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One-carbon metabolism biomarkers and risk of colon and rectal cancers

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Abstract

Background—Folate intake has been associated with reduced colorectal cancer risk; however, few studies have prospectively examined circulating folate or other related one-carbon biomarkers.

Methods—We conducted a nested case-control study within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort of 50−69 year old Finnish men to investigate associations between serum folate, vitamin B6, vitamin B12, riboflavin, and homocysteine and risk of colon and rectal cancers. Controls were alive and cancer free at the time of case diagnosis and matched 1:1 on age and date of baseline fasting serum collection with cases (152 colon and 126 rectal cancers). Multivariate-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using conditional logistic regression.

Results—Serum vitamin B6 was inversely associated with colon cancer (OR=0.30, 95% CI 0.11−0.82 in the highest vs. lowest quintile). An increased risk of colon cancer was suggested for men in the middle quintile of serum folate, but without indication of a dose-response relationship. None of the other serum biomarkers were associated with colon or rectal cancer, and we observed no interactions with alcohol consumption or methionine or protein intake. *A priori* combinations of the five one-carbon serum biomarkers provided no clear evidence to support a collective influence on colorectal cancer risk.

Conclusions—Our results support the hypothesis that higher vitamin B6 status may play a role in inhibiting colon cancer carcinogenesis; however, folate and other one-carbon related biomarkers were not associated with colon or rectal cancer.

Keywords

Colorectal neoplasms; folic acid; vitamin B 6; vitamin B 12; riboflavin; homocysteine

Introduction

Colorectal cancer is the third most common malignancy in the United States other than non-melanoma skin cancer, with an estimated 108,070 and 40,740 new cases of colon and rectal

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cancer, respectively, in 2008 (1). This malignancy has lower incidence in Finland but is also common, with age-adjusted incidence rates of 16.3 (colon) and 12.0 (rectum)/100,000 in men in 2004, and slightly lower rates among women (2). Although many studies focus on large bowel cancers combined, the risk factors for colon and rectal cancer may differ (3,4).

One-carbon metabolism reactions encompass a group of biological processes with two major functions: synthesis of purines and pyrimidines needed for DNA replication and repair, and synthesis of S-adenosylmethionine (SAM), a methyl group donor for a number of methylation reactions, including DNA methylation (5). 5-Methyl tetrahydrofolate provides the folate substrate for the re-methylation conversion of homocysteine to methionine, the precursor to SAM, and vitamin B12 serves as a cofactor for the reaction (6). The resulting tetrahydrofolate (THF) is converted to 5,10-methylene THF in a reaction requiring vitamin B6 (7). The 5,10-methylene THF is either used as a folate substrate for DNA synthesis and repair, or is converted to 5-methyl THF by methylenetetrahydrofolate reductase (MTHFR) with flavin adenine dinucleotide (a riboflavin) as a cofactor (8). In a separate transsulfuration pathway, homocysteine is metabolized to cysteine via vitamin B6-dependent enzymes (6). Low concentrations of these one-carbon related serum nutrients (i.e., folate, vitamin B6, vitamin B12, and riboflavin) may lead to elevated homocysteine, disrupted one-carbon metabolism, and insufficient methyl groups for DNA methylation, synthesis or repair, thus potentially promoting carcinogenesis (7,9).

Data from numerous prospective studies generally support the hypothesis that higher folate intakes are associated with reduced risk of colorectal cancer (10,11), while some studies do not (12,13). Relatively few, inconsistent studies have prospectively examined circulating folate and colorectal cancer risk (14-19). Homocysteine has been examined in only three cohort settings (15,17,20), plasma vitamin B6 in only one (21), and plasma vitamin B12 in only one (22). Dietary intake of vitamin B6 has been examined in a growing number of such studies, with fairly consistent findings of inverse associations (13,21,23-26). Few studies examined vitamin B12 or riboflavin intakes (13,24,25).

We report herein findings from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study cohort, based on 17 years of follow-up and 276 incident cases of colon and rectal cancers. In addition to serum folate, we examined other key biomarkers of one-carbon status -- vitamins B6 and B12, riboflavin, and homocysteine – and evaluated the associations by anatomic location of the tumor (i.e., colon, rectum, proximal colon, distal colon).

