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Folate and Inflammatory Markers Moderate the Association Between *Helicobacter pylori* Exposure and Cognitive Function in US Adults

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Keywords

Helicobacter pylori, cognition, inflammation, folate, C-reactive protein, ferritin.

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Abstract

Background: *Helicobacter pylori* (*H. pylori*) infection is associated with cognitive deficits in humans, an association potentially mediated or moderated by folate concentration or inflammation.

Materials and Methods: We used the National Health and Nutrition Examination Survey (NHANES) datasets to examine whether folate concentration or inflammation mediates or moderates the relationship between *H. pylori* and cognitive function. Models were performed using linear, Poisson, and zero-inflated Poisson regression, and we performed separate analyses for groups aged 20–59 and 60–90 years with sample sizes ranging from 700 to 1700.

Results: We did not find evidence of mediation in either age group. In the 20- to 59-year group, interactions between *H. pylori* and ferritin (*p* values ranging from .004 to .039) were associated with worse processing speed, better working memory, and worse reaction time. Interactions between *H. pylori* and fibrinogen (*p* values ranging from .023 to .045), C-reactive protein (CRP) (*p* = .023), and the inflammatory index (*p* = .045) were associated with worse processing speed. In 60- to 90-year-olds, *H. pylori* interacted with ferritin and the inflammatory index to predict fewer mathematical errors (*p* values of .036 and .023). Interactions with folate (*p* values of .016 and .006) and C-reactive protein (*p* values ranging from <.001 to .048) were inconsistent in directionality.

Conclusions: In this dataset, representative of the US population, inflammation and folate concentrations moderated but did not mediate the association between *H. pylori* seropositivity and cognition.

Helicobacter pylori is a gram-negative bacterium found in the stomach and upper gastrointestinal tract of a significant portion of the worldwide population [1]. Infection occurs primarily through ingestion but may also occur *in utero* [2]. *Helicobacter pylori* locates to less acidic portions of the mucosal lining of the gastrointestinal tract ensuring long-term persistence within the host. Gastritis typically follows the initial infection and inflammation persists until successful treatment [3]. Genomic studies have described a specific strain of *H. pylori* that expresses the *cagA* protein, which can trigger a robust inflammatory response [4].

Beydoun et al. [5] found an association between *H. pylori* seropositivity and reduced cognitive function-

ing in adults. Although Gale et al. [6] did not find significant main effects between *H. pylori* and cognitive function in young to middle-aged adults, they did find significant interactions between *H. pylori* and race-ethnicity, educational attainment, and latent toxoplasmosis that predicted cognitive function after controlling for potential sociodemographic confounds. The association between *H. pylori* and cognitive function is consistent with similar studies showing an association between other infectious diseases and cognitive functioning [7,8].

The mechanism by which *H. pylori* may affect cognitive function is unclear, although inflammation or changes in folate metabolism could be involved

[9–13]. However, few studies have directly investigated the mechanism by which *H. pylori* influences cognition. One potential mechanism is that gastritis from *H. pylori* infection can initiate local and systemic inflammatory responses as well as increased blood levels of inflammatory cytokines and other inflammatory markers that could affect brain function over time [14]. Another potential mechanism is that interference with folate metabolism from *H. pylori* could impair homocysteine breakdown due to the reduced availability of folate metabolites, possibly resulting in cognitive deficits associated with increased concentrations of homocysteine [12].

We sought to better understand the mechanism by which *H. pylori* exposure might be associated with cognition using mediation and interaction analyses between *H. pylori* exposure, folate, inflammatory markers, and cognition. In these analyses, significant mediation would suggest a direct association between *H. pylori* via folate metabolism or inflammation or both and cognitive function. Significant interactions, however, would suggest a moderating relationship, indicating that the association between *H. pylori* and cognitive function is dependent on concentrations of serum folate or inflammatory markers. We hypothesized that folate and inflammation would mediate or moderate the relationship between *H. pylori* and cognition with the relationship being more pronounced in subjects seropositive for *H. pylori* and its *cagA* variant.

Methods

Study Sample

A total of 33,994 subjects were enrolled in the third NHANES (1988–1994), 78% of whom also underwent a medical examination in a mobile examination center [15]. Cognitive testing was administered to two separate groups, each with its own set of cognitive evaluations. The first group consisted of a random half-sample of participants aged 20–59 years. Of the 11,306 subjects aged 20–59 years in the survey, 4,924 were randomly selected for cognitive testing. Individuals 60–90 years old constituted the second group for which cognitive testing data were available, although they were given different cognitive tests than were the 20- to 59-year-olds. Of the total 5,724 subjects in this age group, 4,319 completed the cognitive tests needed for our analyses.

