to 0.58 (23–25).

Laboratory Analyses

All assays were conducted at the Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University. Because PLP is the main active form of vitamin B₆ (21), we used plasma concentrations of PLP to determine vitamin B₆ levels. PLP concentrations were determined using an enzymatic procedure based on radioactive tyrosine and the apoenzyme tyrosine decarboxylase, as described by Shin-Beuhring et al. (26). Plasma concentrations of folate and vitamin B₁₂ were determined using a radioassay kit (Bio-Rad, Richmond, CA), and 25(OH) vitamin D concentrations were measured using radioimmunoassay (27).

Blood samples for the cancer case patients and control subjects were handled together, shipped together in the same batch, and assayed in the same analytical run. However, the adenoma assays were performed in two batches (for case patients and matched control subjects accrued before 1996 and for those accrued between 1996 and 1998). To assess laboratory precision, each batch included masked replicate plasma samples that were labeled in a manner identical to that for the regular sample. All laboratory personnel were blinded with respect to case or control status. The mean coefficients of variation (CV) for PLP concentrations were 6.4% for the cancer analysis and 10.9% or less for the two adenoma batches.

Statistical Analyses

A total of 194 cancer case patients and 350 control subjects were included in the cancer analyses and 410 case patients and 410 control subjects in the adenoma analyses. The total numbers of case patients and control subjects for some biomarkers were slightly lower because of missing data resulting from low plasma volume or laboratory errors. We compared the geometric means of the dietary variables and plasma biomarkers of case patients and control subjects using paired t tests. For other continuous variables, we used the Wilcoxon signed-rank test to evaluate differences. For categorical variables, we used a chi-square test to compare case patients and control subjects. We categorized plasma PLP concentration into quartiles using the distribution among the control subjects. To account for slight variations in PLP laboratory values over time between the two batches of adenoma case patients and control subjects (assays performed in 1998 had higher mean values than those performed in 1996), we generated quartiles for PLP concentration from assays performed in 1996 separately from the assays performed in 1998.

Partial Pearson correlations among the control subjects, adjusted for age, date of blood draw, fasting status, and lab
assay batch, were used to evaluate the associations between plasma vitamin B6 and the dietary intake of folate, vitamin B12, and vitamin D. We evaluated both total dietary intake (including supplements) as well as dietary intake from foods only.

We used conditional logistic regression to calculate relative risks (RRs) and 95% confidence intervals (CIs). To control for potential confounders, we created one multivariable model with known risk factors for colorectal cancer and adenoma, including body mass index (BMI, kg/m²), smoking (pack-years), physical activity (MET hours/week), and alcohol intake; all modeled as continuous variables. We also included aspirin use (less than 10 years or 10 or more years), family history of colon or rectal cancer, intake of beef as a main dish (servings per week as a continuous variable), menopausal status, postmenopausal hormone use, and plasma 25(OH) vitamin D concentrations. We initially evaluated dietary calcium as a potential confounder, but its inclusion had little influence on the effect estimates, and therefore we omitted it from subsequent analyses. To further assess the independent association for PLP concentration, we created a second multivariable model adding total dietary folate and dietary methionine, vitamin B supplement use in 1990 (yes or no), and multivitamin supplement use (yes or no in 1990) to the first multivariable model. Information on the majority of the potential nondietary confounders was taken from averaging responses on the 1980 to 1990 biennial questionnaires. For dietary variables, we averaged reported intakes on the four food frequency questionnaires between 1980 and 1990 to most accurately estimate long-term average intake.

To assess whether specific other factors influenced the relationship between PLP concentration and colorectal neoplasia, we cross-tabulated PLP concentrations with the variable of interest and then fit a conditional logistic regression model with a multiplicative interaction term created by multiplying the medians of the quartiles for each variable. For methionine, we used the median as a dichotomous cutpoint. To increase statistical power for the analysis of advanced and early-stage adenoma, we used unconditional logistic regression including all adenoma control subjects (controlling for the matching factors). All statistical tests were two-sided and performed using SAS, version 8.2 (SAS Institute, Cary, NC). P values of <.05 were considered statistically significant.