Subjects and methods

Study cohort

The ATBC Study was a randomized, double-blind, placebo-controlled, primary prevention trial of daily supplementation with α-tocopherol and/or, β-carotene (27). Between 1985 and 1988, 29,133 men between the ages of 50 and 69 years, who smoked at least five cigarettes per day, were recruited from southwestern Finland. Men with prior cancer or serious illness, and those who reported current use of vitamins E, A, or β-carotene in excess of defined amounts, were ineligible. Participants were randomly assigned to receive either α-tocopherol as *dl*-α-tocopheryl acetate (50 mg/day), β-carotene as *all-trans*-β-carotene (20 mg/day), both supplements, or placebo capsules, based on a 2 × 2 factorial design, for 5−8 years (median 6.1 years), until trial closure (April 30, 1993). The ATBC Study was approved by the institutional review boards of the participating institutions, and written informed consent was obtained from each participant prior to randomization.

Data collection

At baseline, participants completed questionnaires regarding general risk factors, medical history, and dietary intake. The food frequency questionnaire, intended to measure usual consumption over the previous 12 months, asked about the frequency of consumption of 276 common foods and mixed dishes, with a picture booklet to aid in portion size estimation (27, 28). The reproducibility and validity of this questionnaire have been reported (28). Nutrient intake was estimated based on food composition data from the National Public Health Institute.

Case identification and control selection

Cases were defined as incident colon and rectal adenocarcinomas (International Classification of Diseases (ICD) 9, codes 153 and 154, respectively), diagnosed between study baseline and April 30, 2002, with adequate serum. Cancer cases were identified through the Finnish Cancer Registry. For cases diagnosed through April 1999 (n=111 colon and 81 rectum), medical records were reviewed centrally by two study oncologists for diagnostic confirmation and staging, and cases with histolopathologic or cytologic specimens available were also reviewed and confirmed by one or two pathologists. Information on colorectal cancer cases diagnosed since May 1999 (n=41 colon and 45 rectum) was derived only from the Finnish Cancer Registry, which provides almost 100% case ascertainment (29). A total of 152 colon and 126 rectal cases were included. Two cases with both a colon and a rectal diagnosis on the same day were included in both groups. We also divided the colon cases into proximal (ICD9 codes 153.0, 153.1, 153.4, 153.6, 153.7) and distal (ICD9 153.2, 153.3) subsites, with 88 defined as proximal and 57 as distal. The remaining cases did not have data to specify subsite and were therefore excluded from these sub-analyses. One case with both a proximal and a distal colon cancer diagnosis on the same date was included in both groups. Controls were alive and cancer free at the time of case diagnosis and matched to cases (1:1) on age at randomization (+/− 5 years) and date of blood draw (+/− 30 days).

Serum biomarker determination

Fasting serum samples were collected during the pre-randomization baseline visit, and stored at −70 °C. Serum folate and vitamin B12 were determined by radioassay (Bio-Rad Laboratories, Richmond, CA), while pyridoxal 5' phosphate, the principal active form of vitamin B6, was determined by the tyrosine decarboxylase apoenzyme method (30). Homocysteine was determined using a high performance liquid chromatography (HPLC) method (31), and riboflavin was determined using HPLC analysis with fluorimetric detection (32). Each batch contained case and matched control pairs placed adjacently but in random order, as well as six blinded quality control samples derived from a pool of serum. The within-batch coefficients of variation were 6.2% for folate, 7.1% for vitamin B12, 4.5% for vitamin B6, 6.2% for homocysteine, and 4.8% for riboflavin, calculated by logarithmically transforming the quality control data and using a nested components of variance analysis (33).

Statistical analysis

Chi-square tests or Fisher exact tests (for categorical variables) and Wilcoxon rank sum tests (for continuous variables) were used to compare various characteristics of cases and controls. Correlations were estimated using Spearman's rank order coefficient among the control subjects, and for which dietary intakes which were log-transformed and adjusted for energy intake using the residual method (34). Conditional logistic regression models were used to determine odds ratios (OR) and 95 percent confidence intervals (CI) for the association between colorectal cancer and serum biomarkers. Serum biomarker quintiles were based on the distribution among all the controls combined and entered into the models as indicator variables with the lowest quintile as the referent category. Tests for linear trend were obtained by