The samples for these groups were further limited by data missing because of the survey design as well as nonresponse. *Helicobacter pylori* seropositivity was

determined in Phase 1 (1988–1991) of the NHANES cycle for only a subset of the sample. Inflammatory marker data were not available for all Phase 1 participants, and there were a small amount of missing data on other covariates included in the models. Accordingly, the number of subjects included in each model differed based on the variables assessed and varied between approximately 700–1700 subjects.

Helicobacter pylori Status

NHANES assessed *H. pylori* exposure by IgG antibody detection using an enzyme-linked immunoassay (ELISA). Raw IgG results were not published as part of the NHANES datasets. Instead, subjects were considered negative for *H. pylori* infection if the immune status ratio (calculated by dividing optical density of the specimen by the mean optical density of cutoff controls) was within the negative range (0.00–0.90), equivocal if in the 0.91–1.09 range, and positive if the ratio exceeded 1.10 [16]. We treated subjects with equivocal results as negative for the purposes of these analyses. A separate test differentiated between those positive for *H. pylori* who were also positive or negative for the *cagA* strain. *CagA*, an *H. pylori* oncogene, has been reported to be responsible for disruption of multiple cell functions including cell–cell interactions [17]. Anti-*cagA* IgG exposure was measured in subjects using a noncommercial test [16]. For our analyses, we combined *H. pylori* infection status and *cagA* presence/absence status into a categorical variable including subjects seronegative for *H. pylori*, seropositive for *H. pylori* and negative for *cagA*, or seropositive for the *cagA* variant of *H. pylori*. In generating the categorical *H. pylori* and *cagA* status variable, we observed a fourth group in which subjects tested positive for the *cagA* factor but not for *H. pylori*. As this combination of results is likely inaccurate and could be attributed to instrumentation or testing error [4,17], we excluded this category from the analyses. Information about other strains of *H. pylori* was unavailable in the NHANES datasets.

Folate

Serum folate levels in the NHANES dataset were measured using a Quantaphase folate radioassay kit acquired from Bio-Rad Laboratories [16]. Once prepared, solutions containing folate and vitamin B12 compete in binding to immobilized binding proteins, which are eventually concentrated in pellet form. Radioactivity on each pellet was counted, and concentrations were compared to a standard curve.

Inflammatory Markers and Inflammatory Index

We used fibrinogen, ferritin, and C-reactive protein (CRP) concentrations as indicators of inflammation, the only three inflammatory markers available in participants who had both *H. pylori* ELISAs and cognitive testing data. Each of these three markers is associated with inflammation. Serum biochemistry tests determined blood concentrations of each inflammatory marker as described in the NHANES III laboratory documentation [16]. Serum ferritin and CRP levels were available for all participants over 20 years of age while fibrinogen levels were only available for participants between the ages of 40–74 years. Therefore, any analyses including fibrinogen would be limited to this restricted age range. We treated the fibrinogen and ferritin variables as continuous measures of blood concentrations and converted CRP to a categorical measure due to the severe skewness of the data. As no clinical diagnostic value of blood CRP level has been established, we used the minimum detection value of 0.21 mg/dL (reported in the NHANES III documentation) as the cutoff between significant or nonsignificant blood CRP levels. A value of 0 was assigned to CRP levels recorded as 0.21 mg/dL (the minimum detection point) and a value of 1 to any values above that point [16].

To combine the available inflammatory markers into one measure, we created an overall inflammatory index by standardizing the concentration counts for fibrinogen, ferritin, and CRP and summing them to create a standardized composite score. As serum fibrinogen levels were only obtained in subjects between the ages of 40–74, analyses of the inflammatory index only include subjects between the ages of 40–59 in the younger age group and 60–74 in the older age group.

Cognitive Assessments

The NHANES assessed cognitive functioning in the group aged 20–59 years with the serial digit learning test (SDL), symbol digit substitution test (SDS), and simple reaction time test (SRT), which are computer-administered cognitive tests [15]. The SDL is a memory task that involves viewing a series of digits on a screen and then typing them onto a keyboard. Performance on the SDL was determined by number of trials to criterion (SDL-TTC), which is two consecutive trials without error, and by the total number of errors (SDL-ERR). The SDS test evaluates processing speed, which includes coding symbol–number pairs. In this test, the participant sees a key with the symbol–number pairs and must enter the corresponding number as symbols are presented on the screen. SDS scores were divided

into number of errors (SDS-ERR) and latency times (SDS-LAT; in seconds). Finally, the SRT is a simple reaction time test scored as mean reaction times in milliseconds.

For subjects aged 60–90 years, the NHANES evaluated cognitive function using four individual tasks that were verbally administered by an NHANES representative during the interview portion of the survey. A story recall test (SR-CORR) required participants to correctly recall specific aspects of a dictated story. In a word recall test divided into two components (WR-CORR and WR-TRIALS), subjects memorized and repeated back three specific words provided by the experimenter. A math test required participants to perform multiple subtraction computations. Scores consisted of the number of incorrect responses (MATH-ERR). Finally, an orientation test required participants to provide information about the current date and the participants' address as accurately as possible (ORIENT-ERR). For cognitive function measures for both age groups, higher scores on these variables indicate worse functioning.

