Association Between Composite Dietary Antioxidant Index and Helicobacter pylori Infection: A Population-based and Mendelian Randomization Study

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Abbreviation

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

BMI: Body mass index

CDAI: Composite dietary antioxidant index

CKD: Chronic kidney disease

CRP: C-reactive protein

DM: Diabetes Mellitus

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DBP: Diastolic blood pressure

ELISA: Enzyme-linked immunosorbent assay

FPG: Fasting plasma glucose

GWAS: Genome-wide association study

IVW: Inverse variance weighted

MR: Mendelian Randomization

NHANES: National Health and Nutrition Examination Survey

OD: Optical density

OR: Odds ratio

PIR: Poverty income ratio

RCT: Randomized controlled trial

RCS: Restricted cubic spline

ROS: Reactive oxygen species

SCr: Serum creatinine

SD: Standard deviation

SBP: Systolic blood pressure

SNP: Single-nucleotide polymorphism

TAC: Total antioxidant capacity

TC: Total cholesterol

TG: Triglycerides

UCr: Urine creatinine

WHO: World Health Organization

95% CI: 95% confidence interval

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* (*H. pylori*) infection using cross-sectional design. In this study, participants from the 1999-2000 National Health and Nutrition Examination Survey (NHANES) were analyzed using logistic and Cox regression analyses to assess the associations between *H. pylori* infection and CDAI, encompassing vitamin A, vitamin C, vitamin E, carotene, zinc, selenium, and copper. 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 randomization (MR) study was conducted utilizing genome-wide association study (GWAS) summary statistics to explore the causal relationship between antioxidant levels and *H. pylori* infection. We found that the intake of copper was a protective factor in the occurrence of *H. pylori* infection but did not support a causal association between circulating copper 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 socioeconomic status, smokers, diabetes, and those with hypertension. The study suggests that higher CDAI is linked to a 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.

Keywords: Composite dietary antioxidant index; *Helicobacter pylori*; NHANES; Population-based study; Mendelian Randomization analysis

1 Introduction

Helicobacter pylori (*H. pylori*), a gram-negative bacterium, colonizes 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 United States (U.S.), 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 World Health Organization (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 (ROS) 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 (TAC), encompassing various vitamins and minerals with antioxidant properties (vitamin A, C, E, carotene, as well as the minerals selenium, copper and zinc) (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 NHANES database comprises a series of cross-sectional surveys conducted every two years, representing samples of the non-institutionalized civilian population of the U.S. Since 1999, NHANES has gathered a diverse array of data to evaluate the health status of the U.S. population, including demographic information, dietary patterns, socioeconomic factors, health-related questionnaires, physical and physiological examination results, and extensive laboratory findings. This data is 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 randomization (MR) analysis is an emerging epidemiological method that serves as a complementary approach to randomized controlled trials (RCTs). MR analyses are frequently utilized 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 randomized allocation of genetic variants. Consequently, we sought to explore the potential association between CDAI and *H. pylori* infection using data from NHANES 1999-2000 and MR analysis.

2 Materials and methods

2.1 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 Fig. 1.

2.2 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 normalized by subtracting the means and then dividing by their standard deviation (SD), including vitamin A, vitamin C, vitamin E, carotene, zinc, selenium, and copper (15):

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

2.3 *H. pylori* infection status

Serologic test results from human subjects were collected by NHANES using an enzyme-linked immunosorbent assay (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 (OD) of the collected specimens was utilized to determine the immune status ratio of individuals. Standard cut-off values were employed to classify participants as *H. pylori*-positive (ISR≥ 1.1) or *H. pylori*-negative (ISR < 0.9) (19). Ambiguous values (0.9-1.1) were excluded to ensure precise statistical outcomes in subsequent analyses.

2.4 Covariates

Information about covariates was obtained using standardized survey questionnaires and examinations. Items included age, sex, poverty income ratio (PIR), race/ethnicity, education, body mass index (BMI), smoking status, alcohol consumption, diabetes mellitus (DM), hypertension, daily energy intake level, triglyceride (TG), total cholesterol (TC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum creatinine (SCr), urine creatinine (UCr), C-reactive protein (CRP) and CDAI.

