Published in final edited form as:
Cancer Epidemiol Biomarkers Prev. 2009 August ; 18(8): 2195–2201. doi: 10.1158/1055-9965.EPI-09-0141.

Plasma Levels of B Vitamins and Colorectal Cancer Risk: The Multiethnic Cohort Study

Loïc Le Marchand[1], **Kami K. White**[1], **Abraham M.Y. Nomura**[1], **Lynne R. Wilkens**[1], **Jacob S. Selhub**[2], **Maarit Tiirikainen**[1], **Marc T. Goodman**[1], **Suzanne P. Murphy**[1], **Brian E. Henderson**[3], and **Laurence N. Kolonel**[1]

[1]Cancer Research Center of Hawaii, University of Hawaii, Honolulu, HI

[2]USDA Jean Mayer Human Nutrition Research Center on Aging, Tufts University, Boston, MA

[3]Keck School of Medicine, University of Southern California, Los Angeles, CA

Abstract

B vitamins, such as folate, vitamin B6 and vitamin B12, play an important role as co-enzymes in one-carbon metabolism and may affect colorectal cancer (CRC) risk. We aimed to comprehensively investigate the relationships of plasma folate, pyridoxal-5'-phosphate (PLP, the active form of vitamin B6), vitamin B12, methylmalonic acid, homocysteine and cysteine with CRC risk, accounting for suspected modifiers [alcohol intake, *MTHFR* C677T genotype and plasma C-reactive protein (CRP)] and potential confounders. We conducted a case-control study nested within the Multiethnic Cohort study and analyzed prospectively collected blood samples from 224 incident CRC cases and 411 controls matched on age, sex, race/ethnicity, study site, date/time of blood draw, and hours of fasting. We found an inverse association between plasma PLP levels and CRC, with odds ratios and 95% confidence intervals for increasing quartiles of 1.00, 0.84 (0.51–1.40), 0.62 (0.37–1.03), 0.49 (0.29–0.83), p trend: 0.009. This association was not explained by an association with plasma folate, appeared to be stronger at low levels of alcohol intake and among individuals with the *MTHFR* 677TT genotype, and was independent of plasma CRP levels. An inverse association with plasma folate was also observed among individuals with a low level of alcohol intake. These data suggest an independent role for vitamin B6 in reducing CRC risk.

Keywords

Vitamin B; folate; vitamin B6; colorectal cancer

INTRODUCTION

The B vitamins, such as folate, vitamin B6 and vitamin B12, play an important role as co-enzymes in one-carbon metabolism which is essential for nucleotide synthesis and DNA methylation (1). Disruption of this metabolic pathway can result in aberrations in DNA methylation, imbalance in DNA precursors and deficiency in DNA repair which each may contribute to carcinogenesis, especially in the colon (1). Indeed, high folate status has been associated in several studies with a reduced risk of colorectal cancer (CRC) (2). This association has been shown in some studies to be more marked among individuals with the TT genotype

Address reprint requests to: Loïc Le Marchand, MD, PhD, Epidemiology Program, Cancer Research Center of Hawaii, University of Hawaii, 1236 Lauhala Street, Honolulu, HI 96813. Loic@crch.hawaii.edu.

for *MTHFR* C677T, a genetic polymorphism that results in an 50% reduction in enzyme activity, as well as among high consumers of alcohol, a folate antagonist (2,3).

A smaller number of studies have also linked elevated dietary or plasma vitamin B6 levels to a reduced CRC risk (4–8). This association may involve mechanisms additional to one-carbon metabolism. Low levels of vitamin B6 have been associated with elevated C-reactive protein (CRP), a marker of inflammation, and have been shown to increase oxidative stress, nitric oxide synthesis, cell proliferation and angiogenesis (9,10).

Because few prospective studies have examined the association of plasma levels of B vitamins with CRC risk and because these existing studies typically have not considered the correlation that exists in their levels, it remains unclear which ones, if any, have an independent effect on CRC. We conducted a comprehensive investigation of the relationships of plasma levels of folate, pyridoxal-5'-phosphate (PLP, the active form of vitamin B6), vitamin B12 (cobalamin), methylmalonic acid (elevated in cobalamin deficiency), homocysteine and cysteine with CRC risk, accounting for suspected modifiers, using prospectively collected blood samples in the Multiethnic Cohort (MEC) study.

Methods

Study population

In 1993–1996, the MEC study recruited more than 215,000 individuals to investigate the relationships between diet, genetic variation and cancer in Hawaii and Los Angeles, California (11). The study targeted men and women, 45–75 years of age in 1993 and of five racial/ethnic groups (African Americans, Native Hawaiians, Japanese Americans, Latinos, and Whites). Participants completed a 26-page baseline questionnaire that included a quantitative food frequency questionnaire with detailed information on portion sizes, as well as questions on medical history and lifestyle. From that questionnaire, information on family history of colorectal cancer, body mass index, smoking history, physical activity, multivitamin supplement use, and alcohol consumption was incorporated into the analyses for the present study. Additionally, dietary intakes of specific nutrients and food groups were estimated from single items and mixed dishes as measured by the food frequency questionnaire and from questions on supplement use. A short 4-page questionnaire was administered about 5 years after baseline to update the participants' medical and cancer screening histories.

