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Red blood cell folate and plasma folate are not associated with risk of incident colorectal cancer in the Women's Health Initiative Observational Study

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Abstract

The relationship between folate and colorectal cancer (CRC) risk is unclear. We investigated the association of two biomarkers of folate status, plasma folate and red blood cell (RBC) folate with CRC risk using a nested case-control design in the Women's Health Initiative Observational Study (WHI-OS). Postmenopausal women (n=93,676) aged 50–79 years were enrolled in the WHI-OS (1993–1998). A fasting blood draw and extensive health, dietary and lifestyle data were collected upon enrollment. Through 2008, 988 incident CRC cases were reported and confirmed with medical records adjudication. Cases and controls were matched on age (± 3 y), enrollment date (± 1 y), race/ethnicity, blood draw date (± 6 mo) and hysterectomy status. Plasma and RBC folate

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were determined by radioassay. Folate biomarker values were divided into quartiles and conditional logistic regression estimated odds ratios (ORs) and 95% confidence intervals (CI) for the associations of folate with total CRC, by tumor site and by stage at diagnosis. Additional analyses examined whether risks varied across time periods corresponding to the United States folic acid fortification policy: pre-fortification (1994–95), peri-fortification (1996–97) and post-fortification (1998). ORs for overall CRC risk comparing Q4 vs. Q1 were 0.91 (95% CI 0.67–1.24) and 0.91 (95% CI 0.67–1.23) for RBC and plasma folate, respectively. There were no changes in risk attributable to food supply fortification. These results do not support an overall association of folate with CRC risk and suggest that folic acid fortification of the U.S. food supply did not alter the associations in these postmenopausal women.

Keywords

colorectal cancer; folate; postmenopausal women; observational studies

Introduction

Folate is a member of the B-vitamin family and its principal biochemical role is that of a co-enzyme in one-carbon metabolic reactions. [1] These one-carbon reactions are critical for nucleic acid synthesis and proper DNA function. [1] Disturbances in one-carbon metabolism are well-known to cause DNA damage. [2] For example, in the presence of folate deficiency, uracil is misincorporated into DNA. During the subsequent repair by uracil-DNA-glycosylase, DNA strand-breaks are induced, which can cause permanent changes in the DNA sequence or chromosome translocations.[2] This genomic instability enhances carcinogenesis. [3] Since the gastrointestinal tract has a high rate of epithelial cell turnover rate, this folate deficiency-induced DNA instability may be one reason for the observed associations of poor folate status with colorectal cancer (CRC) risk. [3–5] One-carbon donors are also needed for DNA methylation and both hypermethylation and hypomethylation from folate imbalances have been associated with increased colorectal carcinogenesis in *in vitro* and preclinical animal models[2,3] with relevance for humans. [6,7]

Previous cohort studies of the association of folate biomarkers with CRC risk have yielded inconsistent evidence. In a nested case-control study from the Multiethnic Cohort Study [8] (n=224 cases and n=411 controls) there was a suggestive inverse association of plasma folate with CRC risk, but neither the point estimate nor linear trend test was statistically significant (OR=0.61, 95% CI 0.33–1.13). Similarly, the Alpha Tocopherol and Beta-Carotene Cancer Prevention Study cohort [9] (men only) (n=278 cases) and a Japanese population-based cohort study [10] (n=375 cases) also reported no association of plasma folate with colorectal cancer risk and no evidence of a dose-response relationship. The largest study to date, the European Prospective Investigation into Cancer (EPIC) cohort [11], (n=1,367 cases) also reported no association of plasma folate with CRC risk.

Many of these null or mildly suggestive studies [9–11] were conducted in countries without folic acid fortification programs and results may be different in places such as the United States where enriched cereal grains are fortified with 140 μg/100 grams of flour. [12] The

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primary intent of the FDA's folic acid fortification program was to reduce the number of neural-tube defect affected pregnancies, but the program was also expected to benefit the overall health of the U.S. population. However, some concern has arisen that subsets of the population who consume generous portions of fortified foods or dietary supplements may be exceeding the Tolerable Upper Intake Level (UL) set by the Food and Nutrition Board of the National Academy of Sciences. [1] Excessive folate exposure, particularly in the form of synthetic folic acid, may be associated with unintended adverse health events. [13–15] For example, in the Aspirin/Folate Polyp Prevention Study [13] participants with a previous history of colorectal adenomas were randomized to 1 mg/day of folic acid or placebo and separately randomized to aspirin or placebo. Compared to those taking placebo, participants taking the folic acid supplements had a 1.7-fold increased risk of advanced adenomas and a 2.3-fold increased risk of multiple adenomas during the follow-up period. A subsequent trial [16] showed no association of folic acid supplements with colorectal adenoma recurrence, but the dose was half (0.5 mg/d) that used in the Aspirin/Folate Polyp Prevention Trial. In the observational study literature, suggestive adverse effects of excess folic acid in relation to colorectal cancer risk are supported by a nested case-control study [17] using data and specimens from the Nurses' Health Study, the Health Professionals Follow-Up Study and the Physicians' Health Study. The investigators reported that among n=602 incident colorectal cancer cases higher vs. lower plasma folate was associated with a statistically significant 1.5-fold increased risk of colorectal cancer. Importantly, risk estimates were higher (RR= 2.6, 95% CI 1.09–6.02) during the post-fortification period. [17] We also recently reported [18] from the Women's Health Initiative Observational Study (WHI-OS) that dietary folate intake assessed by food frequency questionnaire may be associated with a slight increase in CRC risk, but only in the early years of the fortification program when manufacturer overages were thought to be common.

