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Helicobacter pylori prevalence and circulating micronutrient levels in a low-income United States population

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Abstract

High prevalence of *Helicobacter 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 two 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 *Helicobacter pylori* proteins were assessed using multiplex serology for a sample of 310 African American and white participants, aged 40–79 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 *Helicobacter pylori* status. The mean serum levels of all micronutrients assayed were lower among *Helicobacter pylori*+ individuals than *Helicobacter pylori*- individuals, significantly for beta-carotene, folate, and retinol (decreases of 27.6%, 18.6%, and 9.7%, respectively). Individuals who were sero-positive to the virulent CagA+ *Helicobacter pylori* strains had even lower mean levels of micronutrients, particularly beta-carotene, folate, total carotenoids, and retinol (decreases of 38.9%, 19.1%, 17.0%, and 11.7%, respectively, compared to *Helicobacter pylori*- individuals). However, dietary micronutrient levels as derived from a food frequency questionnaire did not vary between groups defined by *Helicobacter pylori* status. These results provide support for the hypothesis that *Helicobacter pylori* infection impairs nutrient absorption, and suggest a need for future studies to explore the role of *Helicobacter pylori* infection on nutrition and gastric cancer risk in this high-risk population.

Keywords

antioxidant; micronutrient; biomarker; *Helicobacter pylori*; gastric cancer

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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 non-cardia 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 {Horner, 2009 #166}. 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) (5), but a surprising 80% of a bi-racial sample of Southern Community Cohort Study (SCCS) participants, a primarily low-income study population from the southeastern US, were recently found to be sero-positive for *H. pylori* (6).

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 probable evidence that fruit intake protects against gastric cancer (7). However, while a number of randomized trials of vitamin supplementation (including nutrients such as beta-carotene, ascorbic acid, folic acid, alpha-tocopherol, selenium, and zinc) have been conducted, only two of seven studies have reported benefits in the prevention of gastric premalignancy and two of nine studies have found significant protective effects for gastric cancer (8).

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, it has been suggested that infection with *H. pylori* itself reduces the absorption of nutrients (9, 10). 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. plyori* infection affects acid secretion, which is vital for nutrient absorption. Several studies have found that the concentration of certain micronutrients found in plasma, gastric juice, and gastric mucosa are lower in *H. pylori* infected subjects (11).

In the present study, we examined the association between *H. pylori* prevalence and circulating levels of micronutrients in the Southern Community Cohort Study, representing a low-income US population with high prevalence of *H. pylori* infection.

MATERIALS AND METHODS

Study Population

From 2002 to 2009, the Southern Community Cohort Study (SCCS) recruited approximately 86,000 men and women aged 40–79 from 12 southeastern states (12). Those who enrolled in person at community health centers (~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, validated in this population, was used to collect information on usual diet (13, 14). Those recruited by mail (~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 to be centrifuged the next day and stored at −80°C. Using a 2×2×3×3 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 (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 Southern Community Cohort Study were oversampled (including whites, non-smokers, and non-obese participants), it provided a balanced distribution across these factors in consideration of other blood biomarkers being measured in addition to *H. pylori*.