Results

We compared the characteristics of the cancer and adenoma case patients and control subjects with respect to several plasma markers, diet, and other factors (Table 1). Cancer case patients were less likely than control subjects to use postmenopausal hormones in 1990, had lower intake of folate from food sources, had lower total vitamin B6 intake and lower dietary vitamin B12 intake from both total sources and from food only, and had lower vitamin D intake (both total sources and from food only). In terms of plasma markers, cancer case patients had lower plasma 25(OH) vitamin D concentrations and slightly lower PLP concentrations than did control subjects, but plasma folate, vitamin B12, and homocysteine levels did not differ.

Adenoma case patients had smoked more than control subjects (total pack-years), were less frequently users of postmenopausal hormones at baseline, and were more likely to report a family history of colon or rectal cancer than were control subjects. Plasma concentrations of the various nutrients were not statistically significantly different between case patients and control subjects.

We next evaluated the correlations among plasma PLP concentrations, other plasma biomarkers, and the dietary intake of nutrients for all control subjects (Table 2). As expected, plasma PLP was positively correlated with plasma folate, vitamin B12, and vitamin D and inversely correlated with homocysteine. Plasma PLP concentration was more strongly correlated with the average of total vitamin B6 intake from all sources (Spearman’s ρ = .42) than with dietary vitamin B6 from food only (Spearman’s ρ = .15), reflecting the influence of vitamin B6 supplements on plasma PLP concentrations. The correlation between plasma PLP concentration and vitamin B6 intake from supplements only was 0.40 (data not shown in table). Dietary intake of vitamin B6 made up approximately 60% of total vitamin B6 intake (data not shown). Plasma PLP was modestly positively correlated with total intakes of folate, vitamin D, and vitamin B12.

Plasma concentration of PLP was inversely associated with risk for colorectal cancer (RR for those in the highest versus lowest quartiles = 0.54, 95% CI = 0.31 to 0.92) (Table 3). After adjustment for potential confounding factors, the relative risk was only slightly attenuated, with wider confidence intervals (RR = 0.56, 95% CI = 0.31 to 1.01; χ2 trend = .07). Additional adjustment for other dietary factors, including multivitamin use, dietary intake of folate, vitamin B supplement use and intake of methionine, resulted in a slightly stronger and statistically significant relative risk (RR = 0.48, 95% CI = 0.25 to 0.92; P trend = .03). When we modeled plasma folate and plasma PLP concentrations simultaneously, the association between PLP concentration and colon cancer risk was slightly stronger than when PLP concentration was modeled alone (RR = 0.38, 95% CI = 0.19 to 0.76; P trend = .008; data not shown in table). We evaluated any bias that might have occurred due to any prediagnostic cancer influencing plasma concentrations of PLP by conducting separate analyses in which we excluded the first 2 years of follow-up; the results were essentially unchanged (data not shown).

When we carried out separate analyses for colon and rectal cancer, we found that plasma PLP concentration was inversely associated with risk for colon cancer (multivariable RR, highest versus lowest quartiles = 0.42, 95% CI = 0.21 to 0.85; P trend = .02). We observed no clear association between rectal cancer and plasma PLP concentration (RR = 1.29, 95% CI= 0.22 to 7.52), but the number of rectal cancer cases was small (46).