This important issue of whether excess exposure to synthetic folic acid increases risk of colorectal cancer needs further exploration in order to inform the nation's public health policies and practices. Therefore, our objective was to investigate the association of the two principal biomarkers of folate status [plasma folate and red blood cell (RBC) folate] with CRC risk in WHI-OS. An additional objective was to understand the extent to which folic acid fortification influenced these biomarkers in relation to risk.

Methods

Study Population

The WHI-OS [19,20] is a prospective cohort consisting of 93,676 women who were enrolled at 40 U.S. clinical centers between 1993 and 1998. The study design and baseline characteristics of the cohort have been described in detail. [19] Baseline eligibility requirements included post-menopausal status, age between 50 and 79 years at enrollment, and low likelihood of loss to follow-up within three years due to relocation or death resulting from a pre-existing medical condition. For this nested case-control analysis of CRC risk, women were excluded if they had pre-existing intestinal disease, including history of CRC, carcinoma *in situ*, ulcerative colitis, Crohn's disease, or if they were extremely under- or overweight as indicated by measured body mass index ≤15 or ≥50 kg/m^2. Risk-set

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sampling was used to randomly select controls from within the WHI-OS cohort who were alive and free of any type of CRC, invasive or non-invasive, at the time of case diagnosis. Cases and controls were matched based on age (±3 years), race/ethnicity, enrollment date (±1 year), hysterectomy status and time of blood draw (±6 months). The analyses in this report are based on 988 incident cases of colorectal cancer and 988 matched controls. Written informed consent was obtained from all participants at study enrollment and at various follow-up time points. The study was approved by the human subjects review board at the Fred Hutchinson Cancer Research Center where the WHI Clinical Coordinating Center is located. Additional IRB approval was obtained at the University of California, Davis where some analyte measurements for this report were conducted.

Blood Sample Processing and Analysis

Twelve-hour fasting blood samples were collected from all participants at baseline using EDTA collection tubes. Samples were kept at 4°C for up to one hour prior to centrifugation at 4 °C to obtain plasma and red blood cells (RBCs). Samples were tracked and stored at −70°C at a central biorepository (McKesson BioServices, Rockville, MD) until analysis. Plasma and RBC folate concentrations were determined by radioassay (SimulTRAC Radioassay Kit Folate [125I] MP Biomedicals LLC) at the Fred Hutchinson Cancer Research Center and at the University of California Davis Medical Center, respectively. Inter-assay coefficients of variation (CV) for each of the assays were: plasma folate, 4.8%; RBC folate, 10.2%. Intra-assay CVs calculated from 5% blind duplicate samples were: plasma folate 8.6%; RBC folate: 12.0%.

Demographic and Health Data Collection

Demographic characteristics, including age, race/ethnicity, education, and household income, and health-related characteristics, including personal medical history, use of postmenopausal hormones, dietary supplements and other medications, recreational physical activity, and smoking history, were obtained by self-report at baseline using standardized questionnaires completed at the baseline clinic visit. [19] Dietary intake over the previous three months was assessed with a food frequency questionnaire (FFQ) designed and validated specifically for use in WHI. [21] Baseline height and weight were measured by trained staff using a standard protocol and body mass index (BMI) was computed as weight [kg]/height [m]^2.

Outcomes Ascertainment: Colorectal Cancer

All WHI-OS participants completed yearly medical status update questionnaires by mail. Participants responded to the question, “since the date on the front of this form has a doctor told you for the first time that you have a new cancer or malignant tumor?” If the response was “yes” participants were asked to report the type of cancer/malignancy. Cancer diagnoses were confirmed by trained physician adjudicators using medical records and the International Classification of Diseases for Oncology codes, second edition (ICD-O-2) (http://seer.cancer.gov). CRC cases were classified as ‘proximal’ if they were located in the cecum (ICD-O-2 code: C180), ascending colon (C182), hepatic flexure (C183), transverse colon (C184), or splenic flexure (C185); ‘distal’ if they were located in the descending colon (C186) or sigmoid colon (C187); or ‘rectal’ (C199, C209). This classification of the tumors

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followed the National Cancer Institute's Surveillance Epidemiology and End Results (SEER) criteria (http://seer.cancer.gov).

