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Association between composite dietary antioxidant index and *Helicobacter pylori* infection: a population-based and Mendelian randomisation study

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Research Article

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Composite dietary antioxidant index; *Helicobacter pylori*; NHANES; Population-based study; Mendelian randomisation analysis

Abbreviation:
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CDAI, composite dietary antioxidant index; DM, diabetes mellitus; IVW, inverse variance weighted; MR, Mendelian randomisation; NHANES, National Health and Nutrition Examination Survey.

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Abstract

The composite dietary antioxidant index (CDAI) has been identified as a critical factor in the pathogenesis of certain inflammatory diseases. The study aimed to investigate the relationship between CDAI and *Helicobacter pylori* infection using cross-sectional design. In this study, participants from the 1999–2000 National Health and Nutrition Examination Survey were analysed using logistic and Cox regression analyses to assess the associations between *H. pylori* infection and CDAI, encompassing vitamin A, vitamin C, vitamin E, carotene, Zn, Se and Cu. The results demonstrated a negative correlation between CDAI scores and *H. pylori* infection, revealing a non-linear relationship between the odds of *H. pylori* infection and CDAI as a continuous variable. Subsequently, a two-sample Mendelian randomisation study was conducted utilising genome-wide association study summary statistics to explore the causal relationship between antioxidant levels and *H. pylori* infection. We found that the intake of Cu was a protective factor in the occurrence of *H. pylori* infection but did not support a causal association between circulating Cu levels and *H. pylori* infection. The prevalence of *H. pylori* infection was found to be elevated among individuals of older age, lower education levels, limited socio-economic status, smokers, diabetes and those with hypertension. The study suggests that higher CDAI is linked to decreased odds of *H. pylori* infection, and further prospective studies are needed to confirm the association. Our findings may have significant implications for the prevention and management of *H. pylori*-related diseases.

Helicobacter pylori, a gram-negative bacterium, colonises the human stomach and increases the risk of various gastric diseases, including peptic ulcer disease and gastric cancer, affecting half of the global population[1–3]. In the USA, an estimated 35·6 % of the population was reported to have been exposed to *H. pylori* during the period of 2000–2016, with a higher prevalence observed in economically disadvantaged countries[4,5]. In addition, according to the WHO classification, *H. pylori* is assigned to Group 1 carcinogen for gastric cancer, leading to the development of precancerous and cancerous lesions[6]. Oxidative stress is defined as a disparity between the generation of free radicals and reactive oxygen species and their removal by antioxidants[7]. This imbalance can result in harm to crucial biomolecules at the microscopic level, ultimately affecting the entire organism. When infecting humans, *H. pylori* have the potential to induce systemic oxidative stress and inflammatory reactions[8].

Diet has emerged as a significant risk factor for *H. pylori* infection, with evidence suggesting a potential link between diet, inflammation and *H. pylori* exposure[9]. The composite dietary antioxidant index (CDAI) is a comprehensive measure of an individual's dietary total antioxidant capacity, encompassing various vitamins and minerals with antioxidant properties (vitamin A, C, E, carotene, as well as the minerals Se, Cu and Zn)[10]. Previous studies have shown that CDAI is associated with specific inflammatory biomarkers such as TNFα and IL-1β, and a high CDAI score is correlated with a decreased risk of certain malignancies and all-cause mortality[11]. However, research on the relationship between CDAI and *H. pylori* infection is limited.

The National Health and Nutrition Examination Survey (NHANES) database comprises a series of cross-sectional surveys conducted every 2 years, representing samples of the non-institutionalised civilian population of the USA. Since 1999, NHANES has gathered a diverse array of data to evaluate the health status of the USA population, including demographic information, dietary patterns, socio-economic factors, health-related questionnaires, physical and physiological examination results and extensive laboratory findings. These data are publicly available for research purposes. The survey protocol was approved by the Institutional Review Board of the National Center for Health Statistics, and all participants provided written informed consent voluntarily. Mendelian randomisation (MR) analysis is an emerging

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epidemiological method that serves as a complementary approach to randomised controlled trials. MR analyses are frequently utilised to investigate the causal effects of exposures on specific outcomes at the genetic level[12,13]. By virtue of not being influenced by environmental factors, MR results are less prone to residual confounding and help mitigate reverse causation bias due to the randomised allocation of genetic variants[14]. Consequently, we sought to explore the potential association between CDAI and *H. pylori* infection using data from NHANES 1999–2000 and MR analysis.

Materials and methods

Study design and participants

This study included a total of 9965 participants from NHANES 1999–2000 (https://www.cdc.gov/nchs/nhanes/). After selecting participants aged 18 years and older, we further excluded individuals with missing (*n* 801) or equivocal *H. pylori* infection status (*n* 117), missing dietary intake of antioxidant data (*n* 163), unavailable weight values (*n* 17), lacking education levels, BMI, alcohol consumption, smoking status and other covariates (*n* 1152). Consequently, 3198 participants were included in the final analysis. The selection protocol is illustrated in Figure 1.