We categorized 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 days (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 12 drinks. Former drinking included those who had consumed more than 12 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 (FPG) ≥ 126 mg/dL, hemoglobin A1C concentration ≥ 6.5%, or use of diabetes medication, were identified with DM (22). Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg, or as having an intake of antihypertensive medication (23). The biochemical examination profiles such as energy, TG, TC, ALT, AST, SCr, UCr, CRP, and CDAI were included in the baseline data collection.

2.5 **MR 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 online GWASs. 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 utilized data derived from a comprehensive population-based meta-analysis conducted within the UK Biobank cohort to examine GWAS statistics pertaining to *H. pylori* infection (25). Exposure data concerning copper levels were gathered from a cohort comprising 2603 Australian and 2874 British participants. Through GWAS analysis employing inductively coupled plasma mass spectrometry, we pinpointed two significant loci on chromosome 1 associated with erythrocyte copper levels (26). Detailed insights into the GWAS studies incorporated in the MR analysis can be found in Supplementary Table 1.

We included independent SNPs (r2 < 0.001 within 10,000-kb windows), strongly associated ($P \leq 5\times10^{-8}$) with the serological copper level for the main MR analysis. The F- statistic was estimated to quantify instrument strength for each exposure and we excluded SNPs with an F statistic < 10 to reduce the risk of weak instrument bias (27, 28). The remaining 2 SNPs were selected as instrumental variables (Supplementary Table 2). Associations correspond to an OR for the outcome increase in the genetically predicted concentrations of copper. $P < 0.05$ was considered statistically significant.

2.6 **Statistical Analyses**

Data analyzing and graphing were performed using R software (4.1.1), with the value of two-tailed $P < 0.05$ 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 odds ratios (ORs) and 95 % confidence intervals (CIs) for the association between CDAI and *H. pylori* infection status. CDAI was quartile stratified into four categories and analyzed using generalized 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 utilizing 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 (RCS) was utilized to explore the nonlinearity. 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 copper and *H. pylori* infection. As only two instruments were used for copper, 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 $P < 0.05$.

3 Results

3.1 General population characteristics of study subjects according to *H. 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 socioeconomic 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 has the lowest proportion of *H. pylori*-positive individuals, compared to 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 TG and CRP than the *H. pylori*-negative group. However, there were no significant differences between the two groups in terms of TC, ALT, AST, SCr, and UCr 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)).

3.2 Association between CDAI and *H. pylori* Infection

When analyzed 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 ORs 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 to those with CDAI levels in Q1 (-7.62, -2.98), with *P-trend* less than 0.05. Further, ORs 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 to 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 ORs 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 RCS curves (Fig. 2). A two-piecewise linear regression was employed to analyze 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).

3.3 **Subgroup analysis of the influence of various variables on *H. 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 (Fig. 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).

3.4 **Causal relationship between components of CDAI and *H. 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, zinc, and copper with *H. pylori* infection in Model 1. However, after adjusting for all confounders, only copper intake levels showed a negative association with the occurrence of *H. pylori* infection, with an OR of 0.750477 (95% CI: 0.565112, 0.996643; $P = 0.049$).

After identifying a significant negative correlation between copper intake levels and the odds of *H. pylori* infection in the previous multivariable regression analysis, a MR analysis was performed to investigate the causal effects of copper levels on *H. pylori* infection. Following the selection criteria for SNPs, only 2 SNPs met the criteria for further analysis. There was evidence of heterogeneity of IVW analysis for copper (Q = 7.181939; $P < 0.05$). Therefore, the multiplicative random-effects IVW method was utilized to evaluate the causal association while adjusting for measured heterogeneity. The analysis revealed no significant causal relationships between serological copper 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) (Supplementary Fig. 1). Further, we set the cutoff ($P < 1\times10^{-5}$) to include more SNPs in our MR analysis. However, the results were consistent with our previous findings that there was no evidence for association of serum copper levels for *H. pylori* infection, seen in Supplementary Fig. 2-6.

4 **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, zinc, selenium, and copper, 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 copper intake and *H. pylori* infection, after adjusting for all confounding factors. Some research found a significant inverse association between circulating levels of copper and the odds of gastrointestinal infection, where the standard deviation increase in blood levels of copper was associated with an OR of gastrointestinal infections of 0.91 (95% CI: 0.87, 0.97) ($P < 0.01$) (29).