The Biospecimen Subcohort

Recruitment of cohort members for prospective biospecimen collection largely took place between 2001 and 2006. They were contacted by letter, and then by phone, to request biological specimens (blood and urine). For those who agreed, a phone interview was administered that included a short screening questionnaire and an update of a few items, including CRC cancer screening. Blood samples were drawn at a clinical laboratory or in the subjects' home and were processed within four hours of collection. After centrifugation and separation, blood components were stored in 0.5 cc cryotubes in the vapor phase of liquid nitrogen. A total of 67,594 cohort members contributed to the biorepository, from which the cases and controls for this study were selected. 95% of participants who provided the blood samples had fasted (8 or more hours).

Selection of cases and controls

Incident colorectal cancer cases were identified through the Hawaii and California tumor registries of the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute. For this nested case-control study, cases were defined as individuals who had

contributed blood to the biospecimen subcohort prior to their diagnosis of colon or rectal cancer, and whose diagnosis was reported in the 2006 tumor linkage.

For each case, a pool of potential controls was selected from the MEC participants who contributed blood to the biorepository and were alive and free of colorectal cancer at the age of the case's diagnosis, and who matched the case on: sex; birth year (± 1 year); race/ethnicity; location (Hawaii or California); date of blood draw (± 6 months); time of blood draw (± 2 hours); and hours of fasting prior to blood draw (0–<6, 6–<8, 8–<10, and 10+ hours). From each pool, two controls were then randomly chosen.

Of the 263 eligible colorectal cancer cases and 526 eligible controls, 26 cases and 51 controls did not have any plasma B vitamin measurement due to unavailability of a fasting sample and an additional 4 controls were excluded due to the loss of matched sets (cases had no data). Of the remaining 237 cases and 471 controls, 12 cases and 40 controls were excluded because they had missing data on at least one of the adjustment variables. One additional case and 20 controls were excluded to the loss of matched data sets. A total of 224 cases and 411 controls (187 cases matched to 2 controls; 37 cases matched to 1 control) were analyzed. The vitamin B panel was expanded for the second batch of samples sent for laboratory analysis to include cysteine and methylmalonic acid. These analytes were measured on only 118 cases and 211 controls (93 cases matched to 2 controls; 25 cases matched to 1 control). *MTHFR* polymorphism data were available on 197 cases and 358 controls (161 cases matched to 2 controls; 36 cases matched to 1 control).

Laboratory assays

All laboratory assays were conducted blind to the case-control status of the participants. The samples from each matched case-control set were analyzed together as sets within laboratory batches and in random order within sets.

Plasma B Vitamins—Plasma folate and vitamin B12 concentrations were determined by a radioimmunoassay method using a commercially available kit from Biorad (Richmond, CA). Pooled plasma samples are used for internal laboratory quality control purposes and were analyzed as blind duplicates with the study samples. The intra-batch coefficients of variation from 20 blind duplicate pairs were 5.4% for vitamin B12 and 7.1% 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 apoenzyme with the supernatant fraction of TCA-precipitated serum sample and tritium-labeled tyrosine. The intra-batch coefficient of variation for this assay was 6.1% based on 27 duplicate pairs. Methylmalonic acid was quantified by a modification of the LCMS method described by Blom et al. (13) and the intra-batch correlation coefficient was 11.7% based on 16 duplicate pairs. Total homocysteine and cysteine were determined in plasma by an HPLC method derived from that of Araki and Sako (14). The intra-batch coefficient of variation was 4.2% for homocysteine and 3.5% for cysteine based on 15 duplicate pairs.

Plasma C-Reactive Protein (CRP): The C-reactive protein assay was performed using a Cobas MiraPlus CC clinical autoanalyzer (Roche Diagnostics, Indianapolis, IN) and a latex particle enhanced immunoturbidimetry based kit from Pointe Scientific (Lincoln Park, MI) with a functional sensitivity of 0.1 mg/L. The coefficient of variation for CRP was 7.5% based on 34 duplicate pairs.

DNA extraction and genotyping assay: DNA was purified from blood buffy coat using QIAamp DNA Blood Kits (Qiagen, Valencia, CA). The DNA was plated on 384-well plates together with duplicate QC pairs. All assays were run on an AB 7900HT Fast Real-Time System. Genotyping of the *GSTP1* missense single nucleotide polymorphism (SNP) rs1801133 (*MTHFR* C677T) was done using 10ng of genomic DNA and the 5' nuclease TaqMan allelic discrimination assay from AB (Applied Biosystems, Foster City, CA). Ethnic-specific genotype frequencies for *MTHFR* C677T in the controls were compared against Hardy-Weinberg equilibrium (HWE). All frequencies met the HWE criteria at $p > 0.05$. The genotyping call rate was 99.7% and the concordance rate across 78 blind duplicate samples was 100%.