assigning to each biomarker quintile the median value of the controls and treating this as a continuous variable. To assess confounding, each covariate was tested in a bivariate model with each biomarker, for colon and rectum cancers separately. Potential confounders were defined as covariables that produced >10% change in any of the biomarker coefficients. All potential confounders were then considered jointly and removed from the models if their removal produced <10% change in the coefficients. Finally, any variable that was a confounder in any model was retained in all models, and these included age at randomization, body mass index, physical activity (heavy recreational activity vs. not and heavy occupational activity vs. not as two separate variables), and energy-adjusted intakes of vitamin D and iron (including those with missing information as a separate category to retain those who did not complete the dietary questionnaire). Intervention group, urban residence, education, marital status, smoking (number of cigarettes/day and number of years smoked), height, weight, vitamin supplement use, diet history questionnaire completed (yes/no), alcohol intake, total energy intake, and energy-adjusted intake of calcium were not confounders. Effect modification was statistically evaluated by including the cross-product term of the biomarker variables (quintiles) by the effect modifier (split at the median or yes/no). If the p-value for the interaction term was < 0.10, then conditional models with indicator terms to characterize the variables of interest, with a common referent group, were produced to further examine the relationships. Further analyses of each serum biomarker, cut at the median, and crossed with each of the other serum biomarkers, were tested, as were the joint effects of all serum factors combined. We also examined associations in a group with high serum folate, high protein or methionine intake, and low alcohol intake (median cuts) compared with the opposite. For analyses stratified by follow-up time, cases were split at the median of the diagnosis date (6/15/97 for colon and 9/15/98 for rectum). Statistical analyses were performed using SAS software version 9.1.3 (SAS Institute, Inc., Cary, North Carolina) and all p-values were two-sided.

Results

Baseline characteristics of colon and rectum cancer cases and their respective controls are shown in Table 1. Colon cancer cases were more likely than their controls to have a family history of the same malignancy (p=0.002) and had higher body mass index (BMI, p=0.05) and lower leisure time physical activity (p=0.02). Rectal cases did not differ significantly from controls on any of the characteristics. The family history association was significant for colorectal cases combined (p=0.04). It is unclear why rectal cancer controls had significantly higher education levels than did colon controls (p=0.01), but age and BMI did not account for the difference.

Significant correlations were observed among the serum biomarkers, including for folate which was positively correlated with vitamins B6 and B12 and riboflavin, and negatively correlated with homocysteine (Table 2). Energy-adjusted dietary folate (r=0.19), vitamin B12 (r=0.16), and riboflavin (r=0.16) were each weakly, but significantly, correlated with their corresponding serum levels, in contrast to the stronger relationship for vitamin B6 (r=0.46). The correlation between serum homocysteine and folate intake was weakly inverse, but significant (r= −0.14).

For all case and control subjects, 90% had less than adequate serum folate (< 13.6 nmol/L), 66% had less than adequate serum vitamin B6 (< 30 nmol/L), but only 1% had less than adequate serum vitamin B12 (< 148 pmol/L). Serum homocysteine was elevated (> 15 nmol/ml) in 30% of subjects. (There is no defined adequacy level for serum riboflavin.)

Positive and inverse associations were suggested for serum folate and colon and rectal cancers, respectively, but without indication of dose-response relationships (Table 3). Unadjusted results were similar, with odds ratios for colon cancer of 1.00, 1.48, 2.46, 1.59, and 1.70, and for rectal cancer of 1.00, 0.63, 0.61, 0.31, and 0.77. When examined as a threshold effect

(highest 4 quintiles of serum folate combined vs. the lowest quintile) the odds ratio for colon cancer was 1.68 (95% CI 0.82−3.44), p for trend = 0.16).

High serum vitamin B6 was significantly associated with decreased risk of colon, but not rectal cancer, with the overall colorectal cancer association being non-significantly lower (Table 3). Unadjusted odds ratios were 1.00, 0.89, 1.57, 1.23, 0.60 for colon cancer, and 1.00, 0.79, 0.59, 0.79, 1.02 for rectal cancer. Furthermore, risk was significantly lower for distal colon cancer (adjusted OR= 0.07, 95% CI 0.01−0.52 for the highest vs. lowest quintile of serum B6, p-trend = 0.01) but not proximal colon cancer (adjusted OR= 0.81, 95% CI 0.19−3.41, p-trend 0.22). When the vitamin B6/colon cancer association was examined as a threshold effect (highest quintile vs. lowest 4 quintiles combined) the odds ratio was 0.32 (95% CI 0.14−0.71), p for trend = 0.01.