Statistical Analyses

Baseline characteristics of CRC cases and controls were compared by t-tests (for continuous variables) and Chi-square tests (for categorical variables). Conditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (95% CI) for CRC among quartiles of plasma and RBC folate, with the lowest quartiles of both variables serving as the reference groups. Quartiles were established based on the distributions among the controls. OR and 95% CI determinations were made in sequential models with the first model adjusted only for age. Multivariate models were additionally adjusted for several *a priori* baseline variables including BMI (<25, 25–30, >30–35, >35 kg/m^2), race/ethnicity (white, black, or other), history of colonoscopy (yes/no), family history of CRC, smoking status (never, past, or current), recreational physical activity (0–180, >180–705, or >705 minutes/week), and postmenopausal hormone use (never, past, or current). Final multivariate models included only those covariates that affected the risk estimates by more than 10%; these included age, BMI, postmenopausal hormone use, family history of CRC and history of colonoscopy. Tests of linear trend were conducted using the Wald test across increasing quartiles of plasma or RBC folate with the median values of each quartile modeled as a single continuous variable. Further analyses stratified cases by stage at diagnosis and location of tumor. Analyses were also conducted to test effect modification by folic acid fortification. First, we encoded a variable that indicated whether blood draws were obtained pre-fortification (1993–1995), peri-fortification (1996–1997, when initial fortification began, but was not yet mandated) or post-fortification (1998). Second, to account for the lag time in CRC progression after exposure to folic acid fortification, we created a variable indicating time exposed to folic acid fortification, defined as time from August 1, 1997 (the date when many food manufacturers began fortification prior to the mandatory 01/01/98) to the date of CRC diagnosis for cases or date of last follow-up for controls. The variable was then categorized to <3, 3 to <6, 6 to <9, and >= 9 years, parallel to our prior investigation. [18] In addition, we also explored whether alcohol intake (never/past/current drinkers, and non-drinkers/light drinkers/moderate-heavy drinkers) was an effect modifier since it affects folate absorption and metabolism [1], alcohol may be an independent predictor of CRC [22] and because potential effect modification was observed for dietary folate intake from self-report in the WHI-OS. [18] Statistical interactions were evaluated by Wald tests of a product term between the ordinal trend variables (folate biomarkers) and each effect modifier (folic acid fortification period, time exposed to fortification, and alcohol intake). Statistical significance was defined as $P<0.05$ and all statistical tests were two-sided. Analyses were conducted using SAS (v. 9.2, SAS Institute, Inc., Cary, NC, USA).

Results

CRC cases and controls did not differ with respect to age, race/ethnicity, geographic residence in the U.S., education, household income dietary folate intake or family history of CRC (Table 1). Compared to controls, cases had a higher mean BMI (28.1 vs. 27.1 kg/m^2,

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P=0.0001), had a greater mean pack-years of smoking (13.0 vs. 8.9 pack-years, *P* <0.0001), differed by postmenopausal hormone use practices (more likely to be never users) (*P*=0.003), were less physically active (mean 96 vs. 109 minutes/week, *P*=0.04) and were more likely to have had a previous colon polyp removed (24.9% vs. 18.2%, *P*=0.008).

In both age-adjusted and multivariate-adjusted models we observed no statistically significant association of RBC folate with overall CRC risk [Q4 vs. Q1 (referent) OR=0.85, 95% CI 0.65–1.12 and OR=0.91, 95% CI 0.67–1.24, respectively] (Table 2). Risk estimates differed slightly by tumor site where the odds ratio for proximal tumors was close to the null value whereas that for rectal tumors was a non-significant 32% reduction in risk for higher vs. lower RBC folate. We found no association of RBC folate with CRC risk in models stratified by disease stage at diagnosis (local/regional and metastatic). For plasma folate (Table 3), we observed a suggestive inverse association in age-adjusted models for total CRC [Q4 vs. Q1. OR=0.78, 95% CI 0.60–1.02, *P*-trend =0.09] and for rectal cancers [Q4 vs. Q1, OR=0.60, 95% CI 0.32–1.10, *P*-trend =0.09], but following multivariate adjustment the results were attenuated particularly for overall risk.