Oxidative stress, characterized by the imbalance between antioxidant and pro-oxidant production, can exacerbate tissue and organ damage. The buildup of ROS 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 of DNA and RNA, host inflammatory response, chronic inflammation, achlorhydria, failure of antioxidant protection in the mucosa, and synergistic interactions with other carcinogens. *H. pylori* colonization provokes chronic inflammation and the sustained release of ROS 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 ROS 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 (CKD) (35-37). However, there is limited research that explores the relationship between CDAI and the risk of *H. pylori* infection.

In 1994, the WHO and the International Agency for Research on Cancer (IARC) 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 colonization 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 colonization 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, 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 neutralizing gastric acid, thereby facilitating its colonization of the gastric epithelium (42). Components of CDAI contribute to the inhibition of colonization of *H. pylori*. For example, vitamin C suppresses urease activity and stimulates the immune systems. Zinc compounds, including zinc 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 copper 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 copper levels and *H. pylori* infection. Besides the circulating status in the bloodstream, copper is also present in micromolar concentration at the lumen of the stomach (47). Copper toxicity exploited by macrophages, poisons bacteria presumably by inducing Fenton-like reactions which produce hydroxyl radicals (48, 49). These may provide some mechanical supports for our findings which indicated a negative association between the odds of *H. pylori* infection and dietary copper 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 per se, 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 at 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.

5 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.

Availability of data and materials

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

Statements & Declarations

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Conflicts of Interests

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

Author Contributions

Peng Zou and Chengru Chen contributed to the design and conception of this study, as well as data acquisition and analysis. Fu Xiao and Yusong Wei were major contributors to research guidance. Xiaobin Wu supervised and reviewed the article. All authors contributed to the writing and revision of the manuscript.

