

Helicobacter pylori Prevalence and Circulating Micronutrient Levels in a Low-Income United States Population

Meira Epplein¹, Lisa B. Signorello^{1,4}, Wei Zheng¹, Qiuyin Cai¹, Margaret K. Hargreaves³, Angelika Michel⁵, Michael Pawlita⁵, Jay H. Fowke¹, Pelayo Correa², and William J. Blot^{1,4}

Abstract

High prevalence of *Helicobacter pylori* (*H. pylori*), the leading cause of gastric cancer, and low levels of micronutrients have been observed in many developing countries, and the question remains as to the whether an association between the 2 exists. The present study seeks to further our understanding of this potential connection in the Southern Community Cohort Study, representing a low-income population in the United States. Blood levels of antibodies to *H. pylori* proteins were assessed by multiplex serology for a sample of 310 African American and white participants, ages 40 to 79 years. Blood collected at baseline was also assayed for levels of carotenoids, tocopherols, retinol, and folate. Multivariate linear regression was used to calculate least-squares mean micronutrient levels within groups defined by *H. pylori* status. The mean serum levels of all micronutrients assayed were lower among *H. pylori*+ individuals than *H. pylori*- individuals, significantly for β -carotene, folate, and retinol (decreases of 27.6%, 18.6%, and 9.7%, respectively). Individuals who were seropositive to the virulent CagA+ *H. pylori* strains had even lower mean levels of micronutrients, particularly β -carotene, folate, total carotenoids, and retinol (decreases of 38.9%, 19.1%, 17.0%, and 11.7%, respectively, compared with *H. pylori*- individuals). However, dietary micronutrient levels as derived from a food frequency questionnaire did not vary between groups defined by *H. pylori* status. These results provide support for the hypothesis that *H. pylori* infection impairs nutrient absorption and suggest a need for future studies to explore the role of *H. pylori* infection on nutrition and gastric cancer risk in this high-risk population. *Cancer Prev Res*; 4(6); 871–8. ©2011 AACR.

Introduction

Infection with *Helicobacter pylori* (*H. pylori*), a gram-negative spiral bacterium that induces chronic gastritis in half of the world's population, is the leading cause of gastric cancer (1), the second most deadly cancer worldwide (2). Within both high- and low-risk countries, gastric cancer incidence, like *H. pylori* prevalence, is also strongly associated with low socioeconomic status (3). Recently, a study of trends in incidence of noncardia gastric cancer (the type most significantly associated with *H. pylori*) found that incidence was generally decreasing in the United States as expected, although age-specific analyses suggest that the

decline may be beginning to end (4). In the United States, where gastric cancer is uncommon, there is still a large racial disparity, as African Americans have almost twice the likelihood of being diagnosed with gastric cancer as whites (5). Similarly, the overall *H. pylori* prevalence in the United States is estimated to be relatively low at around 30% (although 50%–60% for African Americans; ref. 6), but a surprising 80% of a biracial sample of Southern Community Cohort Study (SCCS) participants, a primarily low-income study population from the southeastern United States, were recently found to be seropositive for *H. pylori* (7).

Because of the generally consistent findings from observational studies that implicate diet in the etiology of gastric cancer, in 2007, an expert panel of the World Cancer Research Fund judged that there is probably an evidence that fruit intake protects against gastric cancer (8). However, although a number of randomized trials of vitamin supplementation (including nutrients such as β -carotene, ascorbic acid, folic acid, α -tocopherol, selenium, and zinc) have been conducted, only 2 of 7 studies have reported benefits in the prevention of gastric premalignancy and 2 of 9 studies have found significant protective effects for gastric cancer (9).

Another hypothesis for the association of nutrient levels and gastric cancer risk relates to the role of *H. pylori*. Instead of high nutrient levels protecting against *H. pylori* infection,

Authors' Affiliations: ¹Division of Epidemiology and ²Gastroenterology, Department of Medicine, Vanderbilt Epidemiology Center and Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine; ³Department of Internal Medicine, Meharry Medical College, Nashville, Tennessee; ⁴International Epidemiology Institute, Rockville, Maryland; ⁵Division of Genome Modifications and Carcinogenesis, Infection and Cancer Program, German Cancer Research Center (DFKZ), Heidelberg, Germany

Corresponding Author: Meira Epplein, Division of Epidemiology, Department of Medicine, Vanderbilt University, 2525 West End Avenue, Suite 600, Nashville, TN 37203. Phone: 615-936-2145; Fax: 615-936-8291. E-mail: meira.epplein@vanderbilt.edu

doi: 10.1158/1940-6207.CAPR-10-0398

©2011 American Association for Cancer Research.

it has been suggested that infection with *H. pylori* itself reduces the absorption of nutrients (10, 11). This hypothesis is based on the fact that chronic *H. pylori* infection is generally begun in childhood, and thus nutrient levels measured among adolescents and adults are more likely to reflect *H. pylori* status, rather than the other way around. Furthermore, while nutrients are not absorbed in the stomach, *H. pylori* infection affects acid secretion that is vital for nutrient absorption. Several studies have found that the concentration of certain micronutrients found in plasma, gastric juice, and gastric mucosa is lower in *H. pylori*-infected subjects (12).