Helicobacter 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 (15). In summary, it is a new antibody detection technology based on fluorescent polystyrene beads (Luminex) and recombinant glutathione *S*-transferase (GST) fusion protein capture (16, 17). For all 15 antigens – the *H. pylori* proteins UreA, Catalase, GroEL, NapA, CagA, CagM, Cagδ, HP0231, VacA, HpaA, Cad, HyuA, Omp, HcpC, HP0305 – previously determined antigen-specific cut-point values were applied 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 sero-positivity to >3 proteins were considered to be *H. pylori* sero-positive 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 above (11 from each of the 36 strata) was assayed for micronutrient levels. Details on the measurement of plasma alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein plus zeaxanthin, lycopene, and alpha-tocopherol levels using assays based on high performance liquid chromatography, as well as the analysis of serum folate levels using a microbiologic assay, have been recently published (14). 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 the present 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 sero-positive for CagA but sero-negative for *H. pylori* (as they were each sero-positive to <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*-positive and *H. pylori*-negative 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 two ways: as a dichotomous variable (*H. pylori*- vs. *H. pylori*+); and as dummy variables, comparing *H. pylori* sero-negative individuals to those sero-positive to *H. pylori* but not to CagA (*H. pylori*+, CagA−), and those sero-positive to both *H. pylori* and CagA (*H. pylori*+, CagA+), as well as comparing mean micronutrient levels between CagA sero-positive and CagA sero-negative individuals among those who are *H. pylori* sero-positive. Multivariate linear regression models were also performed to explore the association of micronutrient levels with sero-positivity 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 alpha-carotene, beta-carotene, lycopene, lutein and zeaxanthin, and beta-cryptoxanthin. Statistical adjustment was made for age at enrollment (continuous), race (African American/white), sex, body mass index (continuous), education (<high school education/high school or GED/>high school education), smoking status (never/former/current of ≤10 cigarettes per day/current of >10 cigarettes per day), regular (i.e., ≥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 performed using micronutrients levels of intake as derived from the food frequency questionnaire, 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 above.

In the results presented in this paper, the mean levels of micronutrients have been back-transformed 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 using the back-transformed values presented.

All statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC).

RESULTS

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

For the combined category of total carotenoids and for 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 to *H. pylori*- individuals were statistically significant for beta-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 one 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. 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 to *H. pylori*- individuals were found with beta-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 beta-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* sero-prevalence by CagA status, the r^2 value of the model for our strongest association, with beta-carotene, increased to 0.27 from 0.23 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 sero-positivity to VacA also appeared 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 sero-positivity, the associations with VacA sero-positivity 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*+ non-smokers, 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). The associations between *H. pylori* status and circulating nutrient levels did not vary when examining the relationships separately by sex or race (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 food frequency questionnaire, 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 above when moving from the categories of *H. pylori*- to *H. pylori*+, CagA− to *H. pylori*+, CagA+ (Table 4).

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 beta-carotene, folate, and retinol.

The few previous studies of the association between *H. pylori* infection and plasma carotenoid and folate levels and have generally found null results (18–21). Compared to the present study, these small (ranging from 44 to 86 individuals), clinic-based studies had less power and were not able to investigate specific *H. pylori* proteins. Inverse associations have, however, been observed between *H. pylori* infection and levels of beta-carotene in gastric juice (19).

The strong associations between *H. pylori* status and blood nutrient levels observed in this study is made more interesting by the fact that the dietary intake of these micronutrients as determined by a food frequency questionnaire 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* sero-negative and sero-positive individuals have similar diets with regard to nutritional content, but their circulating levels of certain micronutrients are significantly different, most strongly for beta-carotene, whereby *H. pylori*+, CagA+ individuals have a mean circulating level of only 7.35 μg/dL, compared to 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 beta-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, while FFQ-estimated intakes have been significantly correlated with blood levels of these nutrients in this population, the correlation coefficients for beta-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 (9, 11). Of course, it is also possible that high levels of such nutrients impair the viability of *H. pylori* (22). A recent study in Korean isolates found that beta-carotene inhibited the expression of inducible nitric oxide synthase and cyclooxygenase-2 in *H. pylori*-infected gastric epithelial cells (23). Furthermore, it has been suggested by *in vivo* and *in vitro* experiments that certain carotenoids exhibit anti-microbial activity against *H. pylori* (22). Previously observed associations between low levels of plasma carotenoids and gastric cancer risk (24–26) in conjunction with our findings of associations between *H. pylori* sero-prevalence 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 beta-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) (26), where serum micronutrients were inversely related to gastric cancer risk, and somewhat higher than that seen in Linxian (6.4 μg/dL) (27), where intervention trials showed reduced gastric cancer incidence following nutrient supplementation with a combination of beta-carotene, selenium, and vitamin E. SCCS beta-carotene levels were significantly lower than those found at baseline in the European Prospective Investigation into Cancer and Nutrition (median, 19.0 μg/dL) (25), and in a Colombian vitamin supplementation trial (baseline pre-supplementation median, 23.9 μg/dL) (28). 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 as well as eradication schemes that are aided by vitamin supplementation.