Composite dietary antioxidant index

NHANES collected participants' dietary data intake of antioxidants and other nutrient components through a 24-hour dietary recall interview. CDAI was estimated by an equal weight of the sum of daily intakes of seven minerals and vitamins normalised by subtracting the means and then dividing by their standard deviation (SD), including vitamin A, vitamin C, vitamin E, carotene, Zn, Se and Cu[15]:

$$\text{CDAI} = \sum_{i=1}^{n=7} (\text{Individual intake} - \text{Mean})/\text{SD}$$

Helicobacter pylori *infection status*

Serologic test results from human subjects were collected by NHANES using an ELISA to quantify IgG antibodies against *H. pylori*[16]. This method demonstrates comparable sensitivity, specificity and reproducibility to other serum antibody-based tests such as immunofluorescence, complement fixation, hemagglutination and radioimmunoassay[17,18]. The optical density of the collected specimens was utilised to determine the immune status ratio of individuals. Standard cut-off values were employed to classify participants as *H. pylori*-positive (Immune Status Ratio (ISR) $\geq 1{\cdot}1$) or *H. pylori*-negative (ISR $< 0{\cdot}9$)[19]. Ambiguous values (0·9–1·1) were excluded to ensure precise statistical outcomes in subsequent analyses.

Covariates

Information about covariates was obtained using standardised survey questionnaires and examinations. Items included age, sex, poverty income ratio, race/ethnicity, education, BMI, smoking status, alcohol consumption, diabetes mellitus (DM), hypertension, daily energy intake level, TAG, total cholesterol, alanine aminotransferase, aspartate aminotransferase, serum creatinine, urine creatinine, C-reactive protein and CDAI.

We categorised participants who self-identified into the following racial groups: non-Hispanic white, Mexican American, non-Hispanic black or other races. The educational level was grouped into three categories: college or above, high school or equivalent and less than high school. Smoking status was divided into three groups: never smokers, former smokers and current smokers. Never smokers were individuals who had either never smoked or had consumed fewer than 100 cigarettes in their lifetime. Former smokers were those who had previously smoked at least 100 cigarettes but had quit smoking. Current smokers were participants who had smoked more than 100 cigarettes in their lifetime and had consumed a nonzero number of cigarettes daily over the past 30 d[20]. To assess participants' alcohol consumption patterns, alcohol consumption was classified into five categories. Never drinking referred to individuals whose lifetime alcohol consumption was less than twelve drinks. Former drinking included those who had consumed more than twelve drinks in the past but had not consumed any alcohol in the past year. Current alcohol consumption was further divided into three patterns: mild, moderate or heavy drinking. Heavy drinkers were defined as women who consumed three or more drinks per day or men who consumed four or more drinks per day and had at least five binge drinking episodes monthly. Moderate drinkers were women who consumed two or more drinks per day or men who consumed three or more drinks per day. The remaining current drinkers were classified as mild drinkers[21].

Participants who self-reported clinically diagnosed DM, fasting plasma glucose $\geq$ 126 mg/dl, haemoglobin A1C concentration $\geq$ 6·5 % or use of diabetes medication, were identified with DM[22]. Hypertension was defined as systolic blood pressure $\geq$ 140 mmHg and/or diastolic blood pressure $\geq$ 90 mmHg, or as having an intake of antihypertensive medication[23]. The biochemical examination profiles, such as energy, TAG, total cholesterol, alanine aminotransferase, aspartate aminotransferase, serum creatinine, urine creatinine, C-reactive protein and CDAI were included in the baseline data collection.

Mendelian randomisation analysis

To deduce the causal association, we developed a two-sample MR analysis on the components independently related to *H. pylori* infection from publicly available data on an online genome-wide association study. In this study, we addressed the potential bias stemming from population admixture by limiting the genetic background of our MR investigation to individuals of European descent[24]. We utilised data derived from a comprehensive population-based meta-analysis conducted within the UK Biobank cohort to examine genome-wide association study statistics pertaining to *H. pylori* infection[25]. Exposure data concerning Cu levels were gathered from a cohort comprising 2603 Australian and 2874 British participants. Through genome-wide association study analysis employing inductively coupled plasma mass spectrometry, we pinpointed two significant loci on chromosome 1 associated with erythrocyte Cu levels[26]. Detailed insights into the genome-wide association study studies incorporated in the MR analysis can be found in the online Supplementary Table 1.