Ethics Approval

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

Consent to publish

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

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Figure legends

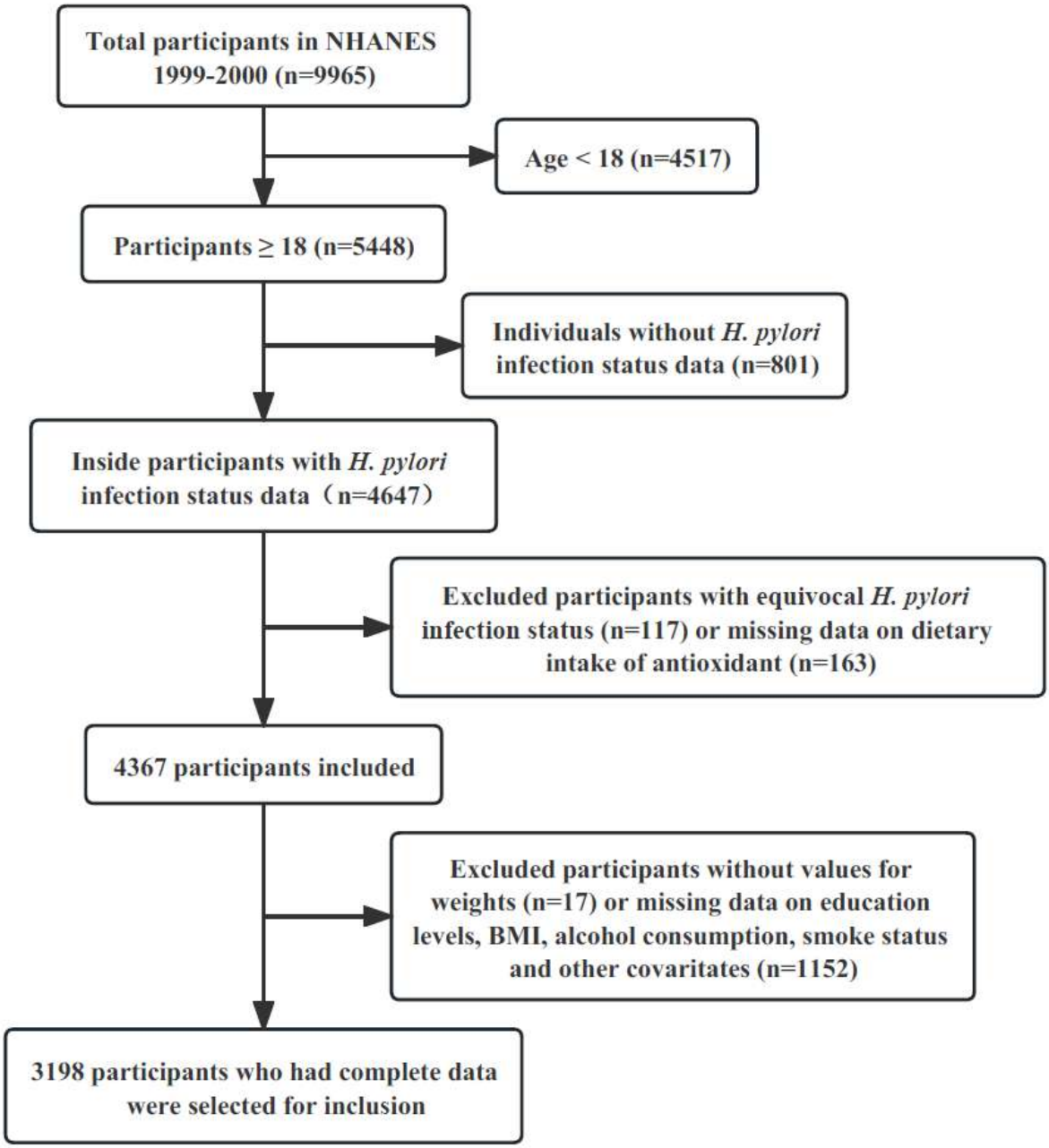


Fig. 1 Flow chart of sample selection from NHANES 1999-2000

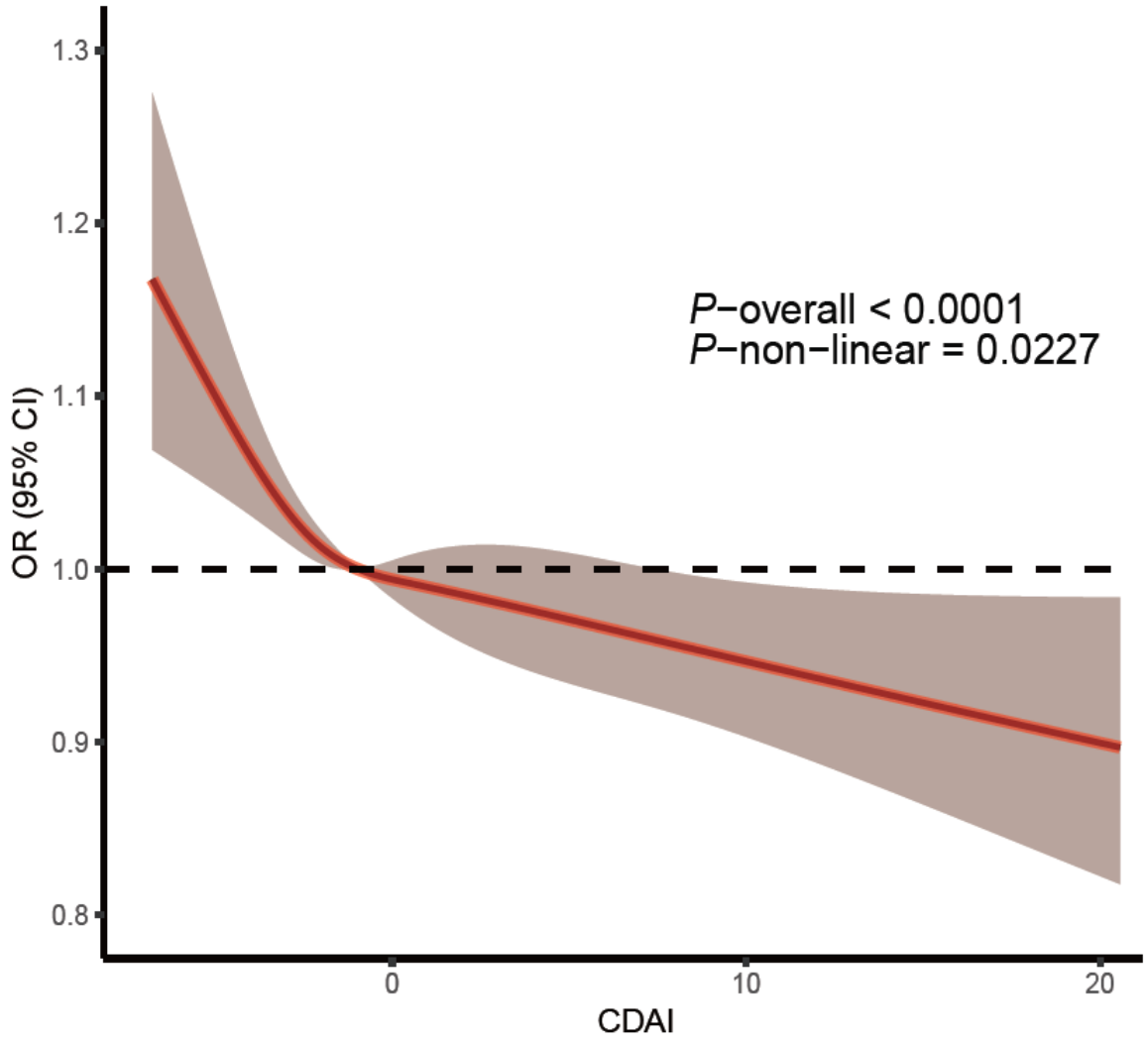


Fig. 2 The restricted cubic spline for the association between CDAI and *H. pylori* infection in Model 3

Dose-response associations of CDAI with *H. pylori* infection. Adjusted for sex, age, race/ethnicity, education, alcohol consumption, and smoking status. $P_{non\text{-}linear} < 0.05$ was regarded as statistically significant.

Subgroups	OR (95%CI)	*P*-value	*P* for interaction
Gender			0.3003
Male	0.97(0.93 to 1.02)	0.2	
Female	0.94(0.87 to 1.01)	0.076	
Age			0.5565
20−39	0.95(0.89 to 1.02)	0.11	
40−59	0.98(0.93 to 1.04)	0.4	