Statistical analyses

To estimate the associations of plasma levels of specific B vitamins and the risk of colorectal cancer, we used conditional logistic regression models to compute odds ratios (ORs) and 95% confidence intervals (CIs), where the matched case-controls sets comprised the strata. We adjusted for age at blood draw and hours of fasting prior to blood draw as continuous variables to account for differences within sets. Quartiles and tertile cutpoints of the overall distribution of plasma levels were used to derive dummy variables, where the lowest quantile served as the reference group. Median cutpoints were also used to derive a dichotomous variable for each B vitamin. To test for linearity in the logit of colorectal cancer risk, a trend variable was assigned the median value of the appropriate quantile. Alcohol and CRP were also dichotomized at the median and *MTHFR* was modeled as a binary variable (TT vs. GG or GT).

In addition to the initial crude regression models, secondary models were created to further adjust the risk estimates for the following risk factors for colorectal cancer: processed meat intake (density, g/1000 kcal/day), ethanol consumption (ever in the past year at least once a month vs never), body mass index (weight, kg/ height, m^2); pack-years of cigarette smoking; hours of moderate or vigorous physical activity; family history of colorectal cancer among parents and siblings, and history of colorectal cancer screening (never versus ever). Plasma folate was also adjusted for in a separate set of models. Other potential confounders, such as intake of red meat, fish and dairy products, were adjusted for in select models. The likelihood ratio test was used to examine interactions between plasma B vitamins and alcohol intake, plasma CRP, the *MTHFR* C677T polymorphism, or plasma folate in relation to colorectal cancer risk. The one degree of freedom test compared a main effects model, including the dichotomous variables of interest, with a model containing the interaction terms. Stratified analyses by sex, race/ethnicity, supplement use, and colon and rectal cancer were also done. Polytomous logistic regression was used to test for differences in the risk estimates for colon and rectal cancer, where sets were broken and matching variables were included as additional adjustment variables. Analyses were performed in SAS, version 9.1.3.

Results

As shown in Table 1, colorectal cancer cases were more likely to be obese (23% vs 11% had BMI ≥ 30 kg/m^2), more likely to consume alcohol (57% vs 52%), and generally consume more red and processed meat and less dairy than controls. Little to no difference was observed for dietary fiber intake between the cases and their matched controls. A higher proportion of cases than controls had a family history of colorectal cancer (12% vs 10%) and had ever smoked (60% vs 54%). Hours spent in moderate or vigorous physical activity were similar. The median levels for the plasma B vitamins were lower for cases than controls, except for methylmalonic acid. CRP levels were higher for cases.

Table 2 presents the crude and adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between each of the plasma B vitamins and the risk of colorectal cancer.

Subjects in the upper quartiles for folate levels tended to show a reduced risk of CRC [adjusted OR (95% CI) for Q4 vs. Q1: 0.61 (0.33–1.13); p for trend: 0.097]. A significant inverse trend was observed for PLP levels, with ORs of 1.00, 0.84 (0.51–1.40), 0.62 (0.37–1.03), 0.49 (0.29–0.83); p for trend: 0.009. Because PLP and folate levels were correlated (Spearman correlation coefficient: 0.40), we further adjusted the risk estimates for plasma folate (last column in Table 2). The association between PLP and CRC remained significant. No significant trends were observed for Vitamin B12, methylmalonic acid or homocysteine. We also ran a model for subjects with low vitamin B12 status, defined as vitamin B12 <148pmol/L or methlymalonic acid >210 nmol/L (223 cases, 407 controls), compared to all others. The adjusted OR for CRC was 1.27 (0.73–2.20). There was a reduced risk suggested for increasing levels of cysteine, but this trend was not statistically significant. Further adjustment for intakes of red meat, fish and dairy did not change the risk estimates.

The main effect for MTHFR C677T was not significant [OR for TT vs. CC+CT genotype: 0.95 (0.52–1.73)]. Similarly, no statistically significant association was found with plasma CRP [multivariate-adjusted ORs for increasing quartiles (≤0.30, 0.31–0.80, 0.81–2.20, >2.2 mg/l): 1.00, 0.88 (0.52–1.49), 1.00 (0.61–1.62) and 1.19 (0.71–2.00), p for trend=0.33] or alcohol intake [multivariate-adjusted ORs for non-drinkers, ≤3.3 g/d, 3.3–16.4 g/d, >16.4: 1.00, 1.24 (0.79–1.96), 1.27 (0.78–2.06), 1.37 (0.81–2.31), p for trend: 0.35] in this nested-case control study.

Table 3 shows the joint effects between B vitamins and, separately, alcohol, CRP, and the *MTHFR* 677TT genotype in relation to the risk of CRC. An interaction between plasma folate and alcohol intake was observed ($p_{interaction}$= 0.050), with a significantly decreased CRC risk observed with a high plasma folate (>15.3 ng/ml) only among subjects with an alcohol intake of ≤0.74 g/day [OR: 0.55 (0.31–0.95), compared to individuals with a low alcohol intake and a lower plasma level] (Table 3). Plasma folate, comparing subjects with high vs. low levels, was not associated with a reduced risk among subjects with an alcohol intake over the median [OR: 1.12 (0.62–2.04)]. This interaction with alcohol intake was also suggested for PLP ($p_{interaction}$= 0.072), where among light drinkers, the association with plasma PLP >51.5 pmol/ml, compared to a lower level [OR: 0.43 (0.26–0.74),] was stronger than among heavier drinkers [OR: 0.84 (0.50–1.40)]. No interaction was found between CRP and PLP, using for PLP either the median value (51.5 pmol/ml) ($p_{interaction}$= 0.42) or the cutpoint usually used for deficiency (30 pmol/ml) ($p_{interaction}$= 0.66). Unexpectedly, we observed a significant interaction between plasma CRP and vitamin B12 ($p_{interaction}$= 0.027), with the suggestion of an increased CRC risk with higher vitamin B12 levels among subjects with high CRP levels [OR: 1.50 (0.92–2.44)]. We also observed the suggestion of an interaction between PLP and *MTHFR* C677T on the risk of CRC ($p_{interaction}$= 0.08), with a stronger reduced risk among TT carriers with plasma PLP above the median, compared to those with PLP ≤ median [OR: 0.25 (0.08–0.80)], than among subjects with the CT or CC genotype [OR: 0.74 (0.47–1.15)]. A similar relationship was suggested for plasma folate, with ORs of 0.37 (0.11–1.21) and 0.92 (0.55–1.51) for high vs. low folate levels among TT carriers and CT/CC carriers, respectively.