Since we were interested in whether the associations of plasma and RBC folate with CRC risk differed in relation to fortification of the U.S. food supply with synthetic folic acid added to enriched cereal grains, we next conducted a series of models to test whether three fortification periods (pre, peri and post-fortification) modified the association of RBC and plasma folate with colorectal cancer risk (Table 4 and Table 5 for RBC folate and plasma folate, respectively), paying particular attention to relationships that could have been masked when not examined by fortification period. While higher vs. lower RBC folate was suggestively associated with a 27% reduced risk for CRC in the peri-fortification period from 1996–1997, the formal interaction test was not statistically significant. Other analyses using both RBC and plasma folate did not reveal any definitive patterns of association between the biomarkers and CRC risk that varied by folic acid fortification period and interactions tests were not statistically significant. In addition, we did not observe any effect modification based on time exposed to folic acid fortification (data not shown). Finally we tested for interactions of alcohol intake with both RBC and plasma folate in relation to CRC risk. However, we observed no interactions and no differences in risk by alcohol intake (data not shown).

DISCUSSION

In the Women's Health Initiative Observational Study neither baseline plasma folate nor RBC folate was associated with subsequent CRC risk. Importantly, there were no significant differences in risk by folic acid fortification period. The latter results are key to public health recommendations as there has been recent controversy about whether the FDA's folic acid fortification program has unintentionally increased the population-level risk of CRC [14,15,22] while achieving the primary goal of the program, which is to reduce the number of neural tube defect-affected pregnancies in the U.S. [23] A 2013 meta-analysis [24] was conducted using data from supplement trials that compared folic acid vs. placebo in order to assess the aggregate cancer risk in relation to folic acid supplementation. It should be noted that for three of the trials examined the primary outcome was colorectal adenomas and for ten trails

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the primary endpoints were cardiovascular disease, coronary heart disease, renal disease, stroke and diabetes. Cancers were secondary outcomes in all the trials. There was no evidence for an increased cancer risk as the summary relative risk was 1.06 (95% CI 0.99–1.13). [24] It was noted that the folic acid doses from these trials was far greater than that obtained from the fortified food supply and that therefore much more modest doses from the food supply would be unlikely to result in increased cancer risk. This statement is supported by the data presented in this report. However, as with any large scale modifications to the food supply, surveillance should continue.

The hypothesis that folate would protect against colorectal cancer was a reasonable one given the biochemistry and the prior observational and laboratory data to support the hypothesis. [2,25–27] Folate plays a critical role as a co-factor in one-carbon metabolic reactions including nucleotide synthesis, maintenance of DNA integrity and synthesis of S-adenosylmethionine (SAM), which is the primary methyl donor for epigenetic DNA modifications. [28–31] Folate deficiency impairs any or all of these critical reactions leaving the host susceptible to DNA damage and subsequent carcinogenesis. Since the gastrointestinal tract of humans has a high epithelial cell turnover rate, the colon has been thought to be particularly susceptible to poor folate status. In 2009, the World Cancer Research Fund noted that the evidence to support a protective association for folate in relation to CRC was reasonably strong. [28] However, the studies reviewed for the WCRF report were primarily based on dietary intake from self-report and not on biomarkers. Since the publication of the WCRF report, other observational studies have been published, including some using data from the post-fortification period and the studies support our findings. The NIH-AARP Diet and Health Study used data from a self-administered food frequency questionnaire plus frequency and duration of folic acid from multivitamins. [32] Analyses tested for differences by the pre-post fortification periods and an inverse association of folate with CRC risk was observed only in the post-fortification period. A report using data from the Nurses' Health Study and the Health Professionals' Follow-Up Study showed that folic acid fortification may attenuate the adverse association of alcohol with CRC risk. [33] These studies all used self-reported intake of folate, not folate biomarkers. Self-reported diet is subject to both random and systematic error [34] whereas biomarker measures of status may more objectively represent nutritional status. Other nested case-control studies [8–11] using biomarkers as the primary folate exposure are consistent with our results from the WHI, which showed no significant association of folate with CRC risk. The only study [17] to report significant findings was that of Lee et al where data were combined from the Nurses' Health Study, the Health Professionals Follow-Up Study and the Physicians' Health Study. These investigators reported a 47% increased CRC risk for the fourth vs. the first quartile of plasma folate and in subgroup analysis the risk estimates were higher during the post-fortification period when combining the top three quartiles of plasma folate compared to the first quartile. [17]

Our findings may to some extent assuage concern about whether the FDA's fortification program has inadvertently increased the incidence of colorectal cancer. In animal models, folic acid supplementation is an effective chemopreventive agent if given prior to the establishment of early gastrointestinal lesions (e.g., aberrant crypt foci), and this relationship has been attributed to adequate supplies for methylation reactions and nucleotide synthesis. [2]