The association between plasma PLP concentration and colorectal cancer did not vary with alcohol intake (P interaction = .58), plasma folate levels (P interaction = .58), or dietary methionine intake (P interaction = .28). The multivariable relative risks for colorectal cancer for each increasing quartile of plasma PLP among women with methionine intake below the median (≤1.8 g/day) were 1.00 (referent), 0.87, 1.33, and 0.43 (95% CI = 0.19 to 1.00); among women with methionine intake above the median (>1.8 g/day), the relative risks were 0.89, 0.57, 0.48, and 0.64 (95% CI = 0.30 to 1.35). In addition, the association between plasma PLP concentration and colon cancer did not vary as much among women with no family history of colon or rectal cancer (119 case patients and 236 control subjects; RR = 0.48, 95% CI = 0.24 to 0.97; P trend = .04) as it did for women with a family history of colon or rectal cancer (23 case patients and 44 control subjects; RR = 0.41, 95% CI = 0.06 to 2.57; P trend = .36), although the statistical power to study these subgroups was limited.
Table 1. Baseline characteristics (mean ± standard deviation or %) and geometric mean (25th–75th percentile) of average dietary intake (1980–1990) and plasma concentrations of biomarkers among women who developed colorectal cancer or distal colorectal adenoma in the Nurses’ Health Study, 1989–90 to 2000 (cancer) or 1998 (adenoma)*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Case patients</th>
<th>Control subjects</th>
<th>P</th>
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<tr>
<td><strong>Cancer</strong></td>
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</tr>
<tr>
<td>N</td>
<td>194</td>
<td>385</td>
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<td>Age, y</td>
<td>60.0±6.5</td>
<td>60.0±6.5</td>
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<td>Cigarette smoking, pack-years</td>
<td>19.1±25.0</td>
<td>16.2±21.0</td>
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<td>BMI, kg/m²</td>
<td>25.2±4.5</td>
<td>25.1±4.3</td>
<td>.76</td>
</tr>
<tr>
<td>Multivitamin use in 1990, %</td>
<td>34</td>
<td>39</td>
<td>.63</td>
</tr>
<tr>
<td>B-vitamin supplement use in 1990, %</td>
<td>9</td>
<td>9</td>
<td>.98</td>
</tr>
<tr>
<td>Aspirin use for 10 y or more, %</td>
<td>8</td>
<td>9</td>
<td>.66</td>
</tr>
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<td>Physical activity, MET-h/wk</td>
<td>15.0±15.0</td>
<td>15.0±15.2</td>
<td>.82</td>
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<tr>
<td>Postmenopausal hormone use, %†</td>
<td>26</td>
<td>34</td>
<td>.06</td>
</tr>
<tr>
<td>Family history of colorectal cancer, %</td>
<td>19</td>
<td>15</td>
<td>.21</td>
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<tr>
<td>History of screening before 1990, %</td>
<td>34</td>
<td>39</td>
<td>.20</td>
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<tr>
<td>Alcohol, g/day</td>
<td>7.6±10.7</td>
<td>6.3±9.5</td>
<td>.15</td>
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<td><strong>Dietary intake‡</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total folate intake, mg/day</td>
<td>370 (278 to 481)</td>
<td>381 (284 to 511)</td>
<td>.43</td>
</tr>
<tr>
<td>Folate from food only, mg/day</td>
<td>275 (233 to 319)</td>
<td>284 (242 to 334)</td>
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<tr>
<td>Total B₉ intake, mg/day</td>
<td>3.1 (1.9 to 3.7)</td>
<td>3.5 (1.9 to 4.2)</td>
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<td>B₉ from food only, mg/day</td>
<td>1.8 (1.6 to 2.0)</td>
<td>1.8 (1.6 to 2.0)</td>
<td>.54</td>
</tr>
<tr>
<td>Total B₁₂ intake, mg/day</td>
<td>8.2 (5.5 to 1.5)</td>
<td>8.9 (6.5 to 12.0)</td>
<td>.10</td>
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<td>B₁₂ from food only, mg/day</td>
<td>6.1 (4.5 to 8.7)</td>
<td>6.6 (5.0 to 8.8)</td>
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</tr>
<tr>
<td>Vitamin D from food only, IU/day</td>
<td>259 (175 to 460)</td>
<td>288 (195 to 449)</td>
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<tr>
<td>Vitamin D, IU/day</td>
<td>164 (131 to 224)</td>
<td>180 (140 to 247)</td>
<td>.02</td>
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<tr>
<td>Methionine, g/day</td>
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<td>1.8 (1.6 to 2.0)</td>
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<td>Calcium, mg/day</td>
<td>854 (660 to 1101)</td>
<td>898 (720 to 1119)</td>
<td>.12</td>
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<tr>
<td><strong>Plasma concn‡</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>25(OH) vitamin D, ng/mL</td>
<td>23.5 (18.4 to 29.5)</td>
<td>25.6 (20.2 to 33.0)</td>
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<tr>
<td>Folate, ng/mL</td>
<td>8.1 (5.4 to 12.4)</td>
<td>7.8 (1.6 to 2.5)</td>
<td>.69</td>
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<tr>
<td>Vitamin B₁₂, pg/mL</td>
<td>422 (321 to 572)</td>
<td>430 (327 to 527)</td>
<td>.60</td>
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<tr>
<td>Vitamin B₆, pmol/mL</td>
<td>47.6 (29.7 to 70.8)</td>
<td>53.2 (33.4 to 83.1)</td>
<td>.07</td>
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<tr>
<td>Homocysteine, nmol/mL</td>
<td>10.8 (8.2 to 13.5)</td>
<td>10.3 (8.2 to 12.2)</td>
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<td>410</td>
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<td>58.4±6.6</td>
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<td>Cigarette smoking, pack-years</td>
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<td>BMI, kg/m²</td>
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<td>24.9±4.0</td>
<td>.49</td>
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<td>Multivitamin use in 1990, %</td>
<td>38</td>
<td>40</td>
<td>.43</td>
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<td>.37</td>
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<td>Physical activity, MET-h/wk</td>
<td>14.5±15</td>
<td>15±17.8</td>
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<tr>
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<td>32</td>
<td>39</td>
<td>.04</td>
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<td>41</td>
<td>36</td>
<td>.13</td>
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<td>Gastrointestinal symptoms as indication for endoscopy, %</td>
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<td>48.3</td>
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<td>Alcohol, g/day</td>
<td>7.0±10.3</td>
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<td><strong>Dietary intake‡</strong></td>
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<td>371 (283 to 486)</td>
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<td>Total B₉ intake, mg/day</td>
<td>281 (239 to 330)</td>
<td>277 (235 to 323)</td>
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<td>B₉ from food only, mg/day</td>
<td>1.7 (1.5 to 1.9)</td>
<td>1.7 (1.4 to 1.9)</td>
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<td>Total B₁₂ intake, mg/day</td>
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<td>8.9 (6.3 to 12.3)</td>
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<td>B₁₂ from food only, mg/day</td>
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<td>Total vitamin D, IU/day</td>
<td>261 (175 to 406)</td>
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<td>Vitamin D from food only, IU/day</td>
<td>168 (129 to 233)</td>
<td>170 (132 to 235)</td>
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<td>Methionine, g/day</td>
<td>1.8 (1.6 to 2.0)</td>
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<td>Calcium, mg/day</td>
<td>911 (720 to 1148)</td>
<td>935 (742 to 1195)</td>
<td>.26</td>
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</tr>
</tbody>
</table>