The other serum biomarkers were not associated with colorectal cancer overall or for specific anatomical subsites. In general, similar patterns were noted for all biomarkers and outcomes when quartile and tertile cuts were used. For example, for vitamin B6 and colon cancer, quartile risks were 1.00, 1.09, 1.54, 0.57, p for trend = 0.10; tertile risks were 1.00, 1.22, 0.54, p for trend = 0.05).

Simultaneous adjustment for the serum biomarkers did not alter these findings. For example, when serum folate, vitamin B12, homocysteine, and riboflavin were included as continuous variables in a multivariate model, the odds ratio for serum vitamin B6 and colon cancer was 0.27, 95% CI 0.09−0.79 for highest vs. lowest quintile, p-trend = 0.01. Residual adjustment of the serum biomarkers for one another also did not change the results.

Smoking (cigarettes/day and years of smoking), BMI, age, vitamin supplement use, or intakes of calcium, vitamin D, or alcohol did not modify the serum biomarker-colorectal cancer associations. Testing an *a priori* hypothesis regarding multifactor causation, we found weak evidence that subjects with low alcohol intake, high serum folate, and high methionine intake had reduced risks of colon cancer when compared to subjects with high alcohol - low serum folate - low methionine (OR = 0.51, 95% CI 0.20−1.31). The reverse was suggested for rectal cancer (OR = 1.72, 95% CI 0.61−4.72), however, but also was not significant. Results for colorectal cancers combined were null (OR = 0.90, 95% CI 0.47−1.73) and substituting total protein for methionine intake yielded similar outcomes.

Biologically-relevant two-way interactions among the one-carbon biomarkers were explored but failed to show strong associations for any of the combinations hypothesized to be important. For example, risk of colon cancer for men with high (above median) folate and low (below median) homocysteine, compared with the converse subgroup, was 1.1 and not significant. Additionally, we examined all combinations of high/low (median split) biomarkers, with participants who were high for homocysteine and low for all other biomarkers as the referent group. The group hypothesized to be at lowest colorectal cancer risk (i.e., high for all except low on homocysteine) was not (OR = 0.91, 95% CI 0.32−2.64). Two other combinations were significantly associated with colorectal cancer risk, however, based on current knowledge, neither of these combinations would be hypothesized to be of specific importance and these findings could be due to chance. Finally, there were no apparent differences of note in the serum biomarker/cancer associations based on time from blood collection to diagnosis.

Discussion

We observed 70% lower risk of colon cancer among men with high (>41.6 pmol/ml) compared with low (<=15.9 pmol/ml) serum vitamin B6 concentrations. Hypothesized protective associations between higher serum folate concentrations and risk of colorectal cancer were not observed, and serum vitamin B12, riboflavin, and homocysteine were also unrelated to risk.

Further, combinations of the five one-carbon serum biomarkers did not provide convincing evidence that these serologic phenotypes collectively influenced colorectal cancer risk in this population.

The colon cancer - serum vitamin B6 association appeared limited to the highest quintile and was stronger for the distal colon, whereas rectal cancer was unrelated to B6. Our finding is another among studies suggesting that the risk factors for colon and rectal cancer may differ (3,4). One other study found significantly lower risk of colon (but not rectal) cancer with higher plasma vitamin B6 concentrations, with significant dose-response trends (21). Mean vitamin B6 concentrations in controls were higher (53.2 pmol/ml) than in our study (39.5 pmol/ml), with corresponding high quantile medians of 131.2 pmol/ml and 65.4 pmol/ml. The threshold association we observed for the highest quintile could be a consequence of the lower than adequate serum vitamin B6 levels in our population. With the exception of one study (13), the prospective epidemiologic evidence for vitamin B6 dietary intake and supplementation appears to be fairly consistent in suggesting an inverse association with colorectal cancer (21,23-26), a conclusion drawn by a recent meta-analysis (35). Interestingly, we observed a fairly strong correlation between serum and dietary vitamin B6 (r=0.46) in the present study, relative to the weaker correlations with the other nutrients examined. The food sources of vitamin B6 in the ATBC cohort included potatoes, rye and wheat products, fish, pork, sausages, milk, fruits, and vegetables, accounting for 76% of the total vitamin B6 intake. Less than 13% of all subjects in this nested study reported intake of supplemental vitamin B6.