We also tested the interaction between plasma folate and PLP on the risk of CRC. The CRC OR for subjects with both PLP and folate levels above the median, compared to those with both levels ≤ median, was 0.55 (0.33–0.93) and the $p_{interaction}$ was 0.93.

Stratified analyses by sex, anatomic subsites (colon, rectum), or race/ethnicity did not reveal any heterogeneity in the association of CRC with plasma PLP. A similar analysis stratified by multivitamin supplement use suggested a stronger effect among non-users [ORs for increasing tertiles of PLP: 1.00, 0.85 (0.41–1.77), 0.34 (0.13–0.92), p trend: 0.03] than users [1.00, 0.72 (0.41–1.26), 0.70 (0.40–1.22), p trend: 0.32]; however, the test for interaction was not

significant ($p_{interaction}$= 0.52). Exclusion of cases diagnosed during the first year of follow-up did not materially change the risk estimates.

Discussion

In this case-control study nested in the MEC study, we found an inverse association between plasma PLP levels and CRC which was not explained by an association with plasma folate, appeared stronger at low levels of alcohol intake and among individuals with the *MTHFR* 677TT genotype, and was independent of plasma CRP levels. An inverse association with plasma folate was also observed which appeared to be stronger among individuals with a low level of alcohol intake and was suggested among subjects with the *MTHFR* 677TT genotype.

Our findings for PLP are consistent with those of Wei et al. (5) who found a 52% reduction in CRC risk comparing the highest to the lowest quartile for plasma PLP (p trend: 0.07) in a nested case-control study in women. They are also consistent with those of Figueiredo et al. (6) who found an inverse association between baseline plasma PLP levels and recurrence of adenoma in a randomized trial of aspirin and folic acid supplementation. Similarly, an inverse association was reported between serum PLP and colon cancer (but not rectal cancer) in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort (7) and between plasma PLP and colorectal cancer in the Physicians' Health Study trial (8). These findings with biomarkers agree with the evidence from dietary studies which also found an inverse association with CRC for vitamin B6 intake from food and/or supplements (4).

Vitamin B6 could reduce CRC risk by preventing disruption in the one-carbon metabolism in which it acts as a co-factor for serine hydroxymethyltransferase that catalyzes the formation of 5,10-methylenetetrahydofolate, an important carrier of one-carbon groups for nucleotide synthesis and DNA repair. Vitamin B6 is also a cofactor for cystathionine-β-synthase, which catalyzes the irreversible conversion of homocysteine to cystathionine, a reaction that affects levels of homocysteine. Vitamin B6 may also protect against CRC through mechanisms independent of the one-carbon metabolism. A study showed that vitamin B6 supplementation reduced cell proliferation and the number of colon tumors induced by a carcinogen administered to mice (15). Vitamin B6 has also been shown to reduce oxidative stress and angiogenesis (10). Although vitamin B6 is widely distributed in foods, there is evidence that many older adults are not obtaining adequate amounts of this nutrient (16). For example, the prevalence of inadequate intakes is approximately 20% for men over age 50, and 40% for women in this age group.

Animal studies have suggested that folate may have a dual role, where a beneficial role may be observed at the early stage of carcinogenesis, before the establishment of neoplastic foci in the intestine (17). However, once preneoplastic lesions are present, folate may enhance the development and progression of colorectal tumors (17). Some recent human data have raised concerns that it may be the case in humans as well (18,19). It has even been suggested that folate intake may be too high in the US as a result of the grain fortification with folic acid initiated in the 1990s (20–22). However, we did not observe any increased CRC risk with plasma folate.

Consistent with several past studies (2,3), we found an inverse association between plasma folate and CRC which was of borderline significance and appeared to be stronger among subjects with the *MTHFR* 677TT genotype. The association with plasma vitamin B6 also appeared stronger in this subgroup in our study. Previous studies have also reported a stronger protective effect of vitamin B6 on adenoma risk with the *MTHFR* 677TT genotype (23–25), although the interaction was significant in only one of these studies (23). These interactions

may reflect the greater flow of one-carbon groups toward nucleotide synthesis with the TT genotype which may be protective only when the overall one-carbon pool is of sufficient size.