Covariates

In an effort toward replication, we chose covariates used by Beydoun et al. [5]: age (continuous), ethnicity–race (non-Hispanic white, non-Hispanic black, Hispanic), gender, a healthy eating index (continuous score derived from reported eating habits), a comorbidity index (a summed index of comorbid medical issues), the poverty-to-income ratio (calculated by dividing earned income by a federally established poverty level), language used in examination, marital status, education (continuous; completed years of education), self-reported health (categorized as “excellent,” “very good,” “good,” “fair,” and “poor”), smoking history, and the body mass index.

Statistical Analysis

We used Stata release 14.1 [18] for all statistical analyses. The NHANES dataset is designed to be representative of the US population through the use of multistage complex sampling methods that utilize weighting and stratification. Traditionally, strata and cluster variables are provided to researchers to account for the survey characteristics. However, certain combinations of variables may lead to multiple strata containing single clusters and thereby compromise this estimation technique, as was the case in our study. As an alternative estimation technique, 52 repeated replicate weights were also provided in the NHANES dataset that, through the use of Fay's method in balanced repeated replication [19],

similarly accounted for the survey design. We conducted all statistical tests in accord with this complex survey design.

We described categorical variables using proportions and standard errors and continuous variables as means and standard errors. We calculated all summary statistics and counts for each age group separately to enable comparisons between the two groups. Finally, we also calculated minimums and maximums for each of the variables included in our analyses.

Similar to the methodology described in Beydoun et al. [5], we used ordinary least squares (OLS) regression for continuous dependent variables (all cognitive tests for the 20–59 age group and the SR-CORR and WR-CORR tests in the 60–90 age group), Poisson regression for WR-TRIALS and ORIENT-ERR in the 60–90 age group because they were distributed as a count, and zero-inflated Poisson regression for MATH-ERR test in the 60–90 age group because this test was distributed as a count with an excess of zeros.

We estimated two separate models for each outcome to test whether folate, individual inflammatory markers, or the combined inflammatory index mediated the relationship between the categorical *H. pylori* variable and cognitive function. In the first model, we regressed a cognitive function measure on the *H. pylori* groups and the covariates and then added a measure of inflammation or folate in the subsequent model. The coefficients for *H. pylori* and *cagA* from these two models were compared using Stata's *suest* command. A significant difference ($p < .05$) between the *H. pylori* or *cagA* coefficients across these two models would indicate that the inflammatory marker or folate mediated the association between *H. pylori* and cognitive function. Although commands designed specifically for mediation analyses are available in Stata, only the *suest* command was able to account for the NHANES complex survey design.

In separate analyses, we examined interactions between *H. pylori* and the folate and inflammatory variables to examine whether these markers of inflammation moderate the relationship of *H. pylori* and its *cagA* variant with cognitive functioning. Results of these analyses are presented graphically using Stata's *margins* and *marginsplot* commands. Briefly, the *margins* command estimates the discrete difference in probability between *H. pylori* positive and *H. pylori* negative groups at different levels of the interacting variable (folate, fibrinogen, ferritin, CRP). The *marginsplot* command displays the results from the *margins* command. Plots depicting significant interactions were shaded gray, whereas plots depicting nonsignificant interactions were left white.

Results

In the 20–59 age group, 10% of the sample was seropositive for *H. pylori* without the *cagA* strain, while 18 percent tested positive for *H. pylori* with the *cagA* strain. In the 60–90 age group, 27% of the sample was seropositive for *H. pylori* without the *cagA* strain and 28% tested positive for *H. pylori* with the *cagA* strain. Other demographic information is shown in Table 1.

In the first part of the analysis, we tested for the association between *H. pylori* infection status and cognitive function. In the adult group aged 20–59 years, we found that *H. pylori* infection status was associated with cognitive function measured by the SDL-TTC ($\beta = 0.53$, $p = .003$; $\beta = 0.34$, $p = .031$) and SDL-ERR ($\beta = 1.01$, $p = .008$) while controlling for potentially confounding variables, although there was no association between *H. pylori* seropositivity and the SDS-LAT, the SDS-ERR, and the SRT (Table 2). In the group aged 60–90 years, *H. pylori* seropositivity was associated with the SR-CORR ($\beta = -0.18$, $p = .035$) and MATH-ERR estimates of cognitive function ($\beta = 0.23$, $p = .011$) (Table 2). We also did this same analysis using the same variable structure as Beydoun et al. [5] and achieved nearly identical results. The results for this analysis are shown in Table S1.

We found no significant mediating relationships between *H. pylori* and folate, the inflammatory index, ferritin, fibrinogen, or CRP in either age group (Table S2).