In this study, we examined the association between *H. pylori* prevalence and circulating levels of micronutrients in the SCCS, representing a low-income U.S. population with high prevalence of *H. pylori* infection.

Materials and Methods

Study population

From 2002 to 2009, the SCCS recruited approximately 86,000 men and women, ages 40 to 79, from 12 southeastern states (13). Those who enrolled in person at community health centers (approximately 86%) completed a comprehensive computer-assisted in-person interview, providing information on demographics, anthropometrics, medical history, and cancer risk factors (questionnaire available at: <http://www.southerncommunitystudy.org>). A food frequency questionnaire (FFQ), validated in this population, was used to collect information on usual diet (14, 15). Those recruited by mail (approximately 14%) completed the same baseline survey, by paper. Race was self-reported with participants instructed to choose all applicable racial/ethnic categories. Venous blood samples (20 mL) were collected from participants at the time of their baseline interview at the community health center, then refrigerated and shipped overnight to Vanderbilt University, Nashville, TN, to be centrifuged the next day and stored at -80°C . By using a $2 \times 2 \times 3 \times 3$ factorial design with 22 individuals selected within each of the 36 strata defined by self-reported race (African American/white), sex, smoking status (current/former/never), and body mass index (BMI, 18–24.9/25–29.9/30–45 kg/m^2), 792 of the 12,162 participants who enrolled in the study from March 2002 to October 2004 and donated a blood sample at baseline were randomly selected. While this design meant that some populations from within the entire SCCS were oversampled (including whites, nonsmokers, and nonobese participants), it provided a balanced distribution across these factors in consideration of other blood biomarkers being measured in addition to *H. pylori*.

H. pylori multiplex serology

For each study subject, 50 μL of serum samples were aliquoted for *H. pylori* assays. *H. pylori* multiplex serology, as performed in this study, has been described recently (16). In summary, it is a new antibody detection technology on the basis of fluorescent polystyrene beads

(Luminex) and recombinant glutathione S-transferase fusion protein capture (17, 18). For all 15 antigens—the *H. pylori* proteins UreA, catalase, GroEL, NapA, CagA, CagM, Cag δ , HP0231, VacA, HpaA, Cad, HyaA, Omp, HcpC, and HP0305—previously determined antigen-specific cut-point values were applied by using a bridging panel of 78 previously characterized sera (which included 38 *H. pylori* negative sera and 40 *H. pylori* positive sera). All sera were analyzed once within a single assay day. Individuals with seropositivity to more than 3 proteins were considered to be *H. pylori* seropositive as this definition had shown good agreement with commercial serological assay classification.

Blood nutrient measurement

To study nutritional biomarkers, plasma or serum for exactly half (396) of the individuals selected by the sampling design mentioned before (11 from each of the 36 strata) was assayed for micronutrient levels. Details on the measurement of plasma α -carotene, β -carotene, β -cryptoxanthin, lutein plus zeaxanthin, lycopene, and α -tocopherol levels using assays based on high-performance liquid chromatography, and the analysis of serum folate levels using a microbiologic assay, have been recently published (15). Retinol was measured by the use of a second channel at a wavelength of 325 nm. Quality control procedures included routine analysis of plasma and serum control pools containing high and low concentrations of each analyte. The coefficients of variation were less than 10% for all analytes and control pools.

Statistical analysis

Of the 396 participants selected for the nutritional biomarker sample, 310 (78.3%) were included in this study. The available serum for *H. pylori* multiplex serology was depleted from other assays performed on this group for 77 (19.4%), samples for 3 (0.8%) individuals could not be assayed for *H. pylori* because of serum handling issues, 2 (0.6%) individuals tested seropositive for CagA but seronegative for *H. pylori* (as they were each seropositive to less than 4 *H. pylori* antigens total), and 4 (1.0%) individuals were missing data on the potential confounders of total daily energy intake and/or vitamin supplement use.