*For continuous variables, P values (two-sided) were calculated using the Wilcoxon signed-rank test. For plasma markers and dietary variables, a paired (two-sided) t test was performed on log-transformed values. For categorical variables, P values (two-sided) were calculated using the chi-square test. BMI = body mass index.

†Percentage of women using estrogen replacement therapy was calculated among postmenopausal women only.

‡Values are geometric means (interquartile range). The total number of case patients and of control subjects was lower because of missing data (low plasma volume or laboratory error): for cancer patients, folate (191 case patients/377 control subjects), vitamin B₁₂ (191 case patients/377 control subjects), and vitamin B₆ (188 case patients/371 control subjects); for adenoma patients, folate (360 case patients/360 control subjects), vitamin B₁₂ (406 case patients/406 control subjects), and vitamin B₆ (408 case patients/408 control subjects).
Table 2. Partial Spearman correlations between plasma vitamin B6 and other plasma and dietary characteristics among control subjects of both cancer and adenoma case patients adjusted for age, date of blood draw, fasting status, and laboratory assay batch.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Spearman correlation with plasma vitamin B6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma levels</td>
<td></td>
</tr>
<tr>
<td>Folate</td>
<td>0.51†‡</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>0.25†‡</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>−0.17†‡</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>0.22†‡</td>
</tr>
<tr>
<td>Avg intake</td>
<td></td>
</tr>
<tr>
<td>Total folate</td>
<td>0.30†‡</td>
</tr>
<tr>
<td>Folate (food only)</td>
<td>0.14†‡</td>
</tr>
<tr>
<td>Total vitamin B6</td>
<td>0.42†‡</td>
</tr>
<tr>
<td>Vitamin B6 (food only)</td>
<td>0.15†‡</td>
</tr>
<tr>
<td>Total vitamin B12</td>
<td>0.25†‡</td>
</tr>
<tr>
<td>Vitamin B12 (food only)</td>
<td>0.01†</td>
</tr>
<tr>
<td>Total vitamin D</td>
<td>0.28†‡</td>
</tr>
<tr>
<td>Vitamin D (food only)</td>
<td>0.11§†‡</td>
</tr>
</tbody>
</table>