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However, once a preneoplastic lesion is present, folate may enhance tumor growth, due to the dependence of rapidly dividing tissues on folate for DNA synthesis. [22] These relationships were first described in animal experiments and various cancers, [35,36] but there is now some evidence from a randomized controlled trial, showing that higher folic acid intakes appear to enhance the recurrence of multiple or larger adenomas. [13] In addition, the folic acid used for fortification is an oxidized form of the vitamin that can accumulate in plasma and be harmful to natural killer cell activity/cell mediated immunity as was shown in one small human study. [37] The synthetic form of folic acid may also result in an excess of accumulation of dihydrofolate (DHF), which then disrupts the remainder of key folate-mediated one-carbon metabolic reactions. For this reason, we [30] and others [15,38,39] have modeled various scenarios by which excess synthetic folic acid could potentially be detrimental to long-term health. In addition to theoretical modeling data, Mason et al. [15] used SEER data from 1986–2002 to show an increase in CRC incidence between 1998–2000 on the order of an excess of 4–6 additional cases per 100,000 people. While it is very difficult to draw causal inferences between the folic acid fortification program and the increase in colorectal cancer incidence, all other things being equal, the Mason et al report was sufficiently strong for some to reconsider the merits of increasing the population's exposure to synthetic folic acid. [40] In contrast, Keum and Giovannucci [39] modeled SEER data over time and found no evidence that secular trends in CRC risk could be attributed to folic acid fortification. Clearly a totality of evidence needs to be examined when considering the risks and benefits of the folic acid fortification program. In this regard, our report from the large well-conducted study in the WHI-OS provides some reassurance that the folic acid fortification program did not lead to increased CRC risk among post-menopausal women. Our follow-up was relatively short-term and adequate population surveillance should continue to monitor long-term effects of fortification.

There are numerous strengths to this study. The WHI-OS is a large and well-characterized cohort of postmenopausal women with nearly 20 years of follow-up time. All data and specimens were collected using standardized protocols and all endpoints were adjudicated by trained physician adjudicators using medical records. Baseline blood specimens were collected from all WHI-OS participants, which ensured that the cases and controls could be well-matched on age, clinical center, race/ethnicity, and blood draw date. In addition, unlike most previous cohort studies that assessed either serum or plasma folate, we were able to evaluate both biomarker measures of folate status. Plasma folate is a short-term measure of folate status and is reflective of extracellular concentrations, whereas RBC folate is a longer-term measure of folate status that reflects intracellular concentrations. Another strength is that because WHI enrollment occurred concurrently with the pre, peri and post-fortification epochs, we were able to investigate the important question of whether risk differed by exposure to synthetic folic acid widely present in the food supply during and after initiation of the mandatory fortification program. Limitations include the smaller sample size of participants who enrolled in the latter years of the WHI enrollment period, which limited the power to detect associations in the post-fortification period. Other biomarkers that we were not able to measure include unmetabolized folic acid. [37] While only one study, to our knowledge, has suggested that folic acid fortification may lead to excess circulating unmetabolized folic acid, the potential health risks from this exposure are unknown and this

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remains an important area of research. Another limitation is that we measured the biomarkers at only one point in time in all participants. As such as we were not able to assess whether individual-level folate status changed over time in relation to risk or fortification status. In addition, the time course relationship between the exposure (e.g., serum and RBC folate) and colorectal carcinogenesis is unknown. Carcinogenesis is a lengthy, complex process and it is possible that longer follow-up time could yield different results. An additional limitation is that we were not able to assess whether the lack of associations observed in the WHI would be modified by genetic variation in folate metabolizing enzymes (e.g., MTHFR). At least one recent study shows an important relationship between MTHFR variation and CRC risk. [41] Further research pooling data and specimens from large cohort studies is needed to have sufficient power to address the question. Finally, as with all observational studies, residual confounding may have occurred from variables that were either unmeasured or measured with poor precision in the WHI-OS.

In conclusion, in this large study of postmenopausal women, we found no significant evidence that either plasma folate or RBC folate was associated with CRC risk. While our sample size was limited for subgroup analysis stratified by folic acid fortification period, we observed no consistent evidence to suggest that risk varied after the fortification of the US food supply with folic acid.