We also observed a stronger effect of folate and vitamin B6 at a low level of alcohol intake. Past CRC studies on plasma folate and alcohol have mostly shown no interaction (7,8,26,27), while a stronger protective effect of dietary or plasma folate has been suggested against colon cancer and other cancers among heavy drinkers in some other studies (28,29). A modifying effect of alcohol could be explained by its effect as a folate antagonist reducing the availability of dietary methyl groups and may vary among populations based on methyl group supply and/or alcohol consumption level. Finally, the interaction that we observed in this study between CRP and plasma vitamin B12 levels was unexpected and may be due to chance.

There are a number of limitations to this study, beside its relatively small sample size. We only performed a single measurement of plasma B vitamins which may not reflect long-term vitamin B status, resulting in a possible attenuation of our risk estimates. However, an Australian study found an intra-individual coefficient of variation of only 8.3% and an Irish study found a reliability coefficient of 0.94 over a one year period (30,31) for plasma folate, suggesting that a single measurement reflects reasonably well long-term B-vitamin status. Another limitation is that the dietary and anthropometric variables were not collected concurrently with the blood draw.

The important strengths of our study are the use of biomarkers, in contrast to questionnaire information, the prospective design in which blood samples were collected and covariates measured before CRC diagnosis, and the direct generalizability of our results due to the broad representativeness of the MEC study. In summary, these findings add to the evidence in support of an independent protective effect of vitamin B6 against CRC and suggest that this effect is stronger than that of folate.

Acknowledgments

The authors thank the MEC and PPG staff, who assembled the cohort and biorepository, as well as Dr. Adrian Franke for performing the CRP assays, and Annette Lum-Jones and Ann Seifried for carrying out the genotyping. We are also indebted to the participants for their invaluable participation.

Financial Support: National Cancer Institute Grants P01 CA33619 and R37 CA54281, and contracts N01-PC-35137 and N01-PC-35139 from the National Institutes of Health, Department of Health and Human Services.

References

1. Selhub J. Folate, vitamin B12 and vitamin B6 and one carbon metabolism. J Nutr Health Aging 2002;6:39–42. [PubMed: 11813080]
2. Giovannucci E. Epidemiologic studies of folate and colorectal neoplasia: a review. J Nutr 2002;132:2350S–2355S. [PubMed: 12163691]
3. Le Marchand L, Wilkens LR, Kolonel LN, Henderson BE. The MTHFR C677T polymorphism and colorectal cancer: the Multiethnic Cohort study. Cancer Epidemiol Biomarkers Prev 2005;14:1198–1203. [PubMed: 15894672]
4. Theodoratou E, Farrington SM, Tenesa A, et al. Dietary vitamin B6 intake and risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2008;17:171–182. [PubMed: 18199722]
5. Wei EK, Giovannucci E, Selhub J, Fuchs CS, Hankinson SE, Ma J. Plasma vitamin B6 and the risk of colorectal cancer and adenoma in women. J Natl Cancer Inst 2005;97:684–692. [PubMed: 15870439]
6. Figueiredo JC, Levine JA, Grau MV, et al. Vitamins B2, B6 and B12 and risk of colorectal adenomas in a randomized trial of aspirin use and folic acid supplementation. Cancer Epidemiol Biomarkers Prev 2008;17:2136–2145. [PubMed: 18708408]
7. Weinstein SJ, Albanes D, Selhub J, et al. One-carbon metabolism biomarkers and risk of colon and rectal cancers. Cancer Epidemiol Biomarkers Prev 2008;17:3233–3240. [PubMed: 18990766]

