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Brief Communication

BRIEF COMMUNICATION

Unmetabolized Folic Acid in Prediagnostic Plasma and the Risk for Colorectal Cancer

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Abstract

Higher folate has been associated with a reduced colorectal cancer (CRC) risk, but excessive folate may promote tumor progression. The role of unmetabolized folic acid (UFA) from high folic acid consumption in carcinogenesis is largely unexplored. We evaluated prediagnostic plasma levels of UFA in relation to CRC risk in nested case-control studies (618 CRC case patients and 1207 matched control) with blood samples collected prior to folic acid fortification. UFA was detected in 21.4% of control UFA levels were not associated with CRC risk. Compared with undetectable levels, the multivariable relative risks (RRs) of CRC were 1.03 (95% confidence interval [CI] = 0.73 to 1.46) for less than 0.5 nmol/L and 1.12 (95% CI = 0.81 to 1.55) for 0.5 nmol/L or more (P_{trend} = .32). A positive association between UFA levels and CRC risk was observed among men (RR = 1.57, 95% CI = 0.99 to 2.49 for ≥0.5 nmol/L vs undetectable, $P_{interaction}$ = .04), and a positive association was also observed among those with the methylene-tetrahydrofolate reductase (*MTHFR*) CT/TT genotype (RR = 2.20, 95% CI = 1.22 to 3.94 for ≥0.5 nmol/L vs undetectable, $P_{interaction}$=0.02). In conclusion, prediagnostic plasma levels of UFA from the prefortification period were not associated with risk of CRC.

Colorectal cancer (CRC) is a major burden worldwide (1). Total folate includes both naturally occurring food folate and the synthetic form (folic acid) used in fortified foods and supplements. Several studies have found inverse associations between higher dietary folate or circulating folate levels and risks of colorectal adenoma or cancer (2–4) and several other cancers (2). However, excessive intake of folic acid may increase cancer risk (5–9). Folic acid needs to be reduced to tetrahydrofolate (THF) to be incorporated into one-carbon metabolism (10). If the body's ability to reduce folic acid is exceeded (approximately >200 ug/d), unmetabolized folic acid (UFA) may accumulate in plasma (11). The health effects of circulating UFA levels are unclear (12–14).

We therefore evaluated the association between prediagnostic plasma UFA in relation to CRC risk in case-control studies nested within the Nurses' Health Study (NHS) (15,16) and Health Professionals Follow-up Study (HPFS) (17). All blood samples were collected prior to the 1998 FDA-mandated fortification of grain products with folate (18). Detailed methods are described in the Supplementary Methods (available online). Confirmed incident CRC case patients diagnosed after blood draw through 2010 were included. Two control per case were matched within each cohort. A total of 618 CRC case patients and 1207 matched control were included in the analysis. Plasma UFA level was measured using a liquid chromatography tandem mass spectrometry method (LC/MS/MS) (19). The detection limit was 0.25 nmol/L. We grouped the participants into three categories, undetectable, less than 0.5 nmol/L, and 0.5 nmol/L or more, and used conditional logistic regression to estimate relative risks (RRs) and 95% confidence intervals (CIs) for CRC after adjusting for multiple CRC risk factors. Tests for trend were performed

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by assigning the median (log_e-transformed plasma levels) of each category of UFA as a continuous variable in the models. All statistical tests were two-sided, and a *P* value of less than .05 was considered statistically significant. We used SAS statistical software (version 9.2; SAS Institute, Cary, NC) for all analyses. Details on other statistical tests and methods used in this study are described in detail in the Supplementary Methods (available online).

The NHS was approved by the institutional review boards (IRBs) of the Brigham and Women's Hospital (Boston, MA); the HPFS was approved by the IRB of the Harvard School of Public Health (Boston, MA). Informed consent was implied by receipt of completed questionnaires and blood samples.