To assess differences in demographic and lifestyle characteristics between *H. pylori*+ and *H. pylori*– individuals, crude linear regression was used for the continuous variables of age and daily energy intake and the Mantel-Haenszel chi-square test was used for the remaining categorical variables. To determine the associations between the prevalence of *H. pylori* infection and blood nutrient levels, multivariate linear regression was used to calculate least-squares mean micronutrient levels within groups defined by *H. pylori* status in 2 ways: as a dichotomous variable (*H. pylori*– versus *H. pylori*+) and as dummy variables, comparing *H. pylori* seronegative individuals to those seropositive to *H. pylori* but not to CagA (*H. pylori*+, CagA–), and those seropositive to both *H. pylori* and CagA (*H. pylori*+, CagA+), and comparing mean micronutrient

levels between CagA seropositive and CagA seronegative individuals among those who are *H. pylori* seropositive. Multivariate linear regression models were also carried out to explore the association of micronutrient levels with seropositivity to each of the 15 individual *H. pylori* antigens assessed with multiplex serology. For the outcome of circulating levels of each micronutrient, blood nutrient levels were log-transformed as they were not normally distributed. A category of total carotenoids was created by summing the log-transformed values for α -carotene, β -carotene, lycopene, lutein and zeaxanthin, and β -cryptoxanthin. Statistical adjustment was made for age at enrollment (continuous), race (African American/white), sex, BMI (continuous), education (less than high school education/high school or GED/greater than high school education), smoking status (never/former/current of less than or 10 cigarettes per day/current of more than 10 cigarettes per day), regular (i.e., \geq once a month) use of vitamin supplements (primarily multivitamins) in the past year (yes/no), and total energy intake (continuous). Additional adjustment for income, fruit and vegetable intake, dietary fat intake, alcoholic drink consumption, health insurance status, family history of gastric cancer, and state where recruited was considered, but these variables were not associated with both the exposure and outcome in the data, and thus were not included in the final models. To evaluate potential effect modification, the association of *H. pylori* infection and serum nutrient levels was explored in separate models stratified by race, sex, and smoking status, and a likelihood ratio test was used to compare models with and without the respective interaction terms.

To examine the association of *H. pylori* status and dietary intake of micronutrients, secondary analyses were done by micronutrients levels of intake as derived from the FFQ, also log-transformed due to a skewed distribution. Multivariate linear regression was again used to calculate least-squares mean dietary micronutrient levels within groups defined by *H. pylori* status, as described before.

In the results presented in this article, the mean levels of micronutrients have been backtransformed so to be scientifically meaningful. The percentage difference in levels as shown in the tables, however, reflects the difference between the log-transformed values, and thus is slightly different from what one would calculate by using the backtransformed values presented.

All statistical analyses were done by SAS 9.2 (SAS Institute).

Results

Of the 310 individuals included in this study, 244 (79%) were seropositive for *H. pylori*. *H. pylori* seropositive individuals were more likely to be African American, have less than a high school education, and a first-degree family history of stomach cancer than *H. pylori* seronegative individuals (Table 1).

For the combined category of total carotenoids and all individual micronutrients, the mean circulating level as

derived from serum assays was higher among *H. pylori*- individuals than *H. pylori*+ individuals. The differences in mean levels for *H. pylori*+ individuals, compared with *H. pylori*- individuals, were statistically significant for β -carotene, folate, and retinol (decreases of 27.6%, 18.6%, and 9.7%, respectively; Table 2).

When examining the associations between circulating nutrient levels and *H. pylori* prevalence by CagA status with 1 exception (lutein and zeaxanthin), *H. pylori*- individuals had the highest mean levels of all micronutrients with lower levels for *H. pylori*+, CagA- individuals, and the lowest levels among *H. pylori*+, CagA+ individuals. By comparing *H. pylori*+, CagA- individuals to *H. pylori*- individuals, statistical significance for the difference in mean levels was achieved only for folate (an 18.1% difference). For *H. pylori*+, CagA+ individuals, however, significant differences in mean levels compared with *H. pylori*- individuals were found with β -carotene, folate, total carotenoids, and retinol (decreases of 38.9%, 19.1%, 17.0%, and 11.7%, respectively). Furthermore, *H. pylori*+, CagA+ individuals had significantly lower levels of β -carotene, lycopene, and total carotenoids than *H. pylori*+, CagA- individuals (decreases of 26.4%, 15.6%, and 13.4%, respectively; Table 3). By including *H. pylori* seroprevalence by CagA status, the r^2 value of the model for our strongest association, with β -carotene, increased from 0.23 to 0.27 for the model without any information on *H. pylori*, a small increase despite the strong and significant effect of *H. pylori* status found in the model.

Of the other *H. pylori* antigens explored, only seropositivity to VacA also seemed to be associated with micronutrient levels. However, VacA is strongly correlated with CagA (Pearson correlation coefficient = 0.38, $P < 0.0001$), and after adjusting for CagA seropositivity, the associations with VacA seropositivity were no longer present.

When stratifying by smoking status (current versus never/former), *H. pylori*+ smokers had lower levels of almost all carotenoids than *H. pylori*+ nonsmokers, and there was a consistent trend of a greater percent decrease in blood carotenoid levels among the *H. pylori*+, CagA+ current smokers than among the *H. pylori*+, CagA+ non-current smokers, when comparing to *H. pylori*- individuals within the same smoking category, but the differences were not statistically significant (see Appendix Table 1). The associations between *H. pylori* status and circulating nutrient levels did not vary when examining the relationships separately by sex or race (data not shown).