≥60	0.94(0.89 to 1.00)	**0.039**	
Race/Ethnicity			0.1222
Non−Hispanic White	0.94(0.89 to 0.99)	**0.025**	
Mexican American	1.01(0.95 to 1.06)	0.8	
Non−Hispanic Black	0.98(0.94 to 1.02)	0.3	
Other Races	0.97(0.91 to 1.05)	0.4	
Education			0.1402
College or above	0.93(0.86 to 1.00)	**0.041**	
High School or equivalent	0.99(0.94 to 1.04)	0.6	
Less than high school	0.97(0.93 to 1.01)	0.12	
Alcohol Consumption			0.1089
Never	0.91(0.85 to 0.99)	**0.031**	
Former	0.94(0.84 to 1.07)	0.3	
Mild	0.96(0.90 to 1.02)	0.15	
Moderate	1.01(0.91 to 1.12)	0.8	
Heavy	0.98(0.91 to 1.04)	0.4	

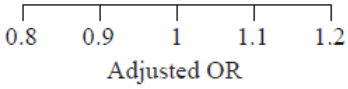
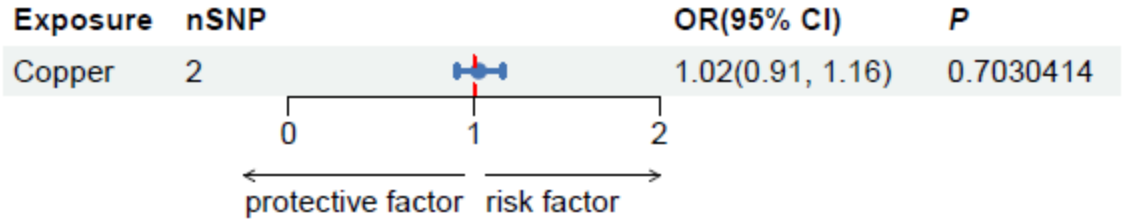


Fig. 3 Subgroup analysis of the association between CDAI and *H. pylori* infection

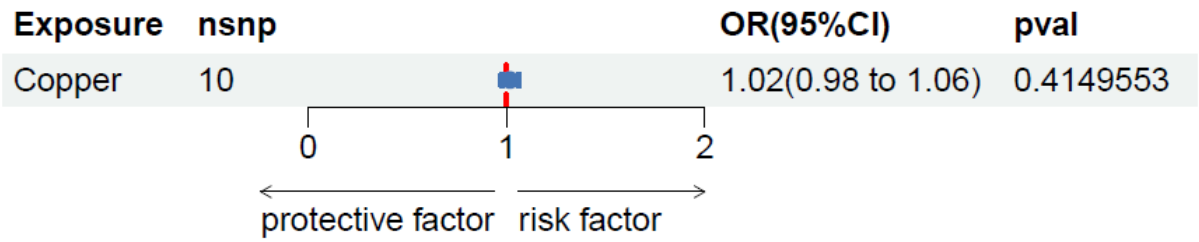
Each stratum was adjusted for age, sex, race/ethnicity, education, alcohol consumption, and smoking status.