UFA was detected in 21.4% of the control and 22.0% of the CRC case patients. Among those detected, the median UFA level was 0.61 nmol/L in both control and case patients. Case patients had a lower prevalence of use of aspirin and lower intake of calcium, vitamin D, folate, and multivitamin use (Table 1). There were 26% of case patients and 31% of control with more than 200 ug/day of supplemental folic acid intake. Among control the median levels of UFA were 2.07 nmol/L for individuals with more than 200 ug/day supplemental folic acid intake and 0.42 nmol/L for those with lower intake. The Spearman correlation coefficient between the UFA levels and supplemental folic acid intake was 0.38 ($P < .001$). The corresponding levels of UFA were 1.93 nmol/L for multivitamin users and 0.17 nmol/L for nonmultivitamin users. Overall, prediagnostic plasma levels of UFA were not associated with CRC risk (Table 2). Compared with those with undetectable UFA, the multivariable relative risks of CRC were 1.03 (95% CI = 0.73 to 1.46) for less than 0.5 nmol/L and 1.12 (95% CI = 0.81 to 1.55) for 0.5 nmol/L or more (P_{trend} = .32). In stratified analyses, we observed a borderline positive association between UFA levels and CRC risk among men (RR = 1.57, 95% CI = 0.99 to 2.49, for ≥0.5 nmol/L vs undetectable) but a borderline inverse association among women (RR = 0.64, 95% CI = 0.39 to 1.05, for the same comparison, $P_{interaction}$ = .04) (Supplementary Table 1, available online). The association was also positive among those with methylene-tetrahydrofolate reductase (*MTHFR*) CT/TT genotype (RR = 2.20, 95% CI = 1.22 to 3.94, for ≥0.5 nmol/L vs undetectable) but suggestively inverse among those with CC genotype (RR = 0.47, 95% CI = 0.21 to 1.03, for the same comparison; $P_{interaction}$ = 0.02). Among control and after excluding undetected values, geometric means of UFA (nmol/L) were 1.18 (95% CI = 0.87 to 1.59) for *MTHFR* CC genotype, 0.77 (95% CI = 0.56 to 1.06) for CT genotype, and 0.95 (95% CI = 0.51 to 1.77) for TT genotype. The associations between UFA levels and CRC risk were similar by other CRC risk factors (Supplementary Table 1, available online).

Few studies have examined health effects of circulating UFA levels. A study among US seniors (1999–2002) found that UFA was associated with increased odds of anemia in alcohol consumers (13). Another study found that higher UFA level was associated with reduced natural killer cell cytotoxicity among healthy postmenopausal women (14).

We found some suggestion that UFA levels may be harmful in men and those with the *MTHFR 677CT/TT* genotype; however, these observations could be because of chance and should be interpreted with caution, especially because the association was suggestively in the opposite direction for women and those with the *MTHFR* 677CC genotype.

Dihydrofolate reductase (DHFR) is an enzyme that converts ingested folic acid to THF, which can be utilized by the cells; folic acid intake of 500 ug/day or more was related to increased prevalence of high circulating UFA levels among those with DHFR deletion polymorphism (20). We had no information on that polymorphism. Any association observed between high circulating UFA and risk of a disease could theoretically reflect that the

Table 1. Baseline characteristics of colorectal cancer case patients and control in a case-control study nested within the Nurses' Health Study and the Health Professionals Follow-up Study

	Case/control status	
Characteristics	Case patients (n = 618)	Control (n = 1207)
Age at blood draw, y, mean*	62.0	61.9
Female, %*	55.7	56.0
Body-mass index, kg/m^2, mean	26.1	25.5
Height, inches	67.2	67.0
Physical activity, METs, h/wk, mean†	22.4	23.5
Current smoker, %	8.7	8.5
Current use of aspirin, %	45.3	49.8
Colorectal cancer in a parent or sibling, %	15.5	13.2
History of endoscopy, %	41.8	49.4
Alcohol consumption, g/d, mean	8.4	7.9
Beef, pork, or lamb as a main dish, servings/d, mean	1.1	1.1
Total calcium intake, mg/d, mean‡	963.9	1008.2
Total vitamin D intake, IU/d, mean‡	385.1	411.1
Total folate intake, µg/d, mean‡	426.3	452.9
Dietary folate intake, µg/d, mean‡	329.8	340.7
Supplemental folic acid intake, µg/d, mean‡§	113.8	131.8
Multivitamin use, %	40.8	44.3
Fasting status, %*	66.0	67.7

* Matching factor.
† MET denotes metabolic equivalent. MET-hours = sum of the average time/wk in each activity x MET value of each activity. One MET, the energy spent sitting quietly, is equal to 3.5 mL of oxygen uptake per kilograms of body weight per minute for a 70 kg adult.
‡ Nutrient values (calcium, vitamin D, and folate) represent the mean of energy-adjusted intakes.
§ Supplemental folic acid intake from folic acid supplements and multivitamins.