Vitamin B6 is a cofactor for an enzyme in the one-carbon remethylation pathway and for two enzymes in the transsulfuration pathway, which metabolizes homocysteine (6,7). Because there are no risk associations or interactions with the other one-carbon biomarkers, the protective association with serum vitamin B6 may be working through the transsulfuration pathway in which homocysteine is degraded to cysteine, a limiting factor in the synthesis of glutathione. Glutathione has numerous functions such as antioxidant defense, detoxification, and reduction of oxidative stress (36). Alternatively, high vitamin B6 concentrations may protect against carcinogenesis directly through hypothesized roles of inhibiting cell proliferation, nitric oxide production, oxidative stress, and angiogenesis, and improving immune function (37-39). Finally, we can not rule out confounding by another highly correlated factor to explain the protective effect of serum vitamin B6.

We found no evidence of a protective association between serum folate and colon or rectal cancer risk – our strongest *a priori* hypothesis. In fact, increased risk of colon cancer for men in the middle quintile was suggested, similar to a pattern observed in a prospective study in Sweden (15). Our study enrolled only men aged 50−69 years at baseline who smoked at least five cigarettes daily at study entry, and research suggests that smokers have reduced biochemical status (tissue and blood) of folate, vitamin B6, and vitamin B12 compared with non-smokers (40), possibly due to lower intakes (especially of folate) or the effects of oxidizing compounds in tobacco smoke which inactivate folate and vitamin B12 cofactors (41,42). Smokers may therefore require higher folate status for colorectal cancer protection; however, the folate concentrations and overall findings we observed were very similar to those in the Swedish cohort study where only 20% of the subjects were active smokers and where younger subjects were included at baseline (15), and we found no evidence that the serum biomarker – colorectal cancer risk associations differed by age or by smoking dose or duration.

Although folate intake and colorectal cancer risk has been extensively examined, the number of prospective studies of folate status are few, and their results have been inconsistent. We previously found no significant association between baseline serum folate and colon and rectal cancer risk in the ATBC Study cohort, based on 144 cases and approximately 8 years of follow-up (14). (The present investigation includes all cases from this previous analysis, but uses only

new and expanded biomarker data). Two other studies were null (18,19), two reported inverse associations (16,17), and one (discussed above) observed elevated risk of colorectal cancer in the third and fourth quintiles of plasma folate (15). The two studies finding protective relationships reported higher mean/median serum folate concentrations (approximately 15 to 22 nmol/L) (16,17), compared with the other studies (14,15,18) and the present analysis (approximately 8 to 10 nmol/L). It is possible that the folate levels in the null studies were not high enough to provide protection against colorectal carcinogenesis. However, a recently published study with higher median plasma folate concentrations (15.4 nmol/L) was also null (19), and a recent trial reported that supplementation with 1 mg/day folic acid did not reduce but may have increased the recurrence of multiple and advanced colorectal adenomas (43). A dual role for folate in carcinogenesis has been suggested to explain these divergent observations, whereby folate may prevent colorectal cancer by reducing DNA damage, but may also promote the growth of preexisting adenomas (44,45). It has been conjectured by some that folic acid fortification in the U.S. and Canada has led to increased rates of colorectal cancer in these countries (46). The lack of a protective effect of folate in the current study could be explained if the subjects had already accumulated colorectal mutations by the time they were recruited into the study.

Alcohol intake can have a negative impact on folate status and metabolism (47), and higher risk of colorectal cancer has been observed among subjects with high alcohol intake and low serum/dietary folate, although not all risk estimates were elevated or statistically significant (12-14,17,19,48-50). We observed no interaction between alcohol, folate status, and colon or rectal cancer, and additional consideration of methionine or protein intake showed no striking interaction. In our earlier analysis, there was no significant elevation in risk of colon cancer for the high alcohol-low serum folate-low protein intake category, while the same analysis using folate intake showed a significantly increased risk in the high risk group (OR=4.8) (14), Only two other studies examined the interaction with folate status rather than intake, and neither reported significant interactions (17,19). In regard to serum vitamin B6 and alcohol, one study observed the most benefit of vitamin B6 intake when alcohol intake was high (26), but two others, in addition to ours, observed no interactions (21,24).