To further explore the association between *H. pylori* status and micronutrient levels, we ran the same models using as the outcome micronutrient intake as determined by the FFQ, rather than the blood levels as used previously. While *H. pylori*+ individuals tended to have lower mean intakes than *H. pylori*- individuals, the differences were neither large nor significant, and there was no trend of decreasing levels as before when moving from the categories of *H. pylori*- to *H. pylori*+, CagA- to *H. pylori*+, CagA+ (Table 4).

Table 1. Demographic and lifestyle characteristics of the study population, a sample of 310 participants from the SCCS

	<i>H. pylori</i> ⁻ individuals (n = 66)	<i>H. pylori</i> ⁺ individuals (n = 244)
Age, y; mean (SD)	52.8 (9.3)	52.9 (9.8)
Sex, n (%)		
Women	41 (62.1)	131 (53.7)
Men	25 (37.9)	113 (46.3)
Race, n (%) ^a		
African American	20 (30.3)	134 (54.9)
White	46 (69.7)	110 (45.1)
Cigarette smoking, n (%)		
Never	16 (24.2)	87 (35.7)
Former	28 (42.4)	81 (33.2)
Current, ≤10 cigarettes per day	9 (13.6)	34 (13.9)
Current, >10 cigarettes per day	13 (19.7)	42 (17.2)
BMI, kg/m ² , n (%)		
18.0–24.9	27 (40.9)	72 (29.5)
25.0–29.9	17 (25.8)	90 (36.9)
30.0–45.0	22 (33.3)	82 (33.6)
Education, n (%) ^a		
Less than high school	7 (10.6)	92 (37.7)
High school or GED	33 (50.0)	92 (37.7)
More than high school	26 (39.4)	60 (24.6)
Household income (\$), n (%)		
<15,000	34 (51.5)	147 (60.7)
≥15,000 to <25,000	16 (24.2)	53 (21.9)
≥25,000	16 (24.2)	42 (17.4)
Regular user of vitamin supplements, n (%)		
Yes	34 (51.5)	99 (40.6)
No	32 (48.5)	145 (59.4)
Average daily serving of fruits and vegetables, n (%)		
0–1	8 (12.1)	49 (20.1)
2–3	34 (51.5)	105 (43.0)
4+	24 (36.4)	90 (36.9)
Average weekly consumption of alcoholic drinks, n (%)		
0	35 (53.0)	127 (52.5)
1–3	17 (25.8)	52 (21.5)
4+	14 (21.2)	63 (26.0)
Have health insurance, n (%)		
Yes	35 (53.0)	149 (61.1)
No	31 (47.0)	95 (38.9)
1° family history of stomach cancer, n (%) ^a		
Yes	0 (0.0)	14 (5.7)
No/do not know/refused	66 (100.0)	230 (94.3)
Dietary fat intake, mean (SD)	92.0 (58.1)	96.8 (58.4)
Total energy intake, kcal/day; mean (SD)	2,407 (1,354)	2,545 (1,439)

^aSignificant difference between *H. pylori*⁻ and *H. pylori*⁺ positive individuals, *P* < 0.05.

Discussion

In this low-income highly *H. pylori*-prevalent population in the southeastern United States, *H. pylori* prevalence,

particularly CagA+ *H. pylori* strains, was associated with lower blood levels of β-carotene, folate, and retinol.

The few previous studies of the association between *H. pylori* infection and plasma carotenoid and folate levels

Table 2. Association of *H. pylori* seroprevalence with blood micronutrient levels

	<i>H. pylori</i> ⁻ (n = 66) mean level (SE) ^a	<i>H. pylori</i> ⁺ (n = 244) mean level (SE) ^a	Difference between <i>H. pylori</i> ⁻ and <i>H. pylori</i> ⁺	
			<i>P</i> value for absolute difference	Percent decrease
Total carotenoids, μg/dL	70.96 (1.06)	63.77 (1.03)	0.12	10.1%
α-carotene, μg/dL	2.18 (1.11)	1.92 (1.05)	0.29	12.1%
β-carotene, μg/dL	11.73 (1.12)	8.49 (1.05)	0.01	27.6%
Lycopene, μg/dL	29.24 (1.08)	26.49 (1.04)	0.28	9.4%
Lutein and zeaxanthin, μg/dL	15.34 (1.06)	15.16 (1.03)	0.87	1.2%
β-cryptoxanthin, μg/dL	6.67 (1.09)	5.90 (1.04)	0.22	11.5%
Retinol, μg/dL	42.88 (1.04)	38.84 (1.02)	0.02	9.7%
α-tocopherol, mg/dL	1.16 (1.05)	1.12 (1.03)	0.50	4.0%
Folate, ng/mL	14.33 (1.08)	11.67 (1.04)	0.02	18.6%

^aAdjusted for age, race, sex, BMI, education, smoking, use of vitamin supplements, and total energy intake.

have generally found null results (19–22). Compared with the present study, these small (ranging from 44 to 86 individuals) clinic-based studies had less power and could not investigate specific *H. pylori* proteins. Inverse associations have, however, been observed between *H. pylori* infection and levels of β-carotene in gastric juice (20).