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Novelty and impact

In this nested case-control study from the Women's Health Initiative, we tested associations of folate with colorectal cancer (CRC) risk using the two principal biomarkers of folate status – serum folate and red blood cell folate. Importantly, we had data and specimens from before, during and after the fortification of the US food supply with folic acid so we were able to test whether associations varied based on fortification. Overall we found no associations of folate biomarkers with CRC risk and risks did not vary by folic acid fortification period. The major impact is that the results suggest that fortification of the US food supply with folic acid did not increase risk of CRC in these postmenopausal women

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Table 1

Characteristics of colorectal cancer cases (n=988) and controls (n=988) in the Women's Health Initiative Observational Study

Characteristics	Cases		Controls		*P*-value
	n	Mean (SD) or %	n	Mean (SD) or %	
Age (years)	988	67 (7)	988	67 (7)	0.50
50 – 54	57	5.8	55	5.6	0.96
55 – 59	106	10.7	104	10.5	
60 – 64	190	19.2	192	19.4	
65 – 69	260	26.3	245	24.8	
70 – 74	230	23.3	246	24.9	
75 – 79	145	14.7	146	14.8	
Body-Mass Index (kg/m^2)**	976	28.1 (6.0)	978	27.1 (5.9)	0.0001
< 25.0	342	35	394	40.3	0.002
25.0 – 29.9	331	33.9	355	36.3	
30 – 34.9	187	19.2	143	14.6	
≥ 35.0	116	11.9	86	8.8	
Race/Ethnicity	988	100	988	100	1.0
White	842	85.2	842	85.2	
Black/African American	88	8.9	88	8.9	
Other	58	5.9	58	5.9	
Household Income ($/yr)	950	100	940	100	0.08
Less than 34,999	457	48.1	421	44.8	
35,000 to 74,999	343	36.1	329	35	
75,000 or more	125	13.2	162	17.2	
Don't know	25	2.6	28	3	
Education (completed high school)	981	100	980	100	0.12
Yes	786	80.1	757	77.2	
No	195	19.9	223	22.8	
Postmenopausal hormone use	987	100	988	100	0.0003
Never user	493	50	418	42.3	

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Characteristics	Cases		Controls		*P*-value
	n	Mean (SD) or %	n	Mean (SD) or %	
Past user	171	17.3	163	16.5	
Current user	323	32.7	407	41.2	
U.S. Geographic Residence	988	100	988	100	
	255	25.8	227	23	0.42
Northeast	223	22.6	240	24.3	
South	228	23.1	222	22.5	
Midwest	282	28.5	299	30.3	
West					
Pack-years smoking	951	13.0 (21.7)	951	8.9 (17.0)	<0.0001
Dietary Folate Equivalents (mcg/day)	988	448 (220)	988	457 (228)	0.39
Minutes of moderate or strenuous activity per week	975	95 (135)	979	109 (143)	0.04
Family History of Colorectal Cancer	910	100	903	100	0.1
Yes	192	21.1	163	18.1	
History of Colonoscopy or Sigmoidoscopy	971	100	975	100	0.0003
Yes	508	52.3	589	60.4	
History of Colon Polyp Removal	494	100	576	100	0.008
Yes	123	24.9	105	18.2	
Tumor location*	990	100			
Proximal	577	58.3			
Distal	211	21.3			
Rectal	184	18.6			
Overlapping lesion/Unknown	18	1.8			
Tumor grade*	990	100			
Well differentiated	75	7.6			
Moderately differentiated	616	62.2			
Poorly differentiated	202	20.4			
Anaplastic	12	1.2			
Unknown/not done	85	8.6			
Tumor stage (SEER staging)*	989	100			

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Characteristics	Cases n	Cases Mean (SD) or %	Controls n	Controls Mean (SD) or %	*P*-value
Localized	431	43.6			
Regional	409	41.3			
Distant	127	12.8			
Unknown/not done	22	2.2			

[1] Includes Hispanic, Asian or Pacific Islander, American Indian or Alaskan Native

* Two participants had both colon cancer and colorectal cancer per adjudications, hence n=990.

** Measured at baseline

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Table 2

Associations of baseline red blood cell folate with colorectal cancer in the WHI-OS.