Serum homocysteine, vitamin B12, and riboflavin were unrelated to risk of colon or rectal cancer. There are very few other prospective studies of these circulating factors and colorectal cancer. One study reported a non-significant elevation in risk with higher serum homocysteine (17), a second found reduced risk with low homocysteine among subjects with the MTHFR valine/valine genotype (20), while a third found no association (15). Circulating vitamin B12, in conjunction with MTHFR and MTR genotypes, was not associated with colorectal cancer risk in one study (20) but was associated with reduced risk of rectal, but not colon, cancer in another (22). In terms of dietary intake, there are also few prospective studies. Vitamin B12 intake was not materially associated with risk of colorectal cancer in three studies (13,24,25). One study found a reduced risk of colorectal cancer with higher riboflavin intake, primarily among subjects with the MTHFR TT genotype (25).

A major strength of the present study is the simultaneous consideration of several key biomarkers of one-carbon metabolism in addition to serum folate, which provided a thorough examination of the hypothesis. Based on these multiple measurements, *a priori* high and low risk combinations of the serum biomarker levels were investigated but provided no clear evidence to support a collective influence on colorectal cancer risk. We utilized a prospective design which minimizes an effect of cancer on the serum biomarker concentrations, and our study encompassed a long follow-up period (17 years). Finally, while folic acid fortification of enriched grain products was mandated in the United States in 1998 (51), there is no such national fortification program in Finland, so our results are free from this perturbation of folate intake. Limitations resulting from the parent study include our investigation of only smokers,

although results are similar to those of other studies which were not limited to smokers (15, 21), and only men. We measured serum vitamin B12, rather than methylmalonic acid, a functional marker of vitamin B12 status. In addition, we had no baseline data on use of aspirin or non-steroidal anti-inflammatory medications. Our use of quintile cuts for the main exposures of interest has the advantage of enabling comparisons between quantiles that are more widely spread apart, thus allowing us to explore potential threshold effects, however risk estimates using quintiles are a bit less stable than using quartiles or tertiles. Finally, our sample size limited our ability to detect interactions among the one-carbon biomarkers and with other factors. Analyses regarding genetic variants in the one-carbon pathway are planned in order to evaluate direct effects of and effect modification by genotypes.

In summary, we observed a significantly reduced risk of colon cancer among men with higher serum concentrations of vitamin B6, and similar evidence for protective associations with vitamin B6 has been accumulating. In addition to other serological studies, investigation of vitamin B6-related genetic polymorphisms may provide additional useful information in this regard. By contrast, the stronger *a priori* hypothesis favoring an inverse folate-colorectal cancer relationship was not observed for the serum biomarker, even in the context of and simultaneous adjustment for other key one-carbon factors. In fact, we observed a suggested increased risk of colon cancer for men in the middle quintile. Thus, although an extensive literature exists regarding a possible benefit for higher folate intake, our study adds to the few studies of serum or plasma folate that have been inconsistent. There were also no associations for other serum one-carbon factors - vitamin B12, riboflavin, and homocysteine - either singly or jointly with folate or vitamin B6. Coupled with the recent trial of folic acid and colorectal adenoma showing no effect or potential harm from supplementation (43), additional careful study of folate and one-carbon biomarker relationships with colorectal cancer, including consideration of genetic variants in the one-carbon pathway and possible isolated associations among genetic subgroups, is warranted.

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TABLE 1

Selected baseline characteristics (medians and interquartile ranges, or percents) for cases and controls