The strong associations between *H. pylori* status and blood nutrient levels observed in this study are made more interesting by the fact that the dietary intake of these micronutrients as determined by an FFQ was not associated with *H. pylori* status, lending support for the theory that *H. pylori* infection impairs the absorption of nutrients. Our data suggest that *H. pylori* seronegative and seropositive individuals have similar diets with regard to nutritional content, but their circulating levels of certain micronutrients are significantly different, most strongly for β-carotene, whereby *H. pylori*⁺, CagA⁺ individuals have a mean circulating level of only 7.35 μg/dL, compared with 12.03 μg/dL for *H. pylori*⁻ individuals (*P* = 0.0004), and 9.99 μg/dL for *H. pylori*⁺, CagA⁻ individuals (*P* = 0.007). In contrast, the mean dietary intake of β-carotene among *H. pylori*⁺, CagA⁺ individuals of 3,181.4 mcg/day is only slightly less than the mean of 3,370.3 mcg/day for *H. pylori*⁻ individuals (*P* = 0.59), and, in fact, is greater than the mean of 3,125.5 among *H. pylori*⁺, CagA⁻ individuals (*P* = 0.84). And although FFQ-estimated intakes have been significantly correlated with blood levels of these nutrients in this population, the correlation coefficients for β-carotene and folate were still only at 0.28 and 0.26, respectively, indicating that there remains much room for divergence (Signorello, 2010 #375).

A number of hypotheses have been proposed as to the mechanism by which *H. pylori* infection reduces nutrient absorption, primarily thorough the bacteria's ability to modify the secretion of hydrochloric acid and the increase in pH (10, 12). Of course, it is also possible that high levels of such nutrients impair the viability of *H. pylori* (23). A recent study in Korean isolates found that β-carotene inhibited the expression of inducible nitric oxide synthase

and cyclooxygenase-2 in *H. pylori*-infected gastric epithelial cells (24). Furthermore, it has been suggested by *in vivo* and *in vitro* experiments that certain carotenoids exhibit antimicrobial activity against *H. pylori* (23). Previously observed associations between low levels of plasma carotenoids and gastric cancer risk (25–27) in conjunction with our findings of associations between *H. pylori* seroprevalence and low levels of circulating micronutrients could reflect both *H. pylori*'s direct association with the disease, by inducing chronic gastritis and its indirect association with lower nutrient levels, through *H. pylori* infection reducing the absorption of antioxidants.

In the present study, the median level of plasma β-carotene among SCCS participants was 9.0 μg/dL (and a significantly lower 7.4 μg/dL among current smokers), similar to that reported in a Shanghai population (9.2 μg/dL; ref. 27), where serum micronutrients were inversely related to gastric cancer risk, and somewhat higher than that seen in Linxian (6.4 μg/dL; ref. 28), where intervention trials showed reduced gastric cancer incidence following nutrient supplementation with a combination of β-carotene, selenium, and vitamin E. SCCS β-carotene levels were significantly lower than those found at baseline in the European Prospective Investigation into Cancer and Nutrition (median, 19.0 μg/dL; ref. 26), and in a Colombian vitamin supplementation trial (baseline presupplementation median, 23.9 μg/dL; ref. 29). Our findings suggest that beyond supplement intervention trials, there are opportunities for both improving nutritional status and reducing gastric cancer incidence in high-risk populations by directly dealing with the potential underlying cause—that of *H. pylori* infection. Methods to eliminate endemic *H. pylori* infection could include vaccine development and eradication schemes that are aided by vitamin supplementation.

The low-income population represented by the SCCS thus seems to have low circulating levels of antioxidants, particularly β-carotene, and a high prevalence of *H. pylori* infection, especially the virulent CagA⁺ *H. pylori* strains,

Table 3. Association of *H. pylori* seroprevalence by CagA status with blood micronutrient levels