We included independent SNP ($r2 < 0{\cdot}001$ within 10 000-kb windows), strongly associated ($P \leq 5 \times 10^{-8}$) with the serological Cu level for the main MR analysis. The F- statistic was estimated to quantify instrument strength for each exposure, and we excluded SNP with an F statistic < 10 to reduce the risk of weak instrument bias[27,28]. The remaining two SNP were selected as instrumental variables (online Supplementary Table 2). Associations correspond

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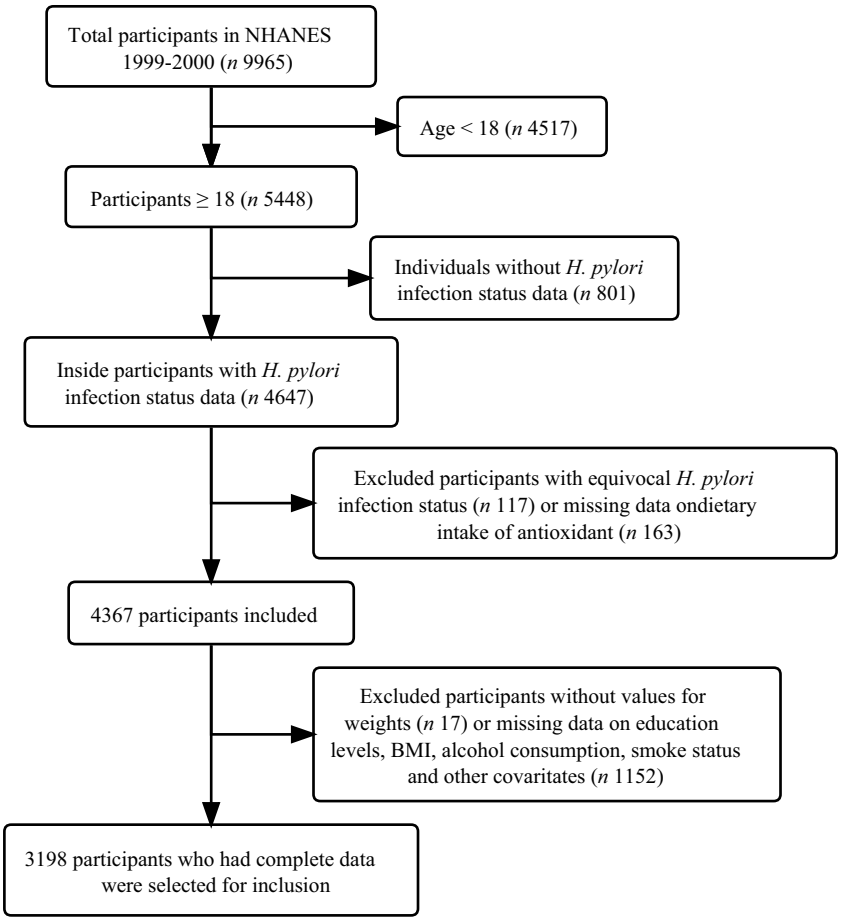


Figure 1. Flow chart of sample selection from National Health and Nutrition Examination Survey 1999–2000.

to an OR for the outcome increase in the genetically predicted concentrations of Cu. $P < 0{\cdot}05$ was considered statistically significant.

Statistical analyses

Data analysing and graphing were performed using R software (4.1.1), with the value of two-tailed $P < 0{\cdot}05$ indicating statistically significant differences. Baseline characteristics of participants were separated into two groups based on the infection status of *H. pylori*. Continuous variables were displayed as weighted means (SD) and compared by *t* test or Wilcoxon rank-sum test, depending on the result of the Kolmogorov–Smirnov normality test. Categorical variables were exhibited as unweighted numbers (weighted percentages) and compared by Chi-square test. Univariate and multivariate logistic regression analyses were used to evaluate the OR and 95 % CI for the association between CDAI and *H. pylori* infection status. CDAI was quartile-stratified into four categories and analysed using generalised linear regression models with the low CDAI group as the reference group. In the regression models, tests for trend (*P*-trend) were undertaken across quartiles utilising the median of CDAI in each quartile as a linear variable. Model 1 was a crude model with no covariate being adjusted; Model 2 was adjusted for age, sex, race and education. Model 3 added to Model 2 the energy, alcohol consumption and smoking status as covariates. The restricted cubic spline was utilised to explore the non-linearity. If non-linear relationships were identified, we used two-piecewise linear regression models to elucidate how the associations differed by the threshold point. The threshold value was estimated by trying all possible values and choosing the threshold point with the highest likelihood.

In the two-sample MR analysis, the inverse variance weighted (IVW) was used as the principal approach to evaluate the causal association between Cu and *H. pylori* infection. As only two instruments were used for Cu, complementary MR analysis methods, including MR-Egger regression, weighted median, simple mode and weighted mode were not applied in the main analysis. Cochrane's Q test was used to assess the potential heterogeneity. Measured heterogeneity was adjusted by a multiplicative random-effect IVW analysis with $P < 0{\cdot}05$.