	Quartiles of RBC folate (ng/mL)				
	1 <427	**2 > 427 – 577**	**3 > 577 – 742**	**4 > 742**	***P* trend**
Overall risk					
No of cases/controls	281/248	227/247	222/247	235/246	
Age-adjusted OR (95% CI)	1.0 (ref)	0.86 (0.67,1.11)	0.81 (0.63,1.06)	0.85 (0.65,1.12)	0.26
Multivariate OR (95%CI)[1]	1.0 (ref)	0.84 (0.63,1.13)	0.85 (0.63,1.13)	0.91 (0.67,1.24)	0.63
By tumor site					
Proximal					
No of cases/controls	153/140	137/158	126/137	146/142	
Age-adjusted OR (95%CI)	1.0 (ref)	0.87 (0.63,1.20)	0.89 (0.64,1.25)	0.96 (0.68,1.37)	0.92
Multivariate OR (95%CI)[1]	1.0 (ref)	0.85 (0.58,1.23)	0.89 (0.60,1.32)	1.01 (0.67,1.52)	0.83
Distal					
No of cases/controls	60/53	43/51	52/53	48/54	
Age-adjusted OR (95% CI)	1.0 (ref)	0.76 (0.42,1.35)	0.87 (0.48,1.57)	0.83 (0.47,1.48)	0.64
Multivariate OR (95%CI)[1]	1.0 (ref)	0.88 (0.44,1.75)	1.11 (0.58,2.14)	1.02 (0.51,2.03)	0.83
Rectal					
No of cases/controls	60/52	44/36	44/51	36/45	
Age-adjusted OR	1.0 (ref)	1.03 (0.55,1.94)	0.73 (0.40,1.31)	0.68 (0.36,1.30)	0.15
Multivariate OR (95%CI)[1]	1.0 (ref)	0.68 (0.31,1.49)	0.52 (0.25,1.09)	0.58 (0.26,1.31)	0.16
By stage at diagnosis					
Local/regional					
No of cases/controls	241/215	193/212	189/206	199/206	
Age-adjusted OR	1.0 (ref)	0.85 (0.65,1.12)	0.82 (0.62,1.09)	0.88 (0.66,1.18)	0.41
Multivariate OR (95%CI)[1]	1.0 (ref)	0.84 (0.62,1.16)	0.82 (0.60,1.14)	0.98 (0.70,1.38)	0.97
Metastatic					
No of cases/controls	33/27	28/30	29/35	31/35	
Age-adjusted OR (95%CI)	1.0 (ref)	0.89 (0.43,1.85)	0.81 (0.39,1.66)	0.73 (0.35,1.52)	0.38

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	Quartiles of RBC folate (ng/mL)				
	1 **<427**	**2** **> 427 – 577**	**3** **> 577 – 742**	**4** **> 742**	***P*** **trend**
Multivariate OR (95%CI)[1]	1.0 (ref)	0.91 (0.39,2.15)	1.14 (0.51,2.58)	0.63 (0.27,1.43)	0.31

[1] Adjusted for age, baseline BMI, postmenopausal HT use, previous colonoscopy, family history of colorectal cancer.

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Table 3

Associations of plasma folate with colorectal cancer risk in the WHI-OS

	Quartiles of plasma folate (ng/mL)				
	1 ≤9.72	**2 >9.72 – 16.85**	**3 >16.85 – 26.85**	**4 >26.85**	***P* trend**
Overall Risk					
No of cases/controls	274/240	236/240	236/240	210/239	
Age-adjusted OR (95% CI)	1.0 (ref	0.87 (0.68,1.12)	0.86 (0.66,1.12)	0.78 (0.60,1.02)	0.09
Multivariate OR (95%CI)[1]	1.0 (ref)	0.84 (0.63,1.12)	0.98 (0.72,1.32)	0.91 (0.67,1.23)	0.80
By tumor site					
Proximal					
No of cases/controls	157/140	128/137	143/143	130/139	
Age-adjusted OR (95%CI)	1.0 (ref)	0.85 (0.60,1.19)	0.92 (0.65,1.28)	0.86 (0.61,1.21)	0.53
Multivariate OR (95%CI)[1]	1.0 (ref)	0.81 (0.56,1.19)	1.03 (0.70,1.51)	0.97 (0.65,1.43)	0.83
Distal					
No of cases/controls	49/50	57/58	55/51	39/44	
Age-adjusted OR (95% CI)	1.0 (ref)	1.04 (0.60,1.80)	1.05 (0.59,1.86)	0.89 (0.47,1.71)	0.71
Multivariate OR (95%CI)[1]	1.0 (ref)	0.93 (0.49,1.77)	1.23 (0.62,2.43)	1.54 (0.71,3.34)	0.20
Rectal					
No of cases/controls	61/47	47/42	35/41	39/51	
Age-adjusted OR (95%CI)	1.0 (ref)	0.81 (0.45,1.44)	0.60 (0.31,1.15)	0.60 (0.32,1.10)	0.09
Multivariate OR (95%CI)[1]	1.0 (ref)	0.76 (0.38,1.54)	0.63 (0.28,1.44)	0.58 (0.27,1.25)	0.16
By stage					
Local/regional					
No of cases/controls	230/208	206/204	198/209	180/195	
Age-adjusted OR (95% CI)	1.0 (ref)	0.93 (0.70,1.22)	0.86 (0.65,1.13)	0.86 (0.64,1.15)	0.29
Multivariate OR (95%CI)[1]	1.0 (ref)	0.86 (0.63,1.18)	0.99 (0.71,1.36)	0.95 (0.68,1.34)	1.00
Metastatic					
No of cases/controls	34/24	24/31	34/27	28/39	
Age-adjusted OR (95% CI)	1.0 (ref)	0.48 (0.21,1.10)	0.94 (0.43,2.06)	0.49 (0.24,1.03)	0.18

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	Quartiles of plasma folate (ng/mL)				
	1 ≤9.72	**2 >9.72 – 16.85**	**3 >16.85 – 26.85**	**4 >26.85**	***P* trend**
Multivariate OR (95%CI)[1]	1.0 (ref)	0.46 (0.17,1.19)	0.82 (0.31,2.15)	0.54 (0.22,1.35)	0.46

[1] Adjusted for age, BMI at baseline, postmenopausal HT use, previous colonoscopy, family history of colorectal cancer.