*P values (two-sided) were calculated using a t test for the Spearman rank correlation coefficient.
†P<.001.
‡Among supplement non-users.
§P=.005.

We also observed associations between the average intake of vitamin B6 from foods and supplements calculated from the four food frequency questionnaires collected between 1980 and 1990 and risk for colorectal cancer (Table 4). Statistically significant inverse trends were stronger for colon cancer risk than for colorectal cancer risk (for colorectal cancer, highest versus lowest quartile, multivariable RR = 0.60, 95% CI = 0.34 to 1.06; for colon cancer, highest versus lowest quartile, multivariable RR = 0.51, 95% CI = 0.27 to 0.97; P_trend = .007), and associations were similar to those observed for plasma PLP concentrations (Table 3). After further adjustment for dietary variables, the association was stronger (for colon cancer, multivariable RR = 0.32, 95% CI = 0.14 to 0.75; P_trend = .003).

We also evaluated associations between plasma PLP concentration and risk of distal colorectal adenoma (Table 5). Women in the highest quartile of plasma PLP had a non-statistically significantly lower unadjusted relative risk than did women in the lowest quartile (RR = 0.66, 95% CI = 0.44 to 1.00; P_trend = .06). Adjustment for potential confounders attenuated the relative risk (RR = 0.72, 95% CI = 0.46 to 1.12; P_trend = .24); additional adjustment for dietary factors did not markedly alter the relative risk (RR = 0.69, 95% CI = 0.41 to 1.15; P_trend = .27). With the addition of plasma folate to the model, the relative risk of adenoma was essentially unchanged (data not shown in table; RR = 0.71, 95% CI = 0.44 to 1.13, P_trend = .29). Comparing adenoma risk by anatomic location, the overall association with PLP concentrations appeared stronger for rectal adenoma (n = 78; RR = 0.15, 95% CI = 0.03 to 0.73) than for sigmoid adenoma (n = 330; RR = 0.91, 95% CI = 0.56 to 1.48).

To evaluate whether the associations varied by adenoma size and histology, we classified adenoma into two categories, advanced (large [≥1 cm], villous or tubulovillous) and early-stage [small and tubular]). A suggestive inverse association between plasma PLP concentration and advanced adenoma (RR = 0.65, 95% CI = 0.37 to 1.11; P_trend = .08) was observed, but there was less evidence of an association with early-stage adenoma (RR = 0.85, 95% CI = 0.52 to 1.38; P_trend = .52). We also observed an association between total vitamin B6 intake and distal colorectal adenoma (data not shown).

The association between plasma PLP concentration and colorectal adenoma risk varied somewhat by plasma folate concentrations (P_interaction = .11) but not with intake of alcohol (P_interaction = .56) or with methionine (P_interaction = .60). To maximize statistical power, we used tertiles to evaluate whether the inverse association between plasma PLP and adenoma varied by plasma folate levels. In fact, we found that the inverse association was particularly evident among women with low plasma folate (multivariable RRs for each increasing tertile of plasma PLP = 1.00 [referent], 0.59, 0.53, among women with low plasma folate; RRs = 0.92, 0.84, and 0.62, among women in the middle tertile; and RRs = 0.74, 0.55, and 0.75, among women with high plasma folate). We observed little difference in the risk of all distal colorectal adenoma by family history of colon or rectal cancer (yes, P_trend = .19; no, P_trend = .28). However, the association

Table 3. Risk of colorectal cancer and colon cancer by quartiles of plasma vitamin B6 among women in the Nurses’ Health Study, 1989–2000*.