8. Lee JE, Li H, Giovannucci E, et al. Prospective study of plasma vitamin B6 and risk of colorectal cancer in men. Cancer Epidemiol Biomarkers Prev 2009;18:1197–1202. [PubMed: 19336555]
9. Friso S, Jacques PF, Wilson PW, Rosenberg IH, Selhub J. Low circulating vitamin B6 is associated with elevation of the inflammation marker C-reactive protein independently of plasma homocysteine levels. Circulation 2001;103:2788–2791. [PubMed: 11401933]
10. Matsubara K, Komatsu S, Oka T, Kato N. Vitamin B6-mediated suppression of colon tumorigenesis, cell proliferation, and angiogenesis (review). J Nutr Biochem 2003;14:246–250. [PubMed: 12832027]
11. Kolonel LN, Henderson BE, Hankin JH, et al. A multiethnic cohort in Hawaii and Los Angeles: baseline characteristics. Am J Epidemiol 2000;151(4):346–357. [PubMed: 10695593]
12. Shin-Buehring Y, Rasshofer R, Edres W. A new enzymatic method for pyridoxal-5'-phosphate determination. J Inherit Metabol Disorders 1981;4:123–124.
13. Blom HJ, van Rooij A, Hogeveen M. A simple high-throughput method for the determination of plasma methylmalonic acid by liquid chromatography-tandem mass spectrometry. Clin Chem Lab Med 2007;45(5):645–650. [PubMed: 17484628]
14. Araki A, Sako Y. Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection. J Chromatography 1987;422:43–52.
15. Komatsu S, Watanabe H, Oka T, Tsuge H, Kat N. Dietary vitamin B6 suppresses colon tumorigenesis, 8-hydroxyguanosine, 4-hydroxynonenal, and inducible nitric oxide synthase protein in azoxymethane-treated mice. J Nutr Sci Vitaminol (Tokyo) 2002;48:65–68. [PubMed: 12026192]
16. Moshfegh, A.; Goldman, J.; Cleveland, L. What We Eat in America, NHANES 2001–2002: Usual Nutrient Intakes from Food Compared to Dietary Reference Intakes. Beltsville MD: US Department of Agriculture, Agricultural Research Service; 2005. [1]
17. Kim Y-I. Folate and colorectal cancer: an evidence-based critical review. Mol Nutr Food Res 2007;51:267–292. [PubMed: 17295418]
18. Ulrich CM, Potter JD. Folate supplementation: too much of a good thing? Cancer Epidemiol Biomarkers Prev 2006:189–193. [PubMed: 16492904]
19. Kim Y-I. Will mandatory folic acid fortification prevent or promote cancer? Am J Clin Nutr 2004:1123–1128. [PubMed: 15531657]
20. Mason JB, Dickstein A, Jacques PF, et al. A temporal association between folic acid and fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis. Cancer Epidemiol Biomarkers Prev 2007;16:1325–1329. [PubMed: 17626997]
21. Sweeney MR, McPartlin J, Weir DG, Scott JM. Measurements of subnanomolar concentrations of unmetabolized folic acid in serum. J Chromatogr B Analyt Technol Biomed Life Sci 2003;788:187–191.
22. Troen AM, Mitchell B, Sorensen B, et al. Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. J Nutr 2006;136:189–194. [PubMed: 16365081]
23. Le Marchand L, Donlon T, Hankin JH, Kolonel LN, Wilkens LR, Seifried A. B-vitamin intake, metabolic genes and colorectal cancer risk (United States). Cancer Causes Control 2002;13:239–248. [PubMed: 12020105]
24. Ulrich CM, Kampman E, Bigler J, et al. Colorectal adenomas and the C677T MTHFR polymorphism: evidence for gene-environment interaction? Cancer Epidemiol Biomarkers Prev 1999;8:659–668. [PubMed: 10744125]
25. Slattery ML, Potter JD, Samowitz W, Schaffer D, Leppert M. Methyltetrahydrofolate reductase, diet and risk of colon cancer. Cancer Epidemiol Biomarkers Prev 1999;8:513–518. [PubMed: 10385141]
26. Kato I, Dnistrian AM, Schwartz M, et al. Serum folate, homocysteine and colorectal cancer risk in women: a nested case-control study. Br J Cancer 1999;79:1917–1921. [PubMed: 10206314]
27. Otani T, Iwasaki M, Sasazuki S, Inouse M, Tsugane S. Plasma folate and risk of colorectal cancer in a nested case-control study: the Japan Public Health Center-based prospective study. Cancer Causes Control 2008;19:67–74. [PubMed: 17943453]

[1]Accessed at: http://www.ars.usda.gov/research/publications/

28. Giovannucci E, Rimm EB, Ascherio A, Stampfer MJ, Colditz GA, Willett WC. Alcohol, low methionine-low folate diets, and risk of colon cancer in men. J Natl Cancer Inst 1995;87:265–273. [PubMed: 7707417]

29. Jiang R, Hu FB, Giovannucci EL, et al. Joint association of alcohol and folate intake with risk of major chronic disease in women. Am J Epidmeiol 2003;158:760–771.

30. Rossi E, Beilby JP, McQuillan BM, Hung J. Biological variability and reference intervals for total plasma homocysteine. Ann Clin Biochem 1999;36(Pt 1):56–61. [PubMed: 10370761]

31. McKinley MC, Strain JJ, McPartlin J, Scott JM, McNulty H. Plasma homocysteine is not subject to seasonal variation. Clin Chem 2001;47:1430–1436. [PubMed: 11468233]