	<i>H. pylori</i> ⁻ (n = 66) mean level (SE) ^a	<i>H. pylori</i> ⁺ , CagA ⁻ (n = 109) mean level (SE) ^a	<i>H. pylori</i> ⁺ , CagA ⁺ (n = 135) mean level (SE) ^a	Difference between <i>H. pylori</i> ⁻ and <i>H. pylori</i> ⁺ , CagA ⁻		Difference between <i>H. pylori</i> ⁻ and <i>H. pylori</i> ⁺ , CagA ⁺		Difference between <i>H. pylori</i> ⁺ , CagA ⁻ and <i>H. pylori</i> ⁺ , CagA ⁺	
				P value ^b	Percent decrease	P value ^b	Percent decrease	P value ^b	Percent decrease
Total carotenoids, µg/dL	71.82 (1.06)	68.83 (1.04)	59.61 (1.04)	0.56	4.2%	0.01	17.0%	0.02	13.4%
α-carotene, µg/dL	2.21 (1.11)	2.07 (1.08)	1.80 (1.08)	0.62	6.3%	0.13	18.5%	0.21	13.0%
β-carotene, µg/dL	12.03 (1.12)	9.99 (1.08)	7.35 (1.08)	0.16	16.9%	0.0004	38.9%	0.007	26.4%
Lycopene, µg/dL	29.67 (1.08)	29.01 (1.06)	24.50 (1.06)	0.82	2.3%	0.06	17.5%	0.04	15.6%
Lutein and zeaxanthin, µg/dL	15.40 (1.06)	15.56 (1.05)	14.82 (1.04)	0.89	-1.0%	0.62	3.7%	0.44	4.7%
β-cryptoxanthin, µg/dL	6.70 (1.09)	6.05 (1.07)	5.78 (1.06)	0.34	9.7%	0.18	13.8%	0.60	4.6%
Retinol, µg/dL	43.15 (1.04)	39.65 (1.03)	38.11 (1.03)	0.88	8.1%	0.01	11.7%	0.33	3.9%
α-tocopherol, mg/dL	1.17 (1.06)	1.17 (1.04)	1.07 (1.04)	0.99	-0.1%	0.18	8.7%	0.10	8.8%
Folate, ng/mL	14.34 (1.08)	11.74 (1.06)	11.60 (1.06)	0.04	18.1%	0.03	19.1%	0.89	1.2%

^aAdjusted for age, race, sex, BMI, education, smoking, use of vitamin supplements, and total energy intake.

^bFor absolute difference.

Table 4. Association of *H. pylori* seroprevalence by CagA status with dietary micronutrient levels, as determined by an FFQ

	<i>H. pylori</i> ⁻ (n = 66) mean intake (SE) ^a	<i>H. pylori</i> ⁺ , CagA ⁻ (n = 109) mean intake (SE) ^a	<i>H. pylori</i> ⁺ , CagA ⁺ (n = 135) mean intake (SE) ^a	Difference between <i>H. pylori</i> ⁻ and <i>H. pylori</i> ⁺ , CagA ⁻		Difference between <i>H. pylori</i> ⁻ and <i>H. pylori</i> ⁺ , CagA ⁺		Difference between <i>H. pylori</i> ⁺ , CagA ⁻ and <i>H. pylori</i> ⁺ , CagA ⁺	
				P value ^b	Percent decrease	P value ^b	Percent decrease	P value ^b	Percent decrease
Total carotenoids, mcg/d	12,014.0 (1.07)	11,275.5 (1.05)	11,414.6 (1.05)	0.45	6.1%	0.56	5.0%	0.87	-1.2%
α-carotene, mcg/d	342.0 (1.12)	362.7 (1.09)	332.6 (1.08)	0.67	-6.0%	0.85	2.8%	0.47	8.3%
β-carotene, mcg/d	3,370.3 (1.09)	3,125.5 (1.07)	3,181.4 (1.06)	0.47	7.3%	0.59	5.6%	0.84	-1.8%
Lycopene, mcg/d	4,526.1 (1.08)	4,324.5 (1.06)	4,025.6 (1.06)	0.63	4.5%	0.23	11.1%	0.38	6.9%
Lutein and zeaxanthin, mcg/d	2,486.1 (1.10)	2,460.1 (1.07)	2,559.9 (1.07)	0.93	1.0%	0.80	-3.0%	0.68	-4.1%
β-cryptoxanthin, mcg/d	161.2 (1.12)	130.2 (1.09)	148.1 (1.09)	0.14	19.3%	0.57	8.1%	0.30	-13.8%
Retinol, mcg/d	454.1 (1.06)	438.0 (1.04)	468.7 (1.04)	0.59	3.5%	0.65	-3.2%	0.24	-7.0%
α-tocopherol, mg/d	7.8 (1.04)	7.4 (1.03)	7.5 (1.03)	0.31	4.9%	0.52	3.3%	0.69	-1.7%
Folate, mcg/d	598.0 (1.04)	558.9 (1.03)	580.8 (1.03)	0.21	6.5%	0.60	2.9%	0.40	-3.9%

^aAdjusted for age, race, sex, BMI, education, smoking, use of vitamin supplements, and total energy intake.

^bFor absolute difference.