Investigating for variables moderating the association between *H. pylori* and cognitive function in the group aged 20–59 years, we found significant interactions between *H. pylori* and ferritin, fibrinogen, CRP, and the inflammatory index but not between *H. pylori* and folate (Fig. 1 and Table S3). Serum ferritin was not associated with SDS latency times in the no infection group but ferritin was positively related to latency times (i.e., worse cognitive functioning with higher concentrations of ferritin) among subjects seropositive for the *cagA* variant of *H. pylori* ($\beta = 0.74$, $p = .015$). In contrast, the no infection group had a weak positive association (worsening cognitive function) between ferritin and SDL-TTC, but SDL-TTC decreased (improved cognitive functioning) as ferritin increased for subjects seropositive for the *cagA* variant of *H. pylori* ($\beta = -0.25$, $p = .039$). There was a similar pattern for SDL-ERR ($\beta = -0.47$, $p < .034$). In addition, while serum ferritin was unrelated to the SRT in uninfected subjects, higher values of ferritin were related to higher SRT in subjects seropositive for the *cagA* variant. ($\beta = 6.66$, $p = .004$). While serum fibrinogen was not related to SDS-LAT in the no

Table 1 Descriptive statistics of study sample: means and proportions of dependent, independent, mediator/moderator, and control variables

	20–59 Years		60–90 Years		Minimum	Maximum
	Mean	SE	Mean	SE		
Cognitive function						
SDS-LAT	12.28	0.18	–	–	6.55	143.30
SDS-ERR	1.43	0.08	–	–	0.00	28.00
SDL-TTC	4.61	0.09	–	–	2.00	9.00
SDL-ERR	4.40	0.19	–	–	0.00	16.00
SRT	228.14	1.17	–	–	158.49	660.06
SR-CORR	–	–	4.13	0.06	0.00	6.00
WR-CORR	–	–	2.60	0.03	0.00	3.00
WR-TRIALS	–	–	1.04	0.01	1.00	3.00
ORIENT-ERR	–	–	0.14	0.01	0.00	2.00
MATH-ERR	–	–	0.55	0.05	0.00	5.00
Helicobacter^a						
No infection	0.72	0.02	0.45	0.02	0.00	1.00
<i>Helicobacter pylori</i>	0.10	0.01	0.27	0.02	0.00	1.00
<i>H. pylori</i> and <i>cagA</i>	0.18	0.01	0.28	0.01	0.00	1.00
Mediators/Moderators						
Fibrinogen	290.91	6.12	291.54	5.87	26.00	916.00
Ferritin	110.92	2.47	110.75	2.45	2.00	1698.00
C-Reactive protein	0.37	0.01	0.37	0.01	0.21	9.90
Folate	5.77	0.14	5.79	0.14	0.60	65.20
Covariates						
Age	36.90	0.36	69.67	0.69	20.00	90.00
Male ^a	0.49	0.01	0.44	0.02	0.00	1.00
Ethnicity^a						
White	0.81	0.02	0.89	0.01	0.00	1.00
Black	0.09	0.01	0.07	0.01	0.00	1.00
Hispanic	0.05	0.00	0.01	0.00	0.00	1.00
Other	0.05	0.01	0.03	0.01	0.00	1.00
English ^a	0.97	0.01	0.98	0.00	0.00	1.00
Married ^a	0.68	0.02	0.65	0.02	0.00	1.00
Smoking ^a	0.43	0.02	0.24	0.02	0.00	1.00
Health status^a						
Excellent	0.25	0.02	0.13	0.01	0.00	1.00
Very good	0.33	0.01	0.25	0.02	0.00	1.00
Good	0.31	0.02	0.35	0.02	0.00	1.00
Fair	0.10	0.01	0.20	0.02	0.00	1.00
Poor	0.05	0.00	0.07	0.01	0.00	1.00
PIR	3.04	0.07	2.94	0.12	0.00	8.41
Education	12.86	0.17	11.43	0.20	0.00	17.00
Comorbidity index	1.51	0.04	3.11	0.07	0.00	12.00
BMI	26.29	0.16	26.73	0.16	15.10	67.30
Healthy eating index	61.72	0.48	68.21	0.54	16.50	99.20

All statistics estimated within the parameters of the NHANES complex survey design.

^aProportions used for categorical variables.

infection group, there was a positive relationship between fibrinogen and SDS-LAT among subjects seropositive for *H. pylori* and the *cagA* variant, although the relationship was only significant for the *H. pylori* group ($\beta = 4.32$, $p = .036$). In subjects seropositive for the *cagA* variant of *H. pylori*, higher

levels of CRP were associated with significant increases in SDS-LAT ($\beta = 2.10$, $p = .023$). Finally, the inflammatory index was not associated with SDS-LAT for the no infection group but positively related for the *H. pylori* and *cagA* groups (only significant for *cagA* group; $\beta = 1.17$, $p = .045$).