	Colon		Rectum	
Characteristic	**Cases N=152**	**Controls N=152**	**Cases N=126**	**Controls N=126**
Age (y)	59 (54−62)	58 (55−61)	58 (54−61)	57 (54−60)
Height (cm)	173 (170−178)	173 (168−178)	174 (171−178)	174 (169−179)
Body mass index (kg/m^2)	26.6 (24.1−29.8)	25.5 (23.9−28.3)*	26.2 (23.7−28.9)	26.0 (23.8−29.4)
Occupational physical activity (% heavy)	5.3	7.2	4.8	10.3
Leisure physical activity (% heavy)	4.0	11.2*	7.1	5.6
Urban residence (% > 20,000 residents)	60.5	63.8	57.9	62.7
Married (%)	78.3	80.3	87.3	84.9
Education (% > elementary)	19.1	15.1	28.6	27.8
Family history of colon or rectal cancer, respectively (%)†	7.2	0‡	2.2	0.9
Smoking (cigarettes/d)	20 (12−25)	20 (15−24)	20 (15−25)	20 (14−25)
Smoking (# years smoked)	39 (31−43)	37 (32−41)	36 (32−42)	37 (31−40)
Dietary intake/d§				
Energy (kcal)	2702 (2239−3253)	2814 (2348−3337)	2717 (2230−3371)	2707 (2294−3241)
Folate (μg)	323 (260−396)	333 (279−395)	324 (279−405)	328 (268−406)
Vitamin B6 (mg)	2.40 (2.00−2.91)	2.58 (2.10−2.94)	2.44 (2.02−2.91)	2.47 (1.92−2.88)
Vitamin B12 (μg)	10.7 (8.1−13.0)	10.6 (8.4−12.8)	10.7 (7.0−14.5)	10.4 (7.6−12.8)
Methionine (mg)	1918 (1606−2362)	2044 (1669−2350)	1973 (1665−2338)	1963 (1601−2349)
Alcohol (g)	10.8 (2.9−27.4)	10.9 (2.7−22.9)	13.1 (3.76−24.8)	13.9 (3.7−26.7)
Vitamin D (ug)	4.92 (3.49−7.32)	4.95 (3.41−7.78)	4.93 (3.17−7.39)	5.13 (3.40−6.96)
Iron (mg)	17.9 (14.3−21.6)	18.1 (14.8−22.4)	17.5 (13.9−22.5)	17.3 (14.7−21.8)
Supplement use				
Any supplement (%)	15.1	23.7	22.2	21.4
Folic acid (%)	4.6	9.2	8.7	7.1
Vitamin B6 (%)	7.2	13.8	15.9	14.3
Vitamin B12 (%)	5.3	10.5	8.7	8.7
Serum biomarkers				
Folate (nmol/L)	8.70 (7.27−10.65)	8.52 (6.44−10.72)	8.65 (6.51−11.42)	8.79 (7.19−10.83)
Vitamin B6 (pmol/ml)	23.7 (17.3−33.8)	22.9 (17.0−38.5)	23.6 (17.0−37.0)	24.0 (17.5−33.1)
Vitamin B12 (pmol/L)	345 (284−429)	370 (287−448)	333 (257−429)	353 (291−434)
Riboflavin (pmol/ml)	8.8 (6.5−12.0)	9.1 (7.0−12.5)	9.6 (7.1−12.0)	8.8 (6.7−11.6)
Homocysteine (nmol/ml)	13.4 (11.1−16.0)	13.3 (11.0−15.7)	13.3 (10.9−15.8)	12.6 (10.8−15.3)

* $p < 0.05$ based on chi-square test or Fisher's exact test for categorical variables, and Wilcoxon test for continuous variables, for site specific case vs. control comparisons

† family history data available on only 79% of these subjects

$^{\neq}$ $p < 0.01$

§ unadjusted intake, excluding supplements. 93% of subjects completed the dietary questionnaire

Table 2

Spearman correlation coefficients for serum biomarkers among controls

	Folate	Vitamin B6	Homocysteine	Vitamin B12	Riboflavin
Folate (nmol/L)	1.00	0.29*	−0.33*	0.19*	0.23*
Pyridoxal-5'-phosphate (pmol/ml)		1.00	−0.16*	0.11	0.17*
Homocysteine (nmol/ml)			1.00	−0.14†	−0.07
Vitamin B12 (pmol/L)				1.00	0.24*
Riboflavin (pmol/ml)					1.00

*$p < 0.01$

†$p < 0.05$

Table 3

Multivariate-adjusted* odds ratios for colorectal, colon, and rectal cancers and serum one-carbon biomarkers