<table>
<thead>
<tr>
<th>Plasma vitamin B6</th>
<th>RR (and 95% CI) by quartile</th>
<th>P_trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Median, pmol/mL</td>
<td>23.9</td>
<td>39.9</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of case patients/no. of control subjects</td>
<td>59 / 92</td>
<td>46 / 94</td>
</tr>
<tr>
<td>Simple matched RR†</td>
<td>1.00 (referent)</td>
<td>0.76 (0.47 to 1.25)</td>
</tr>
<tr>
<td>Multivariable RR‡</td>
<td>1.00 (referent)</td>
<td>0.75 (0.44 to 1.26)</td>
</tr>
<tr>
<td>Multivariable RR§</td>
<td>1.00 (referent)</td>
<td>0.77 (0.45 to 1.31)</td>
</tr>
<tr>
<td>Colon cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of case patients/no. of control subjects</td>
<td>48 / 69</td>
<td>35 / 74</td>
</tr>
<tr>
<td>Simple matched RR†</td>
<td>1.00 (referent)</td>
<td>0.70 (0.40 to 1.21)</td>
</tr>
<tr>
<td>Multivariable RR‡</td>
<td>1.00 (referent)</td>
<td>0.68 (0.38 to 1.22)</td>
</tr>
<tr>
<td>Multivariable RR§</td>
<td>1.00 (referent)</td>
<td>0.69 (0.38 to 1.25)</td>
</tr>
</tbody>
</table>

*P_trend (two-sided) in risk over median values of quartiles among control subjects calculated with conditional logistic regression model using the median values for quartiles of plasma estimates as a continuous variable. RR = relative risk; CI = confidence interval.
†Odds ratio and 95% CI from conditional logistic regression without any covariates in the model.
‡Odds ratio and 95% CI from conditional logistic regression with adjustment for the following covariates: body mass index; physical activity; smoking; menopausal status; postmenopausal hormone use; duration of regular aspirin use; family history of colorectal cancer; intake of alcohol and red meat; plasma vitamin D; and a history of endoscopy.
§Odds ratio and 95% CI from model (‡) above, additionally controlling for intake of folate and methionine, vitamin B supplement use, and multivitamin supplement use.
between plasma PLP and advanced adenoma appeared slightly stronger among women (the highest versus the lowest quartiles) without a family history of colon or rectal cancer (136 case patients and 322 control subjects; multivariable RR = 0.62, 95% CI = 0.32 to 1.18; *P* = .09) than among women with a family history (43 case patients and 67 control subjects; multivariable RR = 0.74, 95% CI = 0.22 to 2.46; *P* = .77).

To determine if the inverse association varied by use of vitamin supplements, we cross-classified PLP quartiles and multivitamin or vitamin B supplement use. We found evidence that increasing concentrations of plasma PLP were associated with lower risk of colorectal cancer both in women who were not taking any supplements and in women who were (multivariable RR for each increasing quartile of PLP among non-supplement users = 1.00 [referent], 0.77, 0.83, and 0.44 [95% CI = 0.17 to 1.15]; multivariable RR for each increasing quartile of PLP among supplement users = 1.69, 1.13, 1.18, and 0.71 [95% CI = 0.36 to 1.40]; *P* for trend = .65).

**DISCUSSION**

In this prospective, nested case–control study of women, a statistically significant inverse association between plasma PLP concentrations and risk of both colorectal cancer and colon cancer, as well as a suggestive inverse association with risk of distal colorectal adenoma, particularly advanced adenoma (those that