Table 2 OLS, Poisson, and zero-inflated Poisson regression of *Helicobacter pylori* and *cagA* seropositivity predicting cognitive functioning in US 20- to 59-year- and 60- to 90- year-olds, NHANES III (1988–1994)

	H. pylori only			H. pylori and cagA		
	β	95% CI	<i>p</i>	β	95% CI	<i>p</i>
Age 20–59						
SDS-LAT	0.90	[−0.06, 1.86]	.066	0.59	[−0.17, 1.37]	.123
SDS-ERR	0.21	[−0.23, 0.65]	.337	0.01	[−0.34, 0.37]	.938
SDL-TTC	0.53	[0.19, 0.88]	.003	0.34	[0.03, 0.65]	.031
SDL-ERR	1.01	[0.28, 1.74]	.008	0.52	[−0.13, 1.17]	.116
SRT	−1.61	[−8.48, 5.26]	.641	4.63	[−3.36, 12.61]	.250
Age 60–90						
SR-CORR	−0.18	[−0.34, −0.01]	.035	−0.09	[−0.25, 0.07]	.264
WR-CORR	0.03	[−0.05, 0.12]	.415	−0.01	[−0.11, 0.08]	.761
WR-TRIALS	−0.01	[−0.04, 0.01]	.246	0.01	[−0.02, 0.04]	.325
OR-ERR	−0.22	[−0.59, 0.15]	.236	−0.02	[−0.33, 0.30]	.922
MATH-ERR						
Count	0.16	[−0.04, 0.36]	.119	0.23	[0.05, 0.40]	.011
Logit	0.12	[−0.25, 0.48]	.523	0.21	[−0.25, 0.66]	.363

All models fully controlled.

CRP, C-reactive protein; SDS-LAT, Symbol digit substitution test – Latency; SDS-ERR, Symbol digit substitution test – Errors; SDL-TTC, Serial digit learning test – Trials to criterion; SDL-ERR, Serial digit learning test – Errors; SRT, Simple reaction time test; SR-CORR, Story recall – Correct; WRCORR, Word recall – Correct; WR-TRIALS, Word recall – Trials; ORIENT-ERR, Orientation – Errors; MATH-ERR, Math – Errors.

For the group aged 60–90 years, there were significant interactions between *H. pylori* exposure and folate, ferritin, CRP, and the inflammatory index, but not fibrinogen, on cognitive function (Fig. 2 and Table S4). Folate concentration was related to lower WR-TRIALS (better functioning) among subjects who were seropositive for the *cagA* *H. pylori* variant ($\beta = -0.06$, $p = .016$). Further, there was a positive, nonlinear relationship between folate and WR-ORIENT for subjects seropositive for *H. pylori* but not the *cagA* variant ($\beta = 0.69$, $p = .006$), with a particularly strong relationship at higher levels of folate. Similar to the results observed in the younger age group, mathematical errors decreased in *H. pylori* infected subjects as ferritin levels increased ($\beta = -0.16$, $p = .036$). While no association was observed between CRP and the WR-TRIALS in the no infection group, CRP was positively related to WR-TRIALS in subjects who were seropositive both for *H. pylori* alone and for *H. pylori* and its *cagA* variant (*H. pylori*, $\beta = 0.04$, $p = .048$; *H. pylori* and *cagA*, $\beta = 0.08$, $p = .011$). In contrast, as CRP levels increased, improved performance on the math test was observed in subjects seropositive for the *cagA* variant ($\beta = -1.51$, $p < .001$). Finally, the inflammatory index was positively associated with MATH-ERR in *cagA* variant seropositive subjects ($\beta = -0.44$, $p = .023$).

Discussion

The main finding of this study is that inflammatory markers and folate appear to moderate but not mediate the association between *H. pylori* seropositivity and cognitive function in both younger and older adults. In this study, we first replicated the findings of Beydoun et al. [5] showing an association between cognitive function and *H. pylori* seropositivity in both younger and older adults. We then evaluated whether folate and inflammatory factors mediate or moderate the association between *H. pylori* seropositivity and cognitive function. We found that *H. pylori* exposure determined by *H. pylori* IgG seropositivity was associated with cognitive function in some but not all of the available cognitive tests in both the group aged 20–59 years and in the group aged 60–90 years, closely replicating the findings of Beydoun et al. [5] This was from the same dataset used by Beydoun et al. [5] but with sample adjustments to include folate, the three inflammatory markers, and an inflammatory index. Further, the *cagA* variant of *H. pylori* may also uniquely affect cognitive function compared to the *cagA* negative group.

We did not find evidence that the available inflammatory markers, the inflammatory index, or folate mediated the association between *H. pylori* seropositivity and cognitive function in either age group.