The low-income population represented by the SCCS thus appears to have low circulating levels of antioxidants, particularly beta-carotene, and a high prevalence of *H. pylori* infection, especially the virulent CagA+ *H. pylori* strains, 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 *Helicobacter pylori* infection on nutrition and gastric cancer risk as the cohort is followed in the coming years.

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Table 1

Demographic and lifestyle characteristics of the study population, a sample of 310 participants from the Southern Community Cohort Study

	H. pylori- individuals (N=66)	*H. pylori*+ individuals (N=244)
Age (y), mean (SD)	52.8 (9.3)	52.9 (9.8)
Sex, n (%)		
Female	41 (62.1)	131 (53.7)
Male	25 (37.9)	113 (46.3)
Race, n (%)*		
African American	20 (30.3)	134 (54.9)
White	46 (69.7)	110 (45.1)
Cigarette smoking		
Never	16 (24.2)	87 (35.7)
Former	28 (42.4)	81 (33.2)
Current, ≤10 cigarettes/day	9 (13.6)	34 (13.9)
Current, >10 cigarettes/day	13 (19.7)	42 (17.2)
Body mass index (kg/m^2)		
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 (%)*		
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		
Yes	34 (51.5)	99 (40.6)
No	32 (48.5)	145 (59.4)
Average daily serving of fruits and vegetables		
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		
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		
Yes	35 (53.0)	149 (61.1)
No	31 (47.0)	95 (38.9)

	***H. pylori*- individuals (N=66)**	***H. pylori*+ individuals (N=244)**
1° family history of stomach cancer*		
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)

* significant difference between *H. pylori*− and *H. pylori*+ positive individuals, $p<0.05$

Table 2

Association of *H. pylori* sero-prevalence with blood micronutrient levels

	***H. pylori*− (N=66)**	***H. pylori*+ (N=244)**	**Difference between *H. pylori*− and *H. pylori*+**	
	mean level (SE)[a]	**mean level (SE)[a]**	***P*-value for absolute difference**	**Percent decrease**
Total carotenoids, μg/dL	70.96 (1.06)	63.77 (1.03)	0.12	10.1%
Alpha-carotene, μg/dL	2.18 (1.11)	1.92 (1.05)	0.29	12.1%
Beta-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%
Beta-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%
Alpha-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%

Abbreviation: SE, standard error

[a] adjusted for age, race, sex, BMI, education, smoking, use of vitamin supplements, and total energy intake

Table 3

Association of *H. pylori* sero-prevalence by CagA status with blood micronutrient levels

	H. pylori− (N=66)	*H. pylori*+, CagA− (N=109)	*H. pylori*+, CagA+(N=135)	Difference between *H. pylori* − and *H. pylori*+, CagA−		Difference between *H. pylori* − and *H. pylori*+, CagA+		Difference between *H. pylori* +, CagA− and *H. pylori*+, CagA+	
	mean level (SE)[a]	mean level (SE)[a]	mean level (SE)[a]	*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%
Alpha-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%
Beta-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%
Beta-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)	0.08	8.1%	**0.01**	11.7%	0.33	3.9%
Alpha-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%

Abbreviation: SE, standard error

[a] adjusted for age, race, sex, BMI, education, smoking, use of vitamin supplements, and total energy intake

[b] for absolute difference

Table 4

Association of *H. pylori* sero-prevalence by CagA status with dietary micronutrient levels, as determined by a food frequency questionnaire