Table 2. Association between unmetabolized folic acid level (nmol/L) and the risk of total and subtypes of colorectal cancer*

Endpoint	Unmetabolized folic acid, nmol/L			P_{trend}
	Undetectable	<0.5	≥0.5	
Median, nmol/L	0	0.10	0.53	
Colorectal cancer				
No. of case patients/control	254/437	233/507	131/263	
Crude OR† (95% CI)	1.00	0.94 (0.67 to 1.31)	1.01 (0.74 to 1.38)	.71
Multivariable† OR (95% CI)	1.00	1.03 (0.73 to 1.46)	1.12 (0.81 to 1.55)	.32
Colon cancer				
No. of case patients	152	147	78	
Crude OR (95% CI)	1.00	1.10 (0.75 to 1.59)	0.81 (0.55 to 1.19)	.25
Multivariable† OR (95% CI)	1.00	1.20 (0.82 to 1.77)	0.90 (0.61 to 1.34)	.75
Proximal colon cancer				
No. of case patients	96	83	55	
Crude OR (95% CI)	1.00	1.27 (0.82 to 1.96)	0.99(0.64 to 1.54)	.89
Multivariable† OR (95% CI)	1.00	1.39(0.89 to 2.19)	1.16 (0.73 to 1.85)	.39
Distal colon cancer				
No. of case patients	55	63	22	
Crude OR (95% CI)	1.00	0.79 (0.42 to 1.48)	0.55(0.28 to 1.08)	.08
Multivariable† OR (95% CI)	1.00	0.86 (0.45 to 1.62)	0.56 (0.28 to 1.11)	.11
Rectal cancer				
No. of case patients	45	46	21	
Crude OR (95% CI)	1.00	0.68 (0.32 to 1.45)	0.94 (0.51 to 1.72)	.08
Multivariable† OR (95% CI)	1.00	0.82 (0.38 to 1.77)	1.10 (0.58 to 2.08)	.63

* Conditional logistic regression was utilized for colorectal cancer, and unconditional logistic regression was used for the subsites of large bowel. Tests for trend were performed by assigning the median (log_e-transformed plasma levels) of each category of unmetabolized folic acid as a continuous variable in the models. All statistical tests were two-sided, and a P value of less than .05 was considered statistically significant. CI = confidence interval; OR = odds ratio.
† Multivariable models are adjusted for age at blood draw (continuous), date of blood draw (continuous), sex (male, female), race (white vs nonwhite), height (continuous), fasting status (yes, no), pack-years of smoking (continuous), body mass index (continuous), physical activity (in quartiles), family history of colorectal cancer (yes or no), history of colonoscopy or sigmoidoscopy (yes or no), alcohol intake (continuous), intake of red and processed meat (in quartiles), vitamin D intake (continuous), calcium intake (continuous), and aspirin use (nonusers vs ever users).

causal factor is the inability to reduce folic acid (eg, low DHFR activity) rather than UFA itself.

Our study has strengths. First, being prospective, we reduced the possibility of reverse causation (ie, CRC affects UFA levels). Second, we adjusted for a wide range of risk factors for CRC. Our study also had limitations. First, because blood samples were collected prior to the Food and Drug Administration's folate fortification, the levels may not reflect those after the fortification, which occurred during follow-up. This may have led to nondifferential misclassification of long-term UFA levels. For example, a proportion of those categorized as having undetectable UFA levels may have had detectable levels following fortification, possibly contributing to the overall null associations. However, the influence of measurement error would depend ultimately on the precise time period where exposure is relevant for risk in colorectal carcinogenesis. The Spearman correlations of total folate intake before and after folic acid fortification (1990 and 2002) were 0.31 in NHS and 0.38 in HPFS. Of note, although the study was conducted prior to fortification, the amount of folic acid in multiple vitamin supplements is substantially higher than typically consumed from fortification. Among control of our study, the percentage of participants using multivitamins on a regular basis was 36% among those with undetectable UFA, 72% for UFA of less than 0.5 nmol/L, and 79% for UFA of 0.5 nmol/L or more. Second, our UFA levels had moderate reproducibility over time, which might have attenuated the overall association.

In conclusion, our study suggests that prediagnostic plasma levels of UFA from the prefortification period in the United States are not associated with CRC risk. There was a suggestion of elevated risk in men and those with *MTHFR* CT/TT genotype, which needs to be explored further in other studies. Because folate intake and blood levels have increased substantially over the past decades due to widespread use of folic acid–containing supplements and folic acid fortification in the United States (21) and other countries have also implemented or are considering implementing nationwide folic acid fortification (22), the health impact of UFA becomes more important. Similar studies need to be conducted in postfortification era, in other populations, and with other cancer sites.

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