<table>
<thead>
<tr>
<th>Plasma vitamin B₆</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th><em>P</em> for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median, pmol/mL</td>
<td>22.5</td>
<td>37.9</td>
<td>63.3</td>
<td>129.0</td>
<td></td>
</tr>
<tr>
<td>Distal colorectal adenoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of case patients/no. of control subjects</td>
<td>124/101</td>
<td>114/102</td>
<td>80/102</td>
<td>90/103</td>
<td></td>
</tr>
<tr>
<td>Simple matched RR‡</td>
<td>1.00 (referent)</td>
<td>0.87 (0.60 to 1.27)</td>
<td>0.60 (0.39 to 0.91)</td>
<td>0.66 (0.44 to 1.00)</td>
<td>.06</td>
</tr>
<tr>
<td>Multivariable RR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Advanced adenoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of case patients/no. of control subjects</td>
<td>52/97</td>
<td>52/96</td>
<td>40/98</td>
<td>35/98</td>
<td></td>
</tr>
<tr>
<td>Multivariable RR§</td>
<td>1.00 (referent)</td>
<td>0.99 (0.60 to 1.63)</td>
<td>0.79 (0.47 to 1.34)</td>
<td>0.65 (0.37 to 1.11)</td>
<td>.08</td>
</tr>
</tbody>
</table>

*P* for trend (two-sided) in risk over median values of quartiles among control subjects calculated using conditional logistic regression model using the median values for quartiles of plasma estimates as a continuous variable. RR = relative risk; CI = confidence interval.

†Odds ratio and 95% CI from conditional logistic regression without any covariates in the model.

‡Odds ratio and 95% CI from conditional logistic regression with adjustment for the following covariates: BMI; physical activity; smoking; menopause status; postmenopausal hormone use; duration of regular aspirin use; family history of colorectal cancer; intake of alcohol and red meat; plasma vitamin D; and a history of endoscopy.

§Odds ratio and 95% CI from adjusted model above, additionally controlling for intake of folate and methionine, vitamin B supplement use, and multivitamin supplement use.
were large [≥1 cm] or had a villous or tubulovillous histology), was observed. Stronger associations between plasma PLP concentration and colorectal neoplasia were suggested among women with low plasma folate, but our power to assess interactions was limited. Total vitamin B₆ intake was also statistically significantly inversely associated with risk of colorectal cancer. We observed 59 cases of colorectal cancer among the 25% of women (approximately 8200) with the lowest plasma vitamin B₆ concentrations over the 10-year follow-up, compared with 33 cases of colorectal cancer in the 25% of women with the highest plasma vitamin B₆ concentrations.

Previously, two case–control studies (13,14) reported statistically significant reductions in risk for colorectal cancer with higher intakes of dietary vitamin B₆. Although results from the Iowa Women’s Health Study cohort (11) did not show an independent association between dietary vitamin B₆ (or folate) and risk of colorectal cancer, they showed a reduced risk for proximal colon cancers (RR = 0.65 [95% CI = 0.50 to 0.84]) among women with high folate and high vitamin B₆ intake. More recently, Larsson et al. (10) observed a 34% statistically significantly reduced risk of colorectal cancer, comparing the highest to lowest quintiles of dietary vitamin B₆ intake among women in the Swedish Mammography Cohort. These associations are consistent with and even slightly stronger than those we report here. The results of an additional case–control study (12) showed no overall inverse association with dietary vitamin B₆ but a statistically significantly reduced risk among those with the TT genotype of the enzyme methylene tetrahydrofolate.

Although the associations we observed were stronger for colorectal cancer than for adenoma, the pattern was similar for both outcomes. We observed slightly different associations by subsite for cancers versus adenoma (i.e., associations were stronger for colon cancer than rectal cancer but stronger for rectal adenomas than colon adenomas); however, we believe these differences are likely due to chance. Furthermore, the stronger inverse risk for advanced adenoma than for early-stage adenoma supports a possible role of vitamin B₆ in progression of adenoma and early cancer. If vitamin B₆ influences risk of colorectal neoplasia via its role in one-carbon metabolism, we would expect plasma PLP concentrations to be more strongly associated with reduced risk among women with low methyl status (low folate, high alcohol, low methionine). In fact, we found some evidence that PLP concentrations were more important among individuals with low plasma folate (for colorectal adenoma) or non-users of multivitamin or B-vitamin supplements (than among supplement users). However, we had limited power to evaluate these associations. Moreover, when we modeled plasma folate and plasma PLP concentrations simultaneously, the inverse association with plasma PLP concentration remained, suggesting that PLP may have an association independent of folate.