Supplementary figure legends

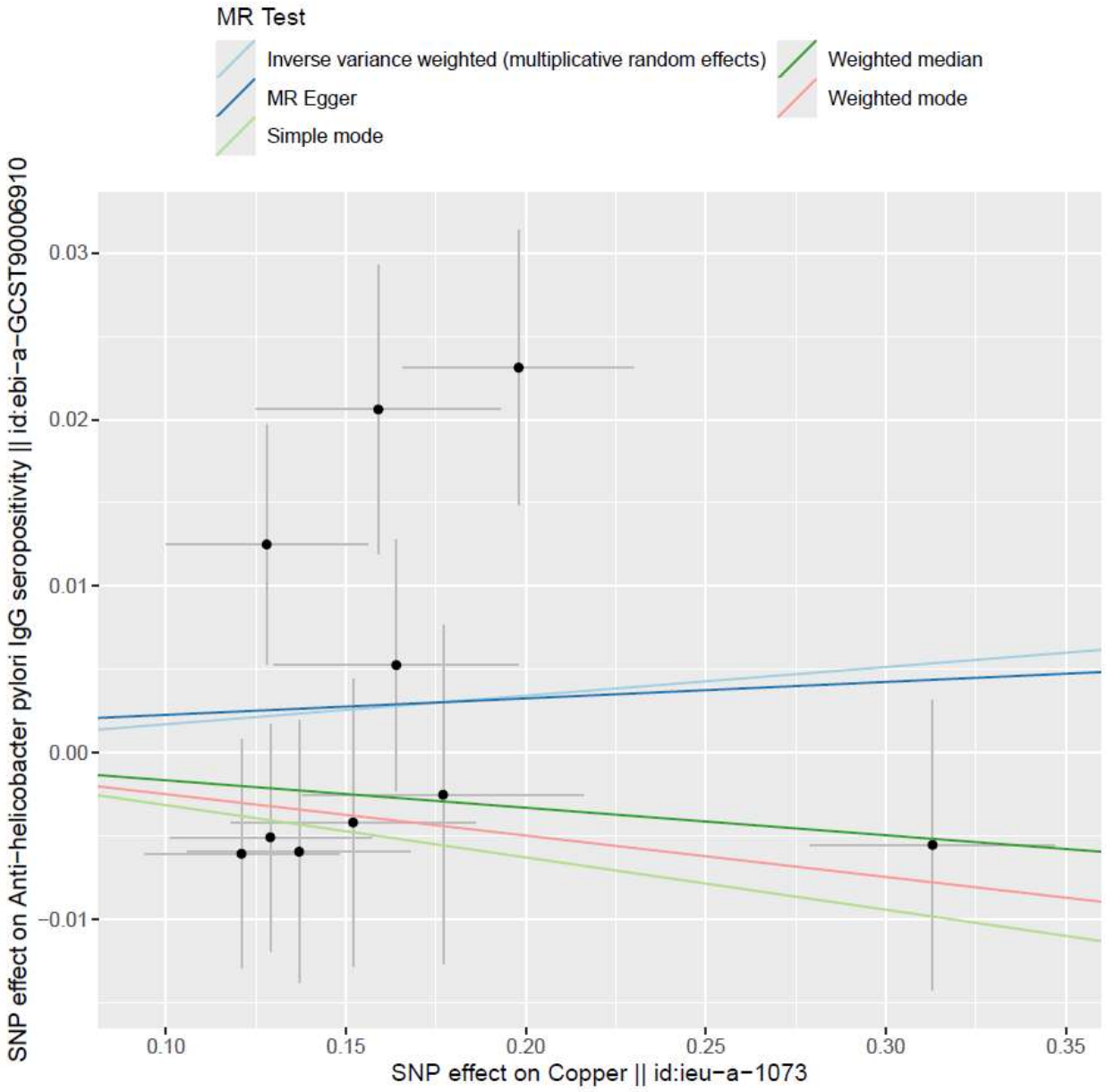


Supplementary Fig. 1 MR results of the association between circulating levels of copper and risk of *H. pylori* infections (threshold: $P < 5\times10^{-8}$)