Appendix Table 1: Association of *H. pylori* seroprevalence by CagA status with blood carotenoid levels, by smoking status

	Mean level (SE) ^a		Difference between <i>H. pylori</i> – and <i>H. pylori</i> + CagA–		Difference between <i>H. pylori</i> – and <i>H. pylori</i> + CagA+		Difference between <i>H. pylori</i> + CagA– and <i>H. pylori</i> + CagA+	
	<i>H. pylori</i> –	<i>H. pylori</i> + CagA–	<i>H. pylori</i> –	<i>H. pylori</i> + CagA–	<i>H. pylori</i> –	<i>H. pylori</i> + CagA+	<i>H. pylori</i> –	<i>H. pylori</i> + CagA+
			P value ^b	Percent decrease	P value ^b	Percent decrease	P value ^b	Percent decrease
Total carotenoids, µg/dL								
Current smokers	71.21 (1.11)	58.10 (1.09)	0.09	19.7%	0.01	28.6%	0.29	11.2%
Never and former smokers	72.25 (1.08)	74.35 (1.05)	0.75	-2.9%	0.22	10.9%	0.05	13.4%
α-Carotene, µg/dL								
Current smokers	2.07 (1.17)	1.48 (1.13)	0.10	28.6%	0.03	36.1%	0.51	10.8%
Never and former smokers	2.30 (1.16)	2.39 (1.11)	0.82	-4.0%	0.56	10.0%	0.32	13.5%
β-Carotene, µg/dL								
Current smokers	10.63 (1.19)	6.41 (1.15)	0.02	39.7%	0.003	48.3%	0.41	14.3%
Never and former smokers	12.45 (1.15)	12.03 (1.10)	0.83	3.4%	0.04	30.9%	0.02	28.5%
Lycopene, µg/dL								
Current smokers	33.4 (1.13)	28.2 (1.10)	0.28	15.7%	0.02	32.3%	0.11	19.7%
Never and former smokers	27.9 (1.10)	29.2 (1.08)	0.71	-4.8%	0.50	8.5%	0.20	12.7%
Lutein and zeaxanthin, µg/dL								
Current smokers	14.9 (1.11)	13.4 (1.09)	0.43	10.2%	0.38	11.5%	0.91	1.4%
Never and former smokers	16.0 (1.08)	16.6 (1.05)	0.70	-3.5%	0.77	2.7%	0.41	6.1%
β-Cryptoxanthin, µg/dL								
Current smokers	6.49 (1.17)	4.71 (1.13)	0.11	27.3%	0.08	30.0%	0.83	3.7%
Never and former smokers	6.76 (1.11)	6.80 (1.08)	0.96	-0.6%	0.73	4.6%	0.62	5.1%

^aAdjusted for age, race, sex, BMI, education, smoking, use of vitamin supplements, and total energy intake; for current smokers, also adjusted for average cigarettes smoked per day.

^bFor absolute difference.

and our data suggest that this association may be due to the ability of *H. pylori* to impair the absorption of nutrients. The presence of this combination of risk factors for gastric cancer also suggests that this population is a high-risk group, with a need for future studies to further explore the role of *H. pylori* infection on nutrition and gastric cancer risk as the cohort is followed in the coming years.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