Results

General population characteristics of study subjects according to Helicobacter pylori *infection*

A total of 3198 American adults were included in the study, and Table 1 provides a summary of their baseline characteristics. The average age of the participants was 42 years[17], with females accounting for 51 % of the cohort. Notably, the *H. pylori*-positive group exhibited a significantly higher average age, lower socio-economic status, lower educational levels, higher proportion of individuals with DM, higher proportion of individuals with hypertension and decreased daily energy intake levels compared to the *H. pylori*-negative group. Non-Hispanic Whites have the lowest proportion of *H. pylori*-positive individuals, compared with

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Table 1. Baseline characteristics of participants with different *Helicobacter pylori* infection status in NHANES 1999–2000 analyses (*n* 3198) (Mean values and standard deviations; numbers and percentages)

			H. pylori Infection Status				
	Overall, *n* 3198 (100 %)		*Hp* Negative, *n* 1834 (71 %)		*Hp* Positive, *n* 1364 (29 %)		
Characteristic	Mean	SD	Mean	SD	Mean	SD	*P*
Age (years)	42	17	43	16	49	17	< **0·001**
	n	%	*N*	%	*n*	%	
Sex							0·6
Female	1680	51 %	986	51 %	694	52 %	
Male	1518	49 %	848	49 %	670	48 %	
PIR	2·96	1·64	3·20	1·59	2·39	1·60	< **0·001**
Race/ethnicity							< **0·001**
Non-Hispanic White	1523	73 %	1170	82 %	353	50 %	
Mexican American	836	5·8 %	279	3·3 %	557	12 %	
Non-Hispanic Black	533	9·2 %	242	6·5 %	291	16 %	
Other races	306	12 %	143	8·2 %	163	22 %	
Education							< **0·001**
College or above	1332	52 %	982	59 %	350	35 %	
High school or equivalent	727	26 %	467	26 %	260	26 %	
Less than high school	1139	22 %	385	14 %	754	39 %	
BMI	28·0	6·3	27·9	6·3	28·1	6·3	0·6
Smoking status							**0·035**
Never smoker	1675	50 %	992	53 %	683	45 %	
Former smoker	866	25 %	493	24 %	373	25 %	
Current smoker	657	25 %	349	23 %	308	30 %	
Alcohol consumption							**0·004**
Never	1047	28 %	559	26 %	488	34 %	
Former	347	7·9 %	178	7·3 %	169	9·4 %	
Mild	832	28 %	522	30 %	310	23 %	
Moderate	398	15 %	261	16 %	137	14 %	
Heavy	574	21 %	314	21 %	260	20 %	
Diabetes mellitus	391	7·9 %	165	6·4 %	226	11 %	**0·008**
Hypertension	1299	33 %	678	31 %	621	37 %	**0·014**
	Mean	SD	Mean	SD	Mean	SD	
Energy (kJ/day (kcal/day))	9267.56 (2215)	4418.30 (1056)	9568.81 (2287)	4338.81 (1037)	8543.73 (2042)	4535.46 (1084)	< **0·001**
TAG (mmol/l)	1·59	1·14	1·56	1·11	1·67	1·20	**0·048**
Total cholesterol (mmol/l)	5·07	1·01	5·06	1·01	5·09	1·04	0·4
ALT (U/L)	27	24	27	25	27	24	> 0·9
AST (U/L)	25	20	25	21	25	16	0·3
Serum creatinine (mg/dl)	0·75	0·48	0·75	0·49	0·75	0·44	> 0·9
Urine creatinine (mg/dl)	135	91	138	93	128	85	0·2
CRP (mg/dl)	0·43	0·74	0·41	0·70	0·48	0·84	**0·042**
CDAI	0·2	4·7	0·6	4·9	−0·6	4·2	< **0·001**

Continuous variables were displayed as weighted means (SD); categorical variables were exhibited as unweighted numbers (weighted percentages); *Hp*, *H. pylori*; PIR, poverty income ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; CDAI, composite dietary antioxidant index.
Bold values highlight those values less than 0.05.

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Table 2. Associations between intake of antioxidant levels and *Helicobacter pylori* infection (OR and 95 % CI)

	H. pylori Infection OR (95 % CI)										
	Continuous variable			Categorical variable							
	CDAI		*P*	Q1	Q2		Q3		Q4		
Variable	OR	95 % CI			OR	95 % CI	OR	95 % CI	OR	95 % CI	*P*-trend
Model 1	0·94	0·91, 0·97	< **0·001**	Referent	0·67	0·43, 1·03	0·61	0·43, 0·87	0·45	0·30, 0·67	< **0·001**
Model 2	0·96	0·93, 0·99	**0·018**	Referent	0·63	0·37, 1·05	0·59	0·3 4, 1·02	0·48	0·29, 0·77	**0·008**
Model 3	0·96	0·92, 1·00	**0·039**	Referent	0·72	0·45, 1·16	0·71	0·44, 1·14	0·56	0·35, 0·89	**0·023**

Model 1 was adjusted for none.
Model 2 was adjusted for sex, age, race and education.
Model 3 was adjusted for sex, age, race, education, energy, alcohol consumption and smoking status.
Bold values highlight those values less than 0.05.

the other races. No significant differences were observed in terms of sex or BMI between the groups. A higher percentage of former smokers, current smokers, never drinkers and former drinkers was observed in comparison to the *H. pylori*-negative group. The *H. pylori*-positive group showed higher mean levels of TAG and C-reactive protein than the *H. pylori*-negative group. However, there were no significant differences between the two groups in terms of total cholesterol, alanine aminotransferase, aspartate aminotransferase, serum creatinine and urine creatinine levels. Individuals in the *H. pylori*-positive group exhibited a lower CDAI score (–0·6 (4·2)) than those in the *H. pylori*-negative group (0·6 (4·9)).