We found little evidence that plasma PLP concentrations were differentially associated with colon cancer or adenoma risk according to family history of colon or rectal cancer, as has been previously reported for dietary folate and multivitamin use (28). However, our results were based on a limited number of case patients so they should be interpreted cautiously.

Although the reduced risk for colorectal cancer was slightly stronger among non-users of supplements than among supplement users, the trend with increasing plasma PLP levels was similar for both non-supplement users and supplement users. This similarity implicates an association between vitamin B₆ and colorectal neoplasia, particularly because diet and supplement use are unlikely to have identical sources of confounding.

This analysis has several limitations. One is the possibility of the occurrence of bias due to measurement error in the laboratory assays. However, the relatively low % CVs of the PLP measurement and the correlation between total vitamin B₆ intake and plasma PLP concentration that we observed suggest that plasma PLP concentration measurements were relatively reliable. Although we do not have information on the stability of PLP under the blood collection or storage conditions we used, any degradation would have occurred non-differentially with respect to case or control status and therefore could not be the main explanation for our findings. Moreover, the range of PLP values in our population is comparable to those in previous reports (29,30). Although we cannot avoid measurement error in dietary intake assessment, the impact of such error should be dampened because we averaged intake reported over four consecutive food frequency questionnaires assessed over 10 years. Further, any measurement error most likely biases our results towards the null. Thus, the true associations would be slightly stronger than what we report here. Another limitation is that our sample size limited our power to evaluate subgroups and statistical interactions.

Strengths of our analysis include the prospective design, high follow-up rates, and relatively large sample size, all of which reduce the possibility that bias influenced our results. Also, due to the extensive information we have on participants’ lifestyle and diet and detailed information on multivitamin and supplement type, brand, and formulation, we were able to control for many potentially confounding factors. We controlled for endoscopy history, including indications for endoscopy, and our finding that higher plasma concentrations of PLP were associated with a reduced risk of adenoma among screened individuals suggests that our results were not due to differential screening by vitamin B₆ status.

The potential role of vitamin B₆ in colorectal carcinogenesis has been studied in much greater detail recently. Several studies have shown that high levels of vitamin B₆ suppress the growth of animal or human cancer cells in vitro (9). Specifically, colon tumorigenesis was suppressed by moderate doses of dietary vitamin B₆ in mice given injections of a carcinogen (azoxymethane) (31). Vitamin B₆ also has been shown to reduce cell proliferation (32), to reduce oxidative stress (33), to suppress nitric oxide (34), and to have antiangiogenic properties (35,36). Vitamin B₆ may also influence risk of colon cancer via inhibition of thymidylate synthase (37,38). Although low activity of thymidylate synthase can increase levels of dUMP (39), high thymidylate synthase activity can lead to cell proliferation (40,41), reduced apoptosis (42), decreased breast cancer and colon cancer survival (43,44,45), and increased colon cancer and colon adenoma risk (43,46). Vitamin B₆ could also be acting via alternative pathways, including as a co-factor in amino acid metabolism, lipid metabolism, and nervous system function (19). Although these preliminary studies suggest several plausible mechanisms by which high dietary or circulating vitamin B₆ may play a role in reducing risk for colorectal cancer, further study of them is necessary.
Increasing evidence indicates that one-carbon metabolism influences colorectal carcinogenesis (1–3). Our results suggest an inverse association between vitamin B6, a key player in one-carbon metabolism, and risk of colorectal neoplasia. Additional animal, epidemiologic, and intervention studies are required to clarify the association between dietary and plasma vitamin B6 and colorectal neoplasia.

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NOTES

The work reported in this manuscript was supported by Public Health Service grants CA87969, CA49449, CA90598 and CA42182 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Dr. Wei is also partially supported by National Research Service Award T32 CA 09001.

We appreciate the leadership and guidance of Dr. Graham Colditz and the dedication and commitment of the participants of the Nurses’ Health Study. We thank Helena Judge-Ellis, Victor Pontes, David Coppola, and Todd Reid for their technical assistance in the preparation of this manuscript.

Manuscript received September 17, 2004; revised March 3, 2005; accepted March 22, 2005.