- Suerbaum S, Michetti P. *Helicobacter pylori* infection. *N Engl J Med* 2002;347:1175–86.
- Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2002: cancer incidence, mortality and prevalence worldwide. IARC CancerBase No. 5, Version 2.0. Lyon: IARC Press; 2004.
- Azevedo NF, Huntington J, Goodman KJ. The epidemiology of *Helicobacter pylori* and public health implications. *Helicobacter* 2009;14 Suppl1:1–7.
- Anderson WF, Camargo MC, Fraumeni JF Jr, Correa P, Rosenberg PS, Rabkin CS. Age-specific trends in incidence of noncardia gastric cancer in US adults. *JAMA* 2010;303:1723–8.
- Horner MJ, Ries LAG, Krapcho M, Neyman N, Aminou R, Howlander N, et al., eds. SEER Cancer Statistics Review, 1975–2006. Bethesda, MD: National Cancer Institute, 2009.
- Everhart JE, Kruszon-Moran D, Perez-Perez GI, Traika TS, McQuillan G. Seroprevalence and ethnic differences in *Helicobacter pylori* infection among adults in the United States. *J Infect Dis* 2000;181:1359–63.
- Epplein M, Signorello LB, Zheng W, Peek RM Jr, Michel A, Williams SM, et al. Race, African ancestry, and *Helicobacter pylori* infection in a low-income United States population. *Cancer Epidemiol Biomarkers Prev* 2011 Feb 25. [Epub ahead of print].
- World Cancer Research Fund/American Institute for Cancer Research. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. Washington, DC: AICR; 2007.
- Plummer M, Vivas J, Lopez G, Bravo JC, Peraza S, Carillo E, et al. Chemoprevention of precancerous gastric lesions with antioxidant vitamin supplementation: a randomized trial in a high-risk population. *J Natl Cancer Inst* 2007;99:137–46.
- Salgueiro J, Zubillaga M, Goldman C, Barrado A, Martinez Sarraasague M, Leonardi N, et al. Review article: is there a link between micronutrient malnutrition and *Helicobacter pylori* infection? *Aliment Pharmacol Ther* 2004;20:1029–34.
- Akcam M. *Helicobacter pylori* and micronutrients. *Indian Pediatr* 2010;47:119–26.
- Annibale B, Capurso G, Fave GD. Consequences of *Helicobacter pylori* infection on the absorption of micronutrients. *Dig Liver Dis* 2002;34 Suppl 2:S72–7.
- Signorello LB, Hargreaves MK, Steinwandel MD, Zheng W, Cai Q, Schlundt DG, et al. Southern Community Cohort Study: establishing a cohort to investigate health disparities. *J Natl Med Assoc* 2005;97:972–9.
- Buchowski MS, Schlundt DG, Hargreaves MK, Hankin JH, Signorello LB, Blot WJ. Development of a culturally sensitive food frequency questionnaire for use in the Southern Community Cohort Study. *Cell Mol Biol (Noisy-le-grand)* 2003;49:1295–304.
- Signorello LB, Buchowski MS, Cai Q, Munro HM, Hargreaves MK, Blot WJ. Biochemical validation of food frequency questionnaire-estimated carotenoid, alpha-tocopherol, and folate intakes among African Americans and non-Hispanic Whites in the Southern Community Cohort Study. *Am J Epidemiol* 2010;171:488–97.
- Michel A, Waterboer T, Kist M, Pawlita M. *Helicobacter pylori* multiplex serology. *Helicobacter* 2009;14:525–35.
- Waterboer T, Sehr P, Michael KM, Franceschi S, Nieland JD, Joos TO, et al. Multiplex human papillomavirus serology based on in situ-purified glutathione s-transferase fusion proteins. *Clin Chem* 2005;51:1845–53.
- Waterboer T, Sehr P, Pawlita M. Suppression of non-specific binding in serological Luminex assays. *J Immunol Methods* 2006;309:200–4.
- Sanderson MJ, White KL, Drake IM, Schorah CJ. Vitamin E and carotenoids in gastric biopsies: the relation to plasma concentrations in patients with and without *Helicobacter pylori* gastritis. *Am J Clin Nutr* 1997;65:101–6.
- Zhang ZW, Patchett SE, Perrett D, Domizio P, Farthing MJ. Gastric alpha-tocopherol and beta-carotene concentrations in association with *Helicobacter pylori* infection. *Eur J Gastroenterol Hepatol* 2000;12:497–503.
- Akcam M, Ozdem S, Yilmaz A, Gultekin M, Artan R. Serum ferritin, vitamin B(12), folate, and zinc levels in children infected with *Helicobacter pylori*. *Dig Dis Sci* 2007;52:405–10.
- Ozturk Y, Buyukgebiz B, Arslan N, Ozer E, Lebe B. Serum vitamin A and total carotene concentrations in well-nourished children with *Helicobacter pylori* infection. *J Pediatr Gastroenterol Nutr* 2003;36:502–4.
- Akyon Y. Effect of antioxidants on the immune response of *Helicobacter pylori*. *Clin Microbiol Infect* 2002;8:438–41.
- Jang SH, Lim JW, Kim H. Beta-carotene inhibits *Helicobacter pylori*-induced expression of inducible nitric oxide synthase and cyclooxygenase-2 in human gastric epithelial AGS cells. *J Physiol Pharmacol* 2009;60 Suppl 7:131–7.
- Persson C, Sasazuki S, Inoue M, Kurahashi N, Iwasaki M, Miura T, et al. Plasma levels of carotenoids, retinol and tocopherol and the risk of gastric cancer in Japan: a nested case-control study. *Carcinogenesis* 2008;29:1042–8.
- Jenab M, Riboli E, Ferrari P, Friesen M, Sabate J, Norat T, et al. Plasma and dietary carotenoid, retinol and tocopherol levels and the risk of gastric adenocarcinomas in the European prospective investigation into cancer and nutrition. *Br J Cancer* 2006;95:406–15.
- Yuan JM, Ross RK, Gao YT, Qu YH, Chu XD, Yu MC. Prediagnostic levels of serum micronutrients in relation to risk of gastric cancer in Shanghai, China. *Cancer Epidemiol Biomarkers Prev* 2004;13 Pt 1:1772–80.
- Blot WJ, Li JY, Taylor PR, Guo W, Dawsey S, Wang GQ, et al. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *J Natl Cancer Inst* 1993;85:1483–92.
- Correa P, Fontham ET, Bravo JC, Bravo LE, Ruiz B, Zarama G, et al. Chemoprevention of gastric dysplasia: randomized trial of antioxidant supplements and anti-*Helicobacter pylori* therapy. *J Natl Cancer Inst* 2000;92:1881–8.

Grant Support

This research was supported by the National Cancer Institute (Grants R01 CA092447 and P30 CA68485) and American Cancer Society—Institutional Research Grant to Vanderbilt University (Grant ACS-IRG-58-009-50).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 23, 2010; revised February 18, 2011; accepted March 7, 2011; published OnlineFirst March 24, 2011.