Association between composite dietary antioxidant index and Helicobacter pylori *infection*

When analysed as a continuous variable, a negative correlation between CDAI and the occurrence of *H. pylori* infection was observed in all three models (Table 2). Specifically, in Model 1, CDAI was found to be associated with a reduced odds of *H. pylori* infection, with an OR of 0·94 (95 % CI: 0·91, 0·97). After adjusting for sex, age, race, education, energy, alcohol consumption and smoking status, individuals with high CDAI scores were less likely to be infected by *H. pylori* (OR: 0·96 (95 % CI: 0·92, 1·00)). When divided into four categories, it was shown that the OR for *H. pylori* infection with CDAI levels in Q2 (–2·98, −0·97), Q3 (–0·97, 1·73) and Q4 (1·73, 55·84) were 0·67 (95 % CI: 0·43, 1·03), 0·61 (95 % CI: 0·43, 0·87) and 0·45 (95 % CI: 0·30, 0·67), respectively, compared with those with CDAI levels in Q1 (–7·62, −2·98), with *P*-trend less than 0·05. Further, OR for *H. pylori* infection with CDAI levels in Q2, Q3 and Q4 were 0·63 (95 % CI: 0·37, 1·05), 0·59 (95 % CI: 0·34, 1·02) and 0·48 (95 % CI: 0·29, 0·77), respectively, compared with those with CDAI levels in Q1 after adjusting for sex, age, race and education in Model 2 (*P*-trend = 0·008). While in Model 3, it was found that the OR for *H. pylori* infection with CDAI levels in Q2, Q3 and Q4 were 0·72 (95 % CI: 0·45, 1·16), 0·71 (95 % CI: 0·44, 1·14) and 0·56 (95 % CI: 0·35, 0·89), respectively, compared to those with CDAI levels in Q1, with *P*-trend less than 0·05.

The association between CDAI and *H. pylori* infection was further examined as non-linear in Model 3 using restricted cubic spline curves (Figure 2). A two-piecewise linear regression was employed to analyse the threshold effect of CDAI on *H. pylori* infection. The cut-off point for CDAI was determined to be 0·324. For CDAI values below 0·324, each unit increase was linked to a 16 % reduction in the odds of *H. pylori* infection ($P < 0·01$), while for CDAI values above 0·324 each unit increase was linked to a 4 % reduction in the odds of *H. pylori* infection, although this relationship was not statistically significant (Table 3).

Subgroup analysis of the influence of various variables on Helicobacter pylori *infection status*

To investigate the consistency of the relationship between CDAI and *H. pylori* infection and to identify potential population-specific factors, a subgroup analysis and interaction tests were conducted, stratified by sex, age, race, education, alcohol consumption and smoking status (Figure 3). Among the subgroups, only participants aged over 60 years, non-Hispanic white, with a college education or higher and who never drank alcohol demonstrated statistical significance ($P < 0·05$) in relation to the association between CDAI and *H. pylori* infection. However, no significant interactions were observed between any of the stratified parameters, suggesting that the relationship between CDAI and *H. pylori* infection is consistent across different subgroups and is independent of sex, age, race, education, alcohol consumption and smoking status (*P* for interaction > 0·05).

Causal relationship between components of CDAI and Helicobacter pylori *infection*

A sensitivity analysis was conducted to assess the association between seven components of CDAI and *H. pylori* infection. The results in Table 4 indicate that statistically significant negative associations were observed between intake levels of vitamin A, vitamin E, Zn and Cu with *H. pylori* infection in Model 1. However, after adjusting for all confounders, only Cu intake levels showed a negative association with the occurrence of *H. pylori* infection, with an OR of 0·750 477 (95 % CI: 0·565112, 0·996643; $P = 0·049$).

After identifying a significant negative correlation between Cu intake levels and the odds of *H. pylori* infection in the previous multivariable regression analysis, an MR analysis was performed to investigate the causal effects of Cu levels on *H. pylori* infection. Following the selection criteria for SNP, only two SNP met the criteria for further analysis. There was evidence of heterogeneity of IVW analysis for Cu ($Q = 7·181\ 939$; $P < 0·05$). Therefore, the multiplicative random-effects IVW method was utilised to evaluate the causal association while adjusting for measured heterogeneity. The analysis revealed no significant causal relationships between serological Cu levels and *H. pylori* infection (OR: 1·02 (95 % CI: 0·91, 1·16); $P = 0·703$), based on the IVW method (multiplicative random effects) (online Supplementary Figure 1). Further, we set the cutoff ($P < 1 \times 10^{-5}$) to include more SNP in our