	Medians	Cases/Controls (n=275 each)	OR	95% CI	Cases/Controls (n=151 each)	OR	95% CI	Cases/Controls (n=126 each)	OR	95% CI
	Quintiles	Colorectal Cancer			Colon Cancer			Rectal Cancer		
Folate (nmol/L)										
1	5.7	53/55	1.00		24/36	1.00		29/19	1.00	
2	7.2	50/55	0.81	(0.44−1.47)	24/27	1.23	(0.47−3.22)	27/28	0.56	(0.24−1.34)
3	8.6	67/55	1.10	(0.63−1.90)	43/27	2.40	(1.03−5.62)	24/28	0.48	(0.20−1.11)
4	10.0	41/55	0.69	(0.35−1.33)	28/31	1.50	(0.58−3.85)	13/24	0.29	(0.09−0.90)
5	12.9	64/55	1.07	(0.60−1.91)	32/30	1.41	(0.62−3.23)	33/27	0.67	(0.27−1.71)
P for Trend				0.68			0.47			0.49
Vitamin B6 (pmol/ml)										
1	12.7	62/55	1.00		34/33	1.00		28/23	1.00	
2	18.9	50/55	0.82	(0.45−1.49)	26/29	0.63	(0.26−1.50)	25/26	0.93	(0.36−2.37)
3	23.6	60/55	1.13	(0.62−2.06)	41/28	1.50	(0.62−3.63)	19/27	0.71	(0.27−1.88)
4	32.8	57/55	0.94	(0.50−1.76)	30/26	1.05	(0.40−2.81)	27/29	0.81	(0.33−2.02)
5	77.7	46/55	0.61	(0.32−1.14)	20/35	0.30	(0.11−0.82)	27/21	0.91	(0.35−2.42)
P for Trend				0.08			0.01			0.93
Vitamin B12 (pmol/L)										
1	243	73/56	1.00		34/33	1.00		39/24	1.00	
2	303	52/55	0.70	(0.40−1.24)	31/30	0.81	(0.36−1.83)	21/25	0.47	(0.19−1.18)
3	353	64/55	1.02	(0.60−1.73)	38/26	1.47	(0.69−3.14)	27/29	0.58	(0.25−1.33)
4	423	41/55	0.65	(0.36−1.16)	22/30	0.76	(0.31−1.84)	20/26	0.50	(0.20−1.25)
5	529	45/54	0.70	(0.39−1.25)	26/32	0.65	(0.27−1.55)	19/22	0.59	(0.23−1.49)
P for Trend				0.21			0.31			0.25
Riboflavin (pmol/ml)										
1	5.4	59/56	1.00		37/28	1.00		22/28	1.00	
2	7.2	50/55	0.83	(0.48−1.45)	23/29	0.51	(0.23−1.16)	27/26	1.56	(0.66−3.68)
3	8.9	43/55	0.75	(0.41−1.36)	27/32	0.44	(0.17−1.09)	16/24	1.27	(0.49−3.33)
4	11.3	67/55	0.94	(0.52−1.68)	33/28	0.51	(0.22−1.21)	35/27	2.36	(0.88−6.29)
5	17.5	56/54	0.89	(0.50−1.57)	31/34	0.52	(0.23−1.17)	26/21	1.79	(0.68−4.73)
P for Trend				0.92			0.30			0.24

	Medians	Cases/Controls (n=275 each)	OR	95% CI	Cases/Controls (n=151 each)	OR	95% CI	Cases/Controls (n=126 each)	OR	95% CI
	Quintiles	Colorectal Cancer			Colon Cancer			Rectal Cancer		
Homocysteine (nmol/ml)										
1	9.2	52/56	1.00		23/28	1.00		29/28	1.00	
2	11.3	43/55	0.76	(0.41−1.39)	26/33	0.77	(0.31−1.89)	18/24	0.84	(0.35−2.04)
3	13.0	67/55	1.26	(0.71−2.23)	37/24	1.82	(0.76−4.36)	30/31	1.04	(0.46−2.35)
4	14.7	46/54	0.77	(0.41−1.46)	27/31	0.68	(0.28−1.68)	20/23	0.85	(0.31−2.32)
5	18.8	67/55	1.22	(0.68−2.17)	38/35	1.21	(0.52−2.80)	29/20	1.29	(0.53−3.12)
P for Trend				0.42			0.70			0.51

* Adjusted for age at randomization, body mass index, occupational and leisure physical activity, and intakes of vitamin D and iron