	H. pylori− (N=66)	*H. pylori*+, CagA− (N=109)	*H. pylori*+, CagA+(N=135)	Difference between *H. pylori* − and *H. pylori*+, CagA−		Difference between *H. pylori* − and *H. pylori*+, CagA+		Difference between *H. pylori* +, CagA− and *H. pylori*+, CagA+	
	mean intake (SE)[a]	mean intake (SE)[a]	mean intake (SE)[a]	*P*-value[b]	Percent decrease	*P*-value[b]	Percent decrease	*P*-value[b]	Percent decrease
Total carotenoids, mcg/day	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%
Alpha-carotene, mcg/day	342.0 (1.12)	362.7 (1.09)	332.6 (1.08)	0.67	−6.0%	0.85	2.8%	0.47	8.3%
Beta-carotene, mcg/day	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/day	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/day	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%
Beta-cryptoxanthin, mcg/day	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/day	454.1 (1.06)	438.0 (1.04)	468.7 (1.04)	0.59	3.5%	0.65	−3.2%	0.24	− 7.0%
Alpha-tocopherol, mg/day	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/day	598.0 (1.04)	558.9 (1.03)	580.8 (1.03)	0.21	6.5%	0.60	2.9%	0.40	−3.9%

Abbreviation: SE, standard error

[a] adjusted for age, race, sex, BMI, education, smoking, use of vitamin supplements, and total energy intake

[b] for absolute difference

Appendix Table

Association of *H. pylori* sero-prevalence by CagA status with blood carotenoid levels, by smoking status

	H. pylori−	*H. pylori*+, CagA−	*H. pylori*+, CagA+	Difference between *H. pylori*− and *H. pylori*+, CagA−		Difference between *H. pylori*− and *H. pylori*+, CagA+		Difference between *H. pylori*+, CagA− and *H. pylori*+, CagA+	
	mean level (SE)[a]	mean level (SE)[a]	mean level (SE)[a]	*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)	50.47 (1.07)	0.09	19.7%	**0.01**	28.6%	0.29	11.2%
Never and former smokers	72.25 (1.08)	74.35 (1.05)	64.38 (1.05)	0.75	−2.9%	0.22	10.9%	0.05	13.4%
Alpha-carotene, μg/dL									
Current smokers	2.07 (1.17)	1.48 (1.13)	1.32 (1.12)	0.10	28.6%	**0.03**	36.1%	0.51	10.8%
Never and former smokers	2.30 (1.16)	2.39 (1.11)	2.07 (1.10)	0.82	−4.0%	0.56	10.0%	0.32	13.5%
Beta-carotene, μg/dL									
Current smokers	10.63 (1.19)	6.41 (1.15)	5.50 (1.12)	**0.02**	39.7%	**0.003**	48.3%	0.41	14.3%
Never and former smokers	12.45 (1.15)	12.03 (1.10)	8.60 (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)	22.6 (1.09)	0.28	15.7%	**0.02**	32.3%	0.11	19.7%
Never and former smokers	27.9 (1.10)	29.2 (1.08)	25.5 (1.07)	0.71	−4.8%	0.50	8.5%	0.20	12.7%
Lutein & zeaxanthin, μg/dL									
Current smokers	14.9 (1.11)	13.4 (1.09)	13.2 (1.08)	0.43	10.2%	0.38	11.5%	0.91	1.4%
Never and former smokers	16.0 (1.08)	16.6 (1.05)	15.6 (1.05)	0.70	−3.5%	0.77	2.7%	0.41	6.1%
Beta-cryptoxanthin, μg/dL									
Current smokers	6.49 (1.17)	4.71 (1.13)	4.54 (1.12)	0.11	27.3%	0.08	30.0%	0.83	3.7%
Never and former smokers	6.76 (1.11)	6.80 (1.08)	6.45 (1.07)	0.96	−0.6%	0.73	4.6%	0.62	5.1%

Abbreviation: SE, standard error

[a] adjusted 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

[b] for absolute difference