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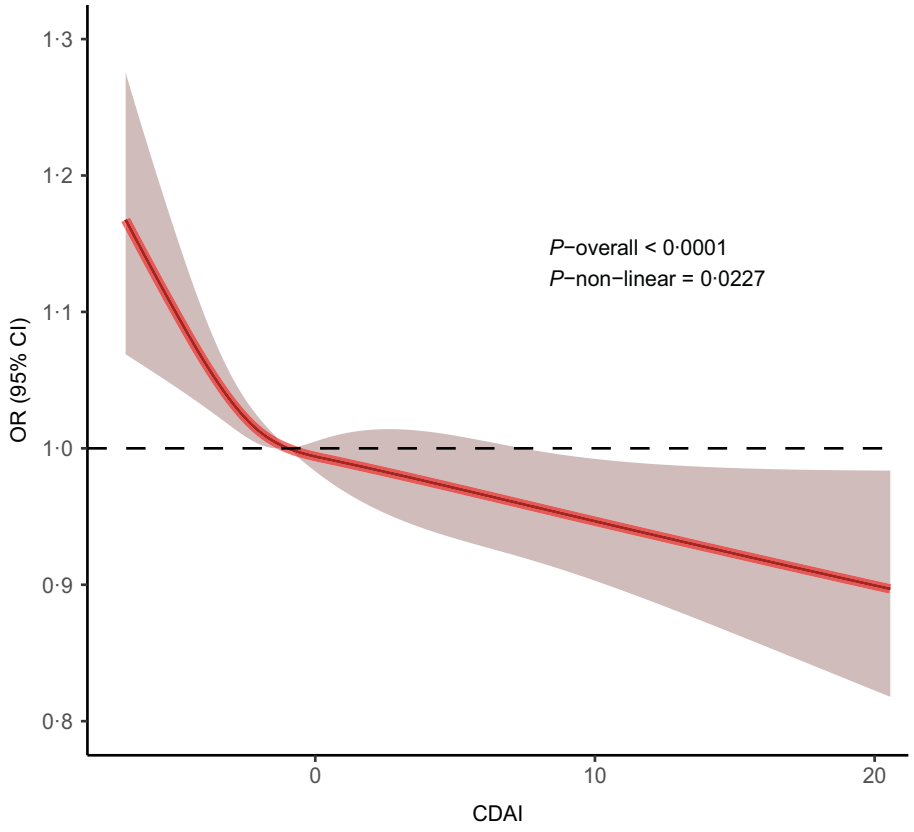


Figure 2. The restricted cubic spline for the association between CDAI and *H. pylori* infection in Model 3. Dose–response associations of CDAI with *H. pylori* infection. Adjusted for sex, age, race/ethnicity, education, alcohol consumption and smoking status. $P_{non\text{-}linear} < 0{\cdot}05$ was regarded as statistically significant. CDAI, composite dietary antioxidant index.

Table 3. Threshold effect analysis of CDAI on *Helicobacter pylori* infection (OR and 95 % CI)

Cut-off point	OR	95 % CI	*P*	logLR	*P* for logLR test
CDAI				4·62	**0·0328**
< **0·324**	0·84	0·75, 0·94	**0·006**		
≥ **0·324**	0·96	0·92, 1·01	0·092		

CDAI, composite dietary antioxidant index; logLR, log-likelihood ratio.
Bold values highlight those values less than 0.05.

MR analysis. However, the results were consistent with our previous findings that there was no evidence for association of serum Cu levels with *H. pylori* infection, as seen in online Supplementary Figures 2–6.

Discussion

In this study, a comprehensive analysis of data from NHANES revealed that dietary intake of antioxidants, such as vitamin A, vitamin C, vitamin E, carotene, Zn, Se and Cu, was inversely associated with the odds of *H. pylori* infection. CDAI emerged as a protective factor against the development of *H. pylori* infection, even after accounting for all covariates. Individuals with higher CDAI scores exhibited a reduced odds of *H. pylori* infection. In addition, further analysis showed an inverse relationship between Cu intake and *H. pylori* infection, after adjusting for all confounding factors. Some research found a significant inverse association between circulating levels of Cu and the odds of gastrointestinal infection, where the standard deviation increase in blood levels of Cu was associated with an OR of gastrointestinal infections of 0·91 (95 % CI: 0·87, 0·97) ($P < 0{\cdot}01$)[29].

Oxidative stress, characterised by the imbalance between antioxidant and pro-oxidant production, can exacerbate tissue and organ damage. The buildup of reactive oxygen species can trigger the oxidation of various molecules, including DNA, proteins, carbohydrates and lipids, ultimately culminating in apoptosis and dysfunction of organs. Exposure to *H. pylori* toxins causes a series of carcinogenic events, including generation of gastric oxidative stress, reactive aldehyde formation, hypermethylation of DNA promoter genes, damage to DNA and RNA, host inflammatory response, chronic inflammation, achlorhydria, failure of antioxidant protection in the mucosa and synergistic interactions with other carcinogens. *H. pylori* colonisation provokes chronic inflammation and the sustained release of reactive oxygen species from gastric tissues, which can eventually lead to the development of peptic ulcers or cancer in a subset of infected hosts[30].