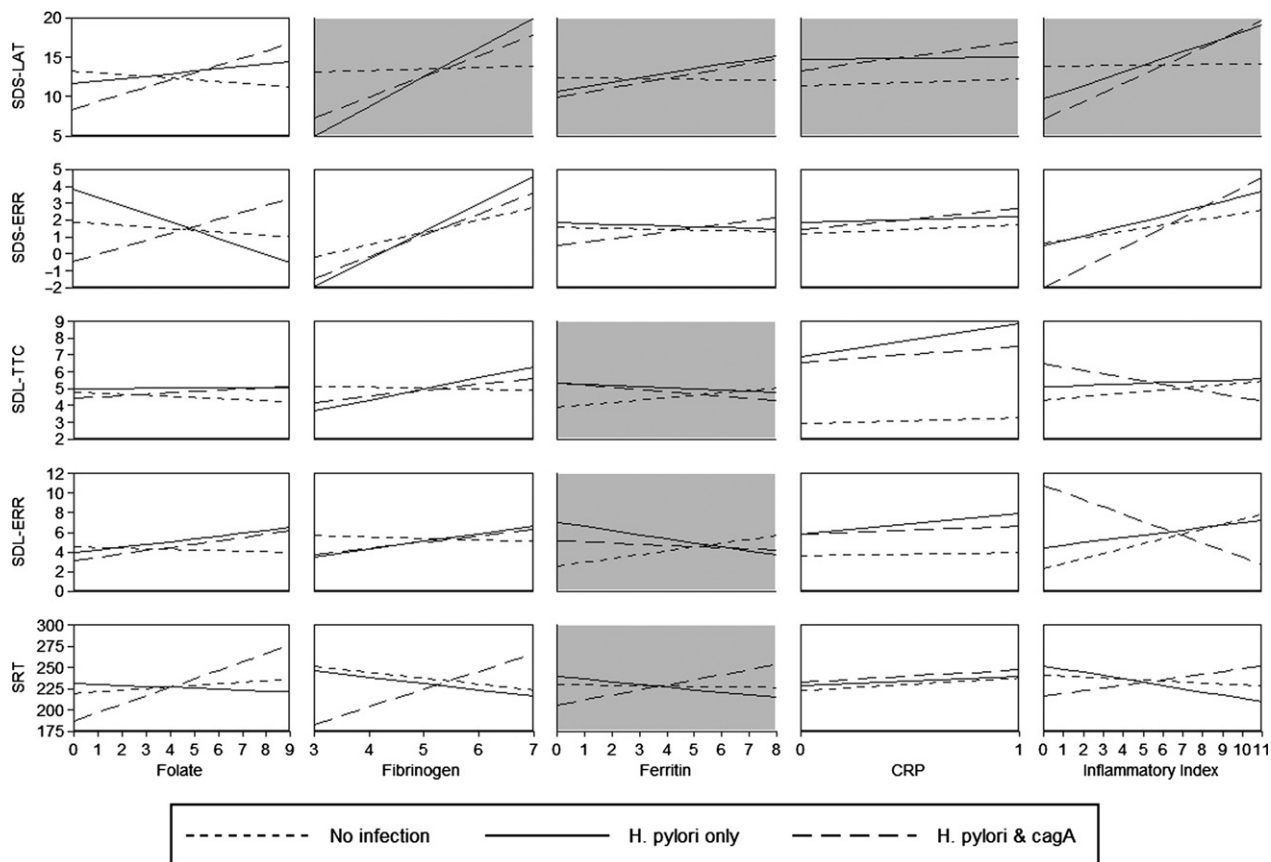


Figure 1 Cognitive performance predicted from the interactions of *Helicobacter pylori* in adults aged 20–59. *Helicobacter pylori* & cagA denotes *H. pylori* and cagA variant seropositivity, *H. pylori* only denotes *H. pylori* seropositivity without cagA, and No infection denotes seronegativity. Plots shaded grey indicate statistically significant interactions. CRP, C-reactive protein; SDS-LAT, Symbol digit substitution test – Latency; SDS-ERR, Symbol digit substitution test – Errors; SDL-TTC, Serial digit learning test – Trials to criterion; SDL-ERR, Serial digit learning test – Errors; SRT, Simple reaction time test. Fibrinogen data was only available for subjects aged 40–59. All models fully controlled.

However, interactions in the group aged 20–59 years between *H. pylori* seropositivity and ferritin, fibrinogen, CRP, and the inflammatory index were associated with increased or decreased cognitive function. Further, in the group aged 60–90 years, interactions between *H. pylori* and folate, ferritin, CRP, and the inflammatory index were also associated with increased or decreased cognitive function. These findings extend the prior work done by Beydoun et al. [5] and suggest that concentrations of inflammatory markers and folate moderate the association between *H. pylori* and cognitive function.