Table 1

Characteristics* of the Colorectal Cancer Cases and Matched Controls

	Cases (n=224)	Controls (n=411)
Age at blood draw (years)†	70.5 (63, 76)	70.0 (63, 76)
Hours of fasting prior to blood draw†	13.1 (12.1, 14.4)	13.1 (12.0, 14.5)
Male, n (%)†	140 (62.5)	257 (62.5)
Ethnicity, n (%)†		
Japanese American	88 (39.3)	166 (40.4)
African American	41 (18.3)	70 (17.0)
Latino	44 (19.6)	78 (19.0)
White	39 (17.4)	73 (17.8)
Native Hawaiian	12 (5.4)	24 (5.8)
Education, n (%)‡		
High school graduate or less	79 (35.4)	133 (32.6)
Some post-secondary education	79 (35.4)	127 (31.1)
College graduate	65 (29.2)	148 (36.3)
Had previous colonoscopy, n (%)	134 (59.8)	278 (67.6)
1° family history of colorectal cancer, n (%)	27 (12.1)	39 (9.5)
Smoking status, n (%)		
Never	89 (39.7)	187 (45.5)
Past	100 (44.6)	175 (42.6)
Current	35 (15.6)	49 (11.9)
Pack-years	3.9 (0, 19.8)	2.0 (0, 16.4)
Moderate or vigorous physical activity (hrs/week)	5.0 (2.5, 11.0)	5.0 (2.5, 10.8)
Body mass index (kg/m^2)	26.0 (23.3, 29.7)	25.2 (23.0, 27.8)
<18.5, n (%)	7 (3.1)	10 (2.4)
18.5–24.9, n (%)	85 (38.0)	187 (45.5)
25–29.9, n (%)	80 (35.7)	167 (40.6)
≥ 30, n (%)	52 (23.2)	47 (11.4)
Multivitamin use, n (%)‡	102 (46.6)	207 (50.9)
Alcohol intake (g/day)	0.8 (0, 10.7)	0.4 (0, 10.0)
Red meat intake, excluding processed meat (g/1000 kcal/d)	17.6 (11.8, 29.7)	17.1 (10.6, 25.8)
Processed meat (g/1000 kcal/d)	6.7 (3.5, 11.3)	6.3 (3.2, 10.0)
Dairy food intake (g/1000 kcal/d)	64.6 (32.3, 120.2)	76.2 (37.7, 141.6)
Fish intake, including shellfish (g/1000 kcal/d)	9.0 (4.4, 16.1)	8.9 (4.7, 14.2)
Dietary fiber (g/1000 kcal/d)	11.2 (7.9, 14.4)	11.3 (8.8, 14.4)
Plasma PLP, pmol/ml	40.0 (22.4, 80.8)	57.0 (29.6, 109.8)
Plasma folate, ng/ml	14.7 (9.3, 24.5)	16.2 (10.2, 27.3)
Plasma vitamin B12, pg/ml	517 (361, 740)	523 (365, 712)
Plasma methylmalonic acid**, pmol/ml	161 (96, 226)	148 (103, 208)
Plasma cysteine**, nmol/ml	255 (234, 284)	261 (237, 288)

	Cases (n=224)	Controls (n=411)
Plasma homocysteine, nmol/ml	9.4 (7.7, 11.9)	9.4 (7.6, 11.6)
C-reactive protein, mg/l	0.9 (0.3, 2.6)	0.7 (0.2, 2.0)
MTHFR 677TT genotype*** , n (%)	22 (11.2)	41 (11.5)

* values are median (25^{th}–75^{th}) unless otherwise specified.

† matching variables

‡ totals do not always sum to 224 and 411 because of missing values.

** measured on only 118 cases and 211 controls.

*** measured on only 197 cases and 358 controls.

Table 2

Odds Ratios (95% Confidence Intervals) for Colorectal Cancer by Levels of Various Plasma B Vitamins

Plasma Level (quartiles)	No. of Cases/ Controls	Crude Model	Multivariate Model 1†	Multivariate Model 2‡
Folate (ng/ml)				
≤9.94	63/98	1.00	1.00	NA
9.95–15.3	54/97	0.87 (0.54–1.41)	0.91 (0.56–1.49)	
15.4–26.1	58/101	0.74 (0.42–1.30)	0.79 (0.44–1.41)	
>26.1	48/111	0.56 (0.31–1.03)	0.61 (0.33–1.13)	
p-value for trend§		0.055	0.097	
PLP (pmol/ml)				
≤26.6	70/90	1.00	1.00	1.00
26.7–51.5	60/95	0.80 (0.49–1.32)	0.84 (0.51–1.40)	0.86 (0.52–1.45)
51.6-10.1	52/105	0.57 (0.34–0.93)	0.62 (0.37–1.03)	0.65 (0.37–1.12)
>101	41/117	0.43 (0.26–0.71)	0.49 (0.29–0.83)	0.52 (0.29–0.92)
p-value for trend		0.001	0.009	0.030
Vitamin B12 (pg/ml)				
≤361	55/101	1.00	1.00	1.00
362–525	59/103	1.00 (0.63–1.58)	1.13 (0.70–1.83)	1.20 (0.73–1.97)
526–722	50/107	0.80 (0.49–1.30)	0.89 (0.54–1.46)	1.00 (0.59–1.69)
>722	59/96	1.10 (0.69–1.76)	1.33 (0.81–2.18)	1.66 (0.97–2.86)
p-value for trend		0.74	0.33	0.082
Methylmalonic acid (pmol/ml)				
≤103	34/51	1.00	1.00	1.00
104–151	21/58	0.52 (0.26–1.07)	0.47 (0.22–1.00)	0.47 (0.22–1.01)
152–213	30/54	1.01 (0.47–2.15)	0.92 (0.41–2.03)	0.92 (0.41–2.05)
>213	32/46	1.34 (0.59–3.04)	1.25 (0.53–2.94)	1.29 (0.54–3.06)
p-value for trend		0.28	0.34	0.31
Homocysteine (nmol/ml)				
≤7.65	55/106	1.00	1.00	1.00
7.66–9.44	61/101	1.17 (0.72–1.88)	1.06 (0.65–1.72)	1.01 (0.61–1.65)
9.45–11.8	48/103	0.89 (0.53–1.51)	0.76 (0.44–1.32)	0.73 (0.42–1.26)
>11.8	59/96	1.21 (0.69–2.12)	1.07 (0.60–1.90)	1.00 (0.56–1.79)
p-value for trend		0.62	0.87	0.96
Cysteine (nmol/ml)				
≤235	32/52	1.00	1.00	1.00
236–259	34/49	0.91 (0.46–1.82)	0.89 (0.44–1.80)	0.91 (0.44–1.85)
260–287	25/55	0.61 (0.28–1.33)	0.58 (0.26–1.31)	0.60 (0.27–1.35)
>287	27/55	0.55 (0.22–1.37)	0.50 (0.20–1.30)	0.55 (0.21–1.48)
p-value for trend		0.15	0.12	0.17

[*] Based on conditional logistic regression, using matched sets as strata. Cases and controls were matched on sex, ethnicity, study site, age at blood draw, fasting duration and date and time of blood draw. Continuous variables for age at blood draw and hours of fasting prior to blood draw were included as adjustment variables.