Diet has been identified as playing a crucial role in the development of *H. pylori* infection by influencing the redox status and providing protection against reactive oxygen species and reactive nitrogen species, as shown in previous studies. Intake of antioxidants may prevent oxidative stress by scavenging oxidants and subsequently maintain a steady biological redox status, preventing inflammation, atherosclerosis, insulin resistance and other medical conditions[31–34]. CDAI is a measure of total antioxidant levels in the diet and has been widely used in numerous studies. High CDAI score has been linked to reduced levels of inflammatory factors and a decreased risk of various diseases, including hypertension, lung cancer, non-alcoholic fatty liver disease, DM, depression and chronic kidney disease[35–37]. However, there is limited research that explores the relationship between CDAI and the risk of *H. pylori* infection.

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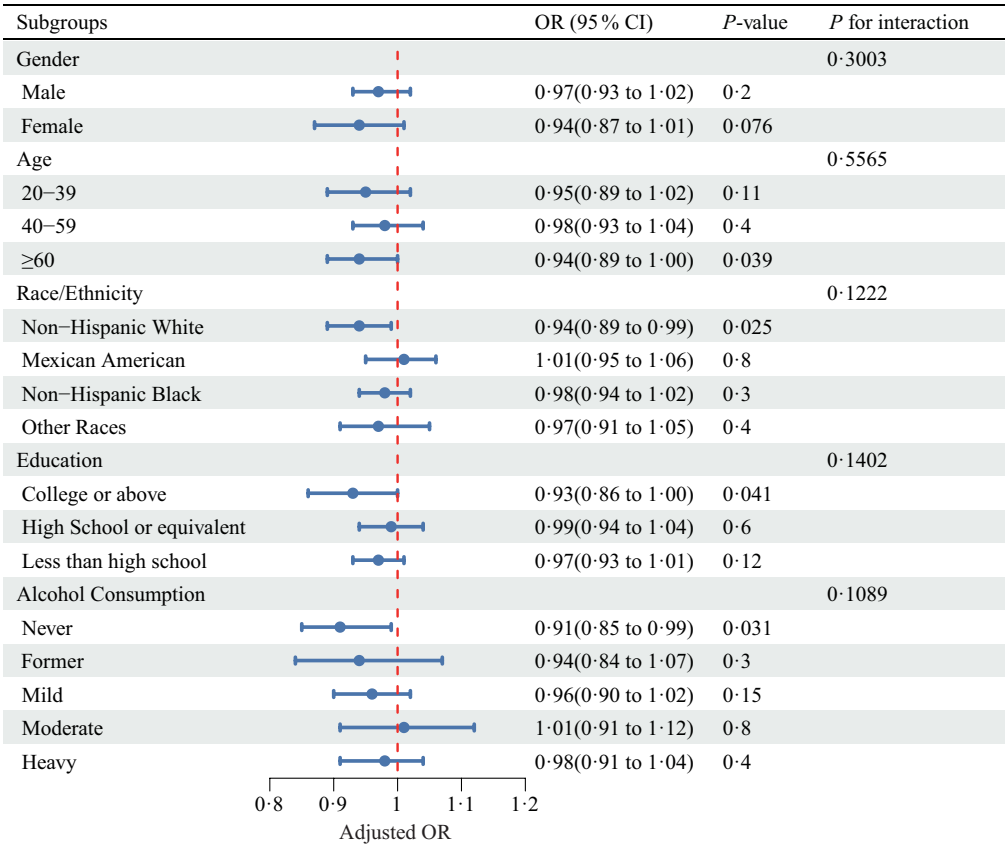


Figure 3. Subgroup analysis of the association between CDAI and *H. pylori* infection. Each stratum was adjusted for age, sex, race/ethnicity, education, alcohol consumption and smoking status. CDAI, composite dietary antioxidant index.

Table 4. Association of seven components of composite dietary antioxidant index and *Helicobacter pylori* infection (OR and 95 % CI)

	Model 1			Model 2			Model 3		
Components	OR	95 % CI	*P*	OR	95 % CI	*P*	OR	95 % CI	*P*
Vitamins A	0·999981	0·999 965, 0·999 997	**0·021**	0·999984	0·999 971, 0·999 996	**0·021**	0·999986	0·999 970, 1·000002	0·073
Vitamins C	0·999290	0·998 295, 1·000286	0·148	0·999153	0·997 917, 1·000391	0·145	0·999544	0·997 764, 1·001328	0·475
Vitamins E	0·96	0·94, 0·98	**0·001**	0·978607	0·952 969, 1·004935	0·093	0·98	0·96, 1·01	0·169
Carotene	0·999850	0·999 685, 1·000016	0·072	0·999868	0·999 733, 1·000002	0·053	0·999888	0·999 716, 1·000059	0·128
Zn	0·97	0·95, 0·99	**0·005**	0·984532	0·966 231, 1·003181	0·088	0·99	0·97, 1·01	0·166
Se	0·998069	0·995 981, 1·000161	0·068	0·999274	0·996 854, 1·001701	0·492	1·000366	0·997 004, 1·003739	0·752
Cu	0·67	0·53, 0·84	**0·002**	0·77	0·61, 0·96	**0·029**	0·750477	0·565 112, 0·996 643	**0·049**

Model 1 was adjusted for none.
Model 2 was adjusted for sex, age, race and education.
Model 3 was adjusted for sex, age, race, education, energy, alcohol consumption and smoking status.
Bold values highlight those values less than 0.05.