General inflammation has been linked to cognitive decline, especially in older adults [20–22]. In this regard, we had expected that the inflammatory index we developed based on serum fibrinogen, ferritin, and C-reactive protein concentrations would interact with *H. pylori* in both age groups to predict worse cognition. While we did find interactions in both age groups, in

subjects seropositive for the cagA variant of *H. pylori* we found instances of both better and worse cognitive performance. How the cagA variant could lead to a potential protective effect from the deleterious effects of inflammation is not clear. It is possible that because there were few inflammatory markers available in these data, and the inflammatory index we used consisted of only three markers, we may not have included the most relevant and influential inflammatory markers in our analysis. Also, the calculated inflammatory index suffers from a significant sample size reduction due to the restricted age range in which fibrinogen data were available (ages 40–74 only).

The significant interactions between *H. pylori* and specific inflammatory markers suggest possible mechanisms by which *H. pylori* may affect cognition. For example, *H. pylori* uses prokaryotic ferritin to adapt to iron availability in the host gastric mucosa [23], taking up available host ferrous iron by specific transport

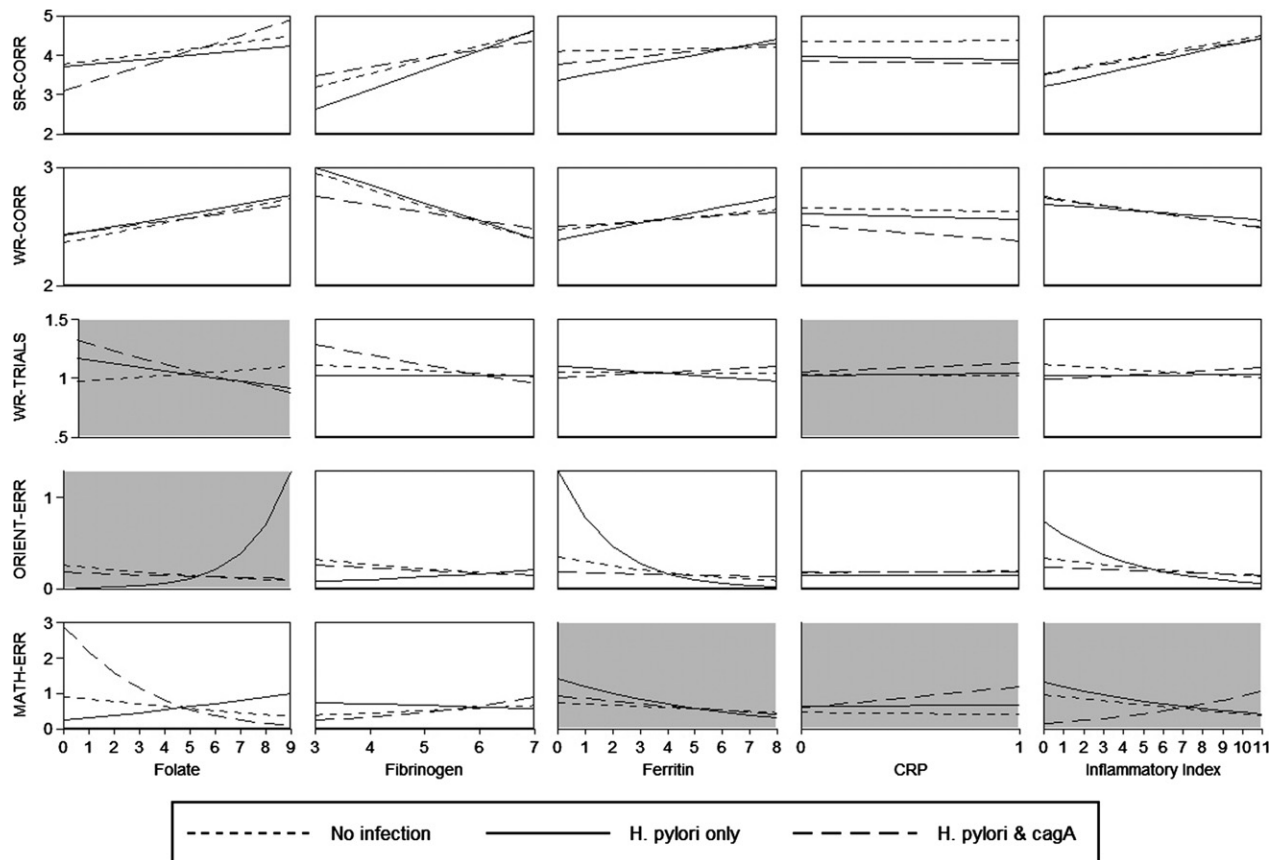


Figure 2 Cognitive performance predicted from the interactions of *Helicobacter pylori* in adults aged 60–90. *Helicobacter pylori* & cagA denotes *H. pylori* and cagA variant seropositivity, *H. pylori* only denotes *H. pylori* seropositivity without CagA, and No infection denotes seronegativity. Plots shaded grey indicate statistically significant interactions. CRP, C-reactive protein; SR-CORR, Story recall – Correct; WR-CORR, Word recall – Correct; WR-TRIALS, Word recall – Trials; ORIENT-ERR, Orientation – Errors; MATH-ERR, Math – Errors. Fibrinogen data was only available for subjects aged 60–74. All models fully controlled.