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Table 4

Effect modification of folic-acid fortification on the association of RBC folate with colorectal cancer risk in the WHI –OS.

	Quartiles of RBC folate (ng/mL)				
Time period	**1 ≤ 427**	**2 > 427 – 577**	**3 > 577 – 742**	**4 > 742**	***P* trend**
Pre-fortification (1993–1995)					
No. of cases/controls	86/92	54/60	52/51	44/43	
Age-adjusted OR (95% CI)	1.0 (ref)	0.96 (0.60,1.54)	1.09 (0.67,1.78)	1.09 (0.65,1.84)	0.67
Multivariate OR (95% CI)[1,2]	1.0 (ref)	1.03 (0.62,1.73)	1.27 (0.75,2.16)	1.40 (0.80,2.47)	0.19
Peri-fortification (1996–1997)					
No. of cases/controls	174/138	127/139	107/133	121/135	
Age-adjusted OR	1.0 (ref)	0.73 (0.52,1.01)	0.64 (0.45,0.90)	0.71 (0.51,1.00)	0.05
Multivariate OR (95% CI)[1,3]	1.0 (ref)	0.77 (0.54,1.10)	0.69 (0.48,1.00)	0.73 (0.51,1.05)	0.10
Post-fortification (1998)					
No. of colon cancer cases	21/18	46/48	63/63	70/68	
Age-adjusted OR	1.0 (ref)	0.82 (0.39,1.74)	0.86 (0.41,1.79)	0.88 (0.42,1.83)	0.96
Multivariate OR (95% CI)[1]	1.0 (ref)	0.79 (0.35,1.80)	1.14 (0.51,2.53)	1.15 (0.52,2.57)	0.36

[1] Adjusted for age, BMI (at baseline), postmenopausal HT use, previous colonoscopy, family history of colorectal cancer. Case-control matching is broken in these subset analyses; as a result models are also controlled for ethnicity and time to diagnosis (the latter designed to assess length of exposure to folic acid).

[2] *P*, interaction = 0.49

[3] *P*, interaction = 0.12

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Table 5

Effect modification of folic-acid fortification on the association of plasma folate with colorectal cancer risk in the WHI –OS[1].

	Quartiles of plasma folate (ng/mL)				
	1 ≤9.72	**2 > 9.72 – 16.85**	**3 > 16.85 – 26.85**	**4 > 26.85**	***P* trend**
Pre-fortification (1994–1995)					
No. of cases/controls	85/74	57/61	47/40	45/67	
Age-adjusted OR (95% CI)	1.0 (ref)	0.81 (0.51,1.31)	1.02 (0.60,1.73)	0.58 (0.36,0.95)	0.05
Multivariate OR (95% CI)[1,2]	1.0 (ref)	0.91 (0.55,1.52)	1.23 (0.69,2.19)	0.90 (0.52,1.53)	0.82
Peri-fortification (1996–1997)					
No. of cases/controls	170/144	135/129	122/149	97/104	
Age-adjusted OR (95% CI)	1.0 (ref)	0.89 (0.64,1.24)	0.70 (0.50,0.97)	0.80 (0.56,1.15)	0.14
Multivariate OR (95% CI)[1,3]	1.0 (ref)	0.99 (0.70,1.41)	0.80 (0.56,1.14)	0.90 (0.60,1.33)	0.41
Post-fortification (1998)					
No. of cases/controls	19/22	44/50	67/51	68/68	
Age-adjusted OR	1.0 (ref)	1.02 (0.49,2.14)	1.53 (0.75,3.11)	1.16 (0.58,2.35)	0.68
Multivariate OR (95% CI)[1]	1.0 (ref)	0.85 (0.38,1.91)	1.59 (0.72,3.54)	1.24 (0.57,2.70)	0.33

[1] Adjusted for age, BMI (at baseline), postmenopausal HT use, previous colonoscopy, family history of colorectal cancer. Case-control matching is broken in these subset analyses; as a result models are also controlled for ethnicity and time to diagnosis (the latter designed to assess length of exposure to folic acid).

[2] *P*, interaction = 0.45

[3] *P*, interaction = 0.12