In 1994, the WHO and the International Agency for Research on Cancer classified *H. pylori* as a class 1 carcinogen[6]. *H. pylori* is transferred by the oral–oral and fecal–oral routes, leading to intergenerational spread in families. It was reported that the prevalence of *H. pylori* in economically limited countries was closely correlated to the social status of individuals, as poor nutrition, overcrowding and inadequate sanitation contributed to the increase in colonisation rates[38]. Thus, an appropriate dose of antioxidants should be administered with caution. The judicious prescription of dietary antioxidants could potentially be a beneficial strategy against *H. pylori* infection.

CDAI was used to estimate the combined exposure of seven dietary antioxidants, revealing a potential non-linear correlation between antioxidant intake and *H. pylori* infection. The odds of *H. pylori* infection gradually decreased with the higher CDAI according to our outcomes. What's more, the results of the two-piecewise linear regression suggested that the intake of antioxidants could significantly decrease the odds of *H. pylori* infection in those with lower CDAI scores. Several studies state that vitamin C may avoid initial colonisation by *H. pylori* organisms in the stomach but may also be valuable in eradication therapy for established *H. pylori* gastritis[39]. In a former case–control study,

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high intakes of these antioxidant vitamins (vitamins A, C and E) showed a tendency to decrease gastric cancer risk regardless of *H. pylori* infection[40].

The antioxidants with free radical scavenging activities inhibit the growth of *H. pylori*[41]. Due to the abundant synthesis of urease, *H. pylori* is capable of neutralising gastric acid, thereby facilitating its colonisation of the gastric epithelium.[42] Components of CDAI contribute to the inhibition of colonisation of *H. pylori*. For example, vitamin C suppresses urease activity and stimulates the immune system. Zn compounds, including Zn L-carnosine and polaprezinc, prevent *H. pylori* adhesion to the gastric epithelial cells and potentially reduce its virulence[43,44].

However, establishing causal relationships between CDAI and *H. pylori* infection is challenging due to the intricate nature of their association. Thus, not only did we use the large-scale representative observational cohort, but we also performed the MR analysis to identify a possible causal association. Our MR results validated that genetically predicted higher circulating Cu levels may not reduce the odds of *H. pylori* infection. Studies also showed that no significant difference was found between *H. pylori* infection and non-infection in adults[45,46]. These results are compatible with our study, which revealed that there was no significant causal relationship between serological Cu levels and *H. pylori* infection. Besides the circulating status in the bloodstream, Cu is also present in micromolar concentration at the lumen of the stomach[47]. Cu toxicity exploited by macrophages, poisons bacteria presumably by inducing Fenton-like reactions, which produce hydroxyl radicals[48,49]. These may provide some mechanical support for our findings, which indicated a negative association between the odds of *H. pylori* infection and dietary Cu intake levels. On the other hand, infection may indirectly influence the CDAI if it changes a person's food intake patterns during illness, leading to lower antioxidant consumption. However, the CDAI itself is not altered by the infection because the index is based on dietary intake as reported by the individuals[15].

To the best of our knowledge, this large, nationally representative study was the first investigation to explore the association between CDAI and *H. pylori* infection. Furthermore, using the national sample data from NHANES, this study can provide antioxidant intake recommendations for public health. However, there are still limitations in our study. First, only the dietary data of individuals with baseline characteristics were investigated. Recalled bias may be inevitable because of the variety of self-reported dietary status or lifestyle. Secondly, the presence of residual or unmeasured confounding variables, which cannot be eliminated, could influence the relationship between CDAI and *H. pylori* infection. Moreover, the study's inability to differentiate between current and past *H. pylori* exposure using serological tests poses a challenge, as antibodies can persist for months even after *H. pylori* eradication therapy. Finally, as the *H. pylori* infection data were only available for 1999 and 2000 in NHANES, a larger sample size and more recent data would be further necessary to substantiate the outcomes.

Conclusion

We explored the non-linear negative correlation between CDAI and *H. pylori* infection, using the representative sample data from NHANES. Our conclusions highlight that the CDAI provides valuable insights for the assessment of the dietary strategies of individuals for the protection of *H. pylori* infection. Conducting prospective large-scale research is crucial to offer more robust evidence supporting the relationship between CDAI and *H. pylori* infection.

Supplementary material. For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114525103693

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The authors declare that there are no commercial relationships that can be construed as conflicts of interest in this research.

Ethical review and approval are unnecessary for this study since all the data from NHANES and MR are publicly accessible.

All authors have reviewed the final version of the manuscript and agreed on the publication.

The raw data supporting the conclusions of this article are available at: https://www.cdc.gov/nchs/nhanes/ and https://gwas.mrcieu.ac.uk.

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