proteins through the bacterial membrane, possibly leading to an acute decrease in host eukaryotic ferritin levels as free ferrous iron is lost to the invading bacterium. Indeed, previous studies have reported decreased serum ferritin levels in individuals infected by *H. pylori* [24,25]. The action of *H. pylori* in altering host iron levels may affect cognition. In contrast, interactions with C-reactive protein predicted worse cognitive functioning in both age groups, consistent with findings suggesting that inflammation is associated with cognition [26]. It is therefore possible that the variability in the relationship between *H. pylori* infection status, the inflammatory index, and cognitive performance is due to the specific and potentially opposing effects of the individual inflammatory markers.

In addition to inflammation, impaired folate metabolism has also been suggested as a possible means by which *H. pylori* infection might affect cognitive functioning [9]. Although a number of studies have attempted to link *H. pylori* infection with folate, B12,

and homocysteine levels, the results have been inconsistent [3,10,12]. Despite this, a possible model of the underlying mechanism has been theorized whereby an initial inflammatory response from *H. pylori* infection results in mild to moderate gastritis, which impairs folate metabolism. As less folate is absorbed, homocysteine levels tend to increase, potentially leading to impaired cognitive function over time [13,27]. Unfortunately, only folate, not B12 or homocysteine levels, was available in the NHANES III dataset we used in the current study. Nonetheless, we found significant interactions between serum folate levels and *H. pylori* infection in predicting cognitive functioning in the 60–90 age group. In this group, less available folate was associated with worse cognitive functioning, and this effect was further exacerbated by *H. pylori* infection status suggesting a mechanistic relationship between folate availability and *H. pylori* infection status in predicting cognitive function in older adults.

Limitations of this study include its cross-sectional design, the inability to directly compare cognitive performance in the younger (20–59 years) and older (60–90 years) age groups because of the different cognitive evaluations used, and the lack of vitamin B12 data. Although we were able to examine the relationship of serum folate levels in the association between *H. pylori* and cognition, *H. pylori* might more directly affect serum vitamin B12 levels, a cofactor also involved in the reduction of blood homocysteine. Furthermore, we could not determine the time of the initial infection. Initial and subsequent infection with *H. pylori* may affect cognition differently depending upon brain development at the time of infection. An additional limitation is that the cognitive testing available in the NHANES dataset was limited to only certain cognitive domains, providing for an incomplete assessment of *H. pylori*'s association with cognition. Future research could include consistent cognitive measures across age groups as well as include vitamin B12 levels.

This study also has a number of strengths. Namely, the NHANES dataset allowed access to data for a large number of subjects. Additionally, complex sampling and weighting techniques made the sample more representative of the US population. Replication of findings from a prior study acts as a check on the methods we used [5]. A further strength is the availability of multiple means of assessing infectious burden interactions including separate analyses testing the effects of each inflammatory marker as well as a general inflammatory index.

In conclusion, in this dataset representative of the US population, exposure to *H. pylori* ascertained by IgG seropositivity was associated with cognitive function in adults aged 20–90 years, consistent with the report of Beydoun et al. [5]. Further, inflammation and serum folate concentrations moderated but did not mediate the association between *H. pylori* seropositivity and cognitive function in adults aged 20–90 years. The direction of moderation observed for folate and the inflammatory index was, in general, inconsistent across multiple tests of cognitive function. In contrast, interactions between ferritin and *H. pylori* tended to be associated with better cognitive function while interactions between CRP and *H. pylori* were associated with worse cognitive function. Although we were not able to determine the exact mechanism by which *H. pylori* affects cognitive function, the results of this study demonstrate that folate and inflammation may each contribute to this association. Future research is indicated to better understand how inflammation and folate status contribute to cognitive function in the presence of *H. pylori* exposure.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. OLS, Poisson, and zero-inflated Poisson regression of *H. pylori* & CagA seropositivity and cognitive functioning in U.S. 20 to 59 and 60 to 90 year-olds, NHANES III (1988–1994)

Table S2. Mediation analysis of *Helicobacter pylori* seropositivity and cognitive functioning in US 20 to 90 year-olds, NHANES III (1988–1994)

Table S3. Interaction analysis of *Helicobacter pylori* seropositivity and cognitive functioning in US 20 to 59 year-olds, NHANES III (1988–1994): unstandardized coefficients from weighted OLS regression

Table S4. Interaction analysis of *Helicobacter pylori* seropositivity and cognitive functioning in US 60 to 90 year-olds, NHANES III (1988–1994): unstandardized coefficients from weighted OLS regression