[†] Further adjusted for: hours of moderate or vigorous physical activity, processed meat, pack-years, BMI, ethanol, family history of colorectal cancer, and history of colorectal cancer screening.

[‡] Further adjusted for plasma folate.

[§] Trend based on the median of each quartile.

Table 3

Interactions of Alcohol, CRP and *MTHFR* C677T with Selected Plasma B-Vitamins in Relation to Risk of Colorectal Cancer

	Alcohol Intake ≤ 0.74 g/d		Alcohol Intake > 0.74 g/d		
Plasma Level (median)	**No. of Cases/ Controls**	**OR* (95% CI)**	**No. of Cases/ Controls**	**OR (95% CI)**	**P for interaction**
Folate (ng/ml)					
≤ 15.3	56/85	1.00	61/110	0.86 (0.53–1.41)	
> 15.3	54/126	0.55 (0.31–0.95)	52/86	0.97 (0.53–1.78)	0.0497
PLP (pmol/ml)					
≤ 51.5	69/94	1.00	62/93	0.91 (0.56–1.46)	
> 51.5	41/119	0.43 (0.26–0.74)	52/105	0.76 (0.44–1.31)	0.072
Vitamin B12 (pg/ml)					
≤ 525	50/91	1.00	64/116	1.09 (0.67–1.76)	
> 525	60/122	0.90 (0.56–1.46)	50/82	1.26 (0.73–2.17)	0.46
Methylmalonic Acid (pmol/ml)					
≤ 151	26/46	1.00	29/63	0.80 (0.39–1.63)	
> 151	29/44	1.57 (0.69–3.55)	33/56	1.24 (0.55–2.82)	0.97
	Plasma CRP ≤ 0.80 mg/l		**Plasma CRP > 0.80 mg/l**		
Folate (ng/ml)					
≤ 15.3	49/96	1.00	68/99	1.16 (0.69–1.97)	
> 15.3	59/133	0.77 (0.44–1.34)	47/78	0.86 (0.47–1.58)	0.93
PLP (pmol/ml)					
≤ 51.5	51/89	1.00	80/98	1.24 (0.76–2.00)	
> 51.5	57/140	0.69 (0.42–1.13)	36/83	0.65 (0.37–1.15)	0.42
Vitamin B12 (pg/ml)					
≤ 525	63/110	1.00	51/ 97	0.79 (0.48–1.30)	
> 525	45/119	0.71 (0.44–1.14)	65/84	1.18 (0.73–1.91)	0.027

Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2010 November 11.

	Alcohol Intake ≤ 0.74 g/d		Alcohol Intake > 0.74 g/d		
Plasma Level (median)	**No. of Cases/ Controls**	**OR* (95% CI)**	**No. of Cases/ Controls**	**OR (95% CI)**	**P for interaction**
Methylmalonic Acid (pmol/ml)					
≤ 151	24/56	1.00	31/53	1.25 (0.60–2.61)	
> 151	22/45	1.50 (0.67–3.35)	40/55	1.93 (0.87–4.29)	0.95
	***MTHFR* 677CC/CT**		***MTHFR* 677TT**		
Folate (ng/ml)					
≤ 15.3	82/145	1.00	13/13	1.53 (0.62–3.81)	
> 15.3	92/168	0.92 (0.55–1.51)	9/28	0.56 (0.23–1.35)	0.15
PLP (pmol/ml)					
≤ 51.5	101/154	1.00	14/12	1.83 (0.75–4.47)	
> 51.5	74/163	0.74 (0.47–1.15)	8/29	0.47 (0.20–1.09)	0.078
Vitamin B12 (pg/ml)					
≤ 525	86/166	1.00	12/17	1.26 (0.57–2.79)	
> 525	91/151	1.27 (0.87–1.87)	10/24	0.87 (0.37–2.04)	0.28
Methylmalonic Acid (pmol/ml)					
≤ 151	39/76	1.00	7/10	1.16 (0.40–3.39)	
> 151	42/71	1.38 (0.66–2.91)	6/8	1.20 (0.26–5.47)	0.75

*Based on conditional logistic regression, using matched sets as strata. Cases and controls were matched on sex, ethnicity, study site, age at blood draw, fasting duration and date and time of blood draw. Continuous variables for age at blood draw and hours of fasting prior to blood draw were included as adjustment variables, along with hours of moderate and vigorous physical activity, processed meat, pack-years, BMI, ethanol and family history of colorectal cancer, and history of colorectal cancer screening. *MTHFR* analyses did not adjust for family history of colorectal cancer.

Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2010 November 11.