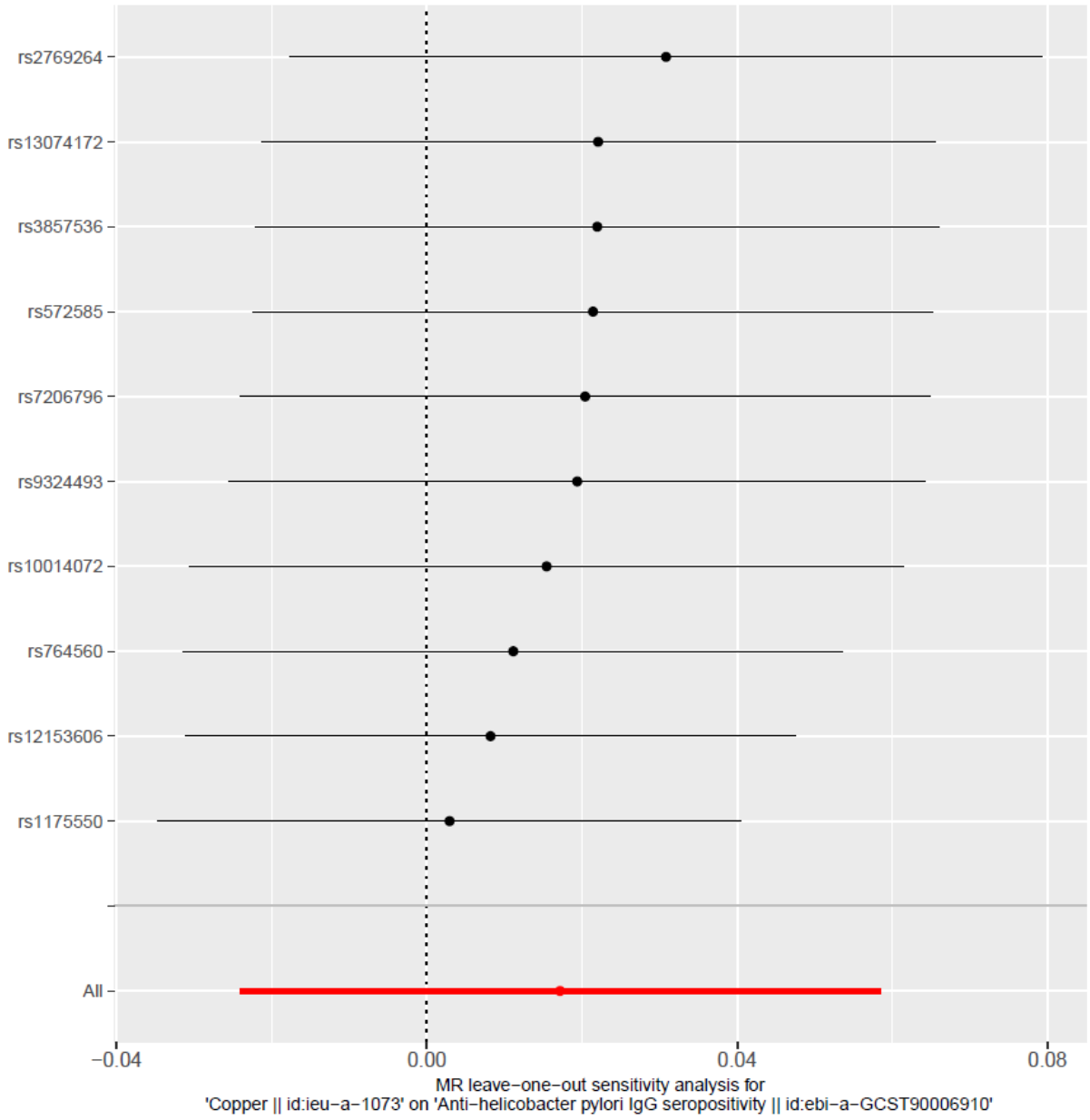
MR, Mendelian randomization; nSNP, Number of single nucleotide polymorphisms; OR, Odds Ratio; CI, confidence interval.



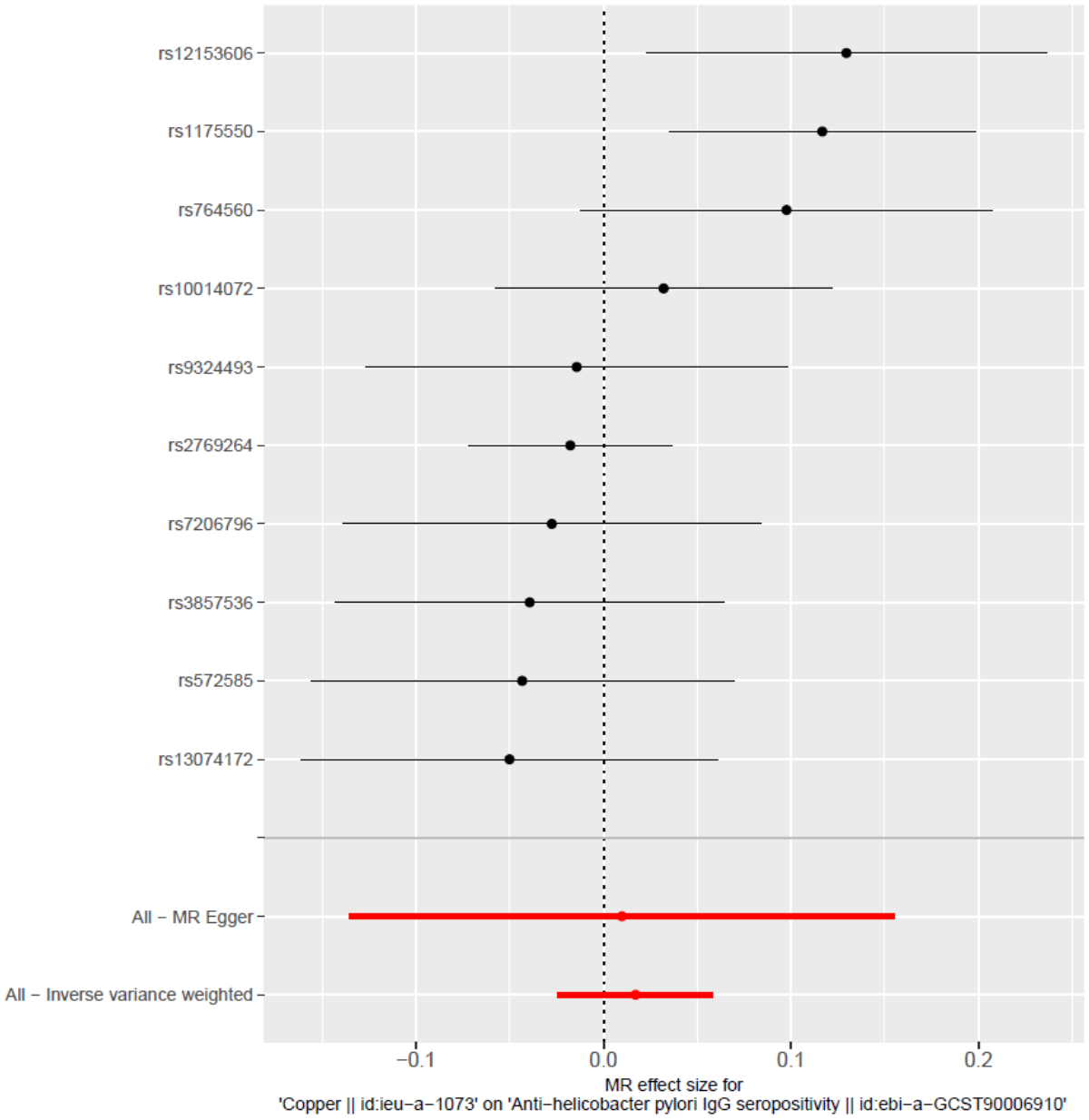
Supplementary Fig. 2 MR results of the association between circulating levels of copper and risk of *H. pylori* infections (threshold: $P < 1\times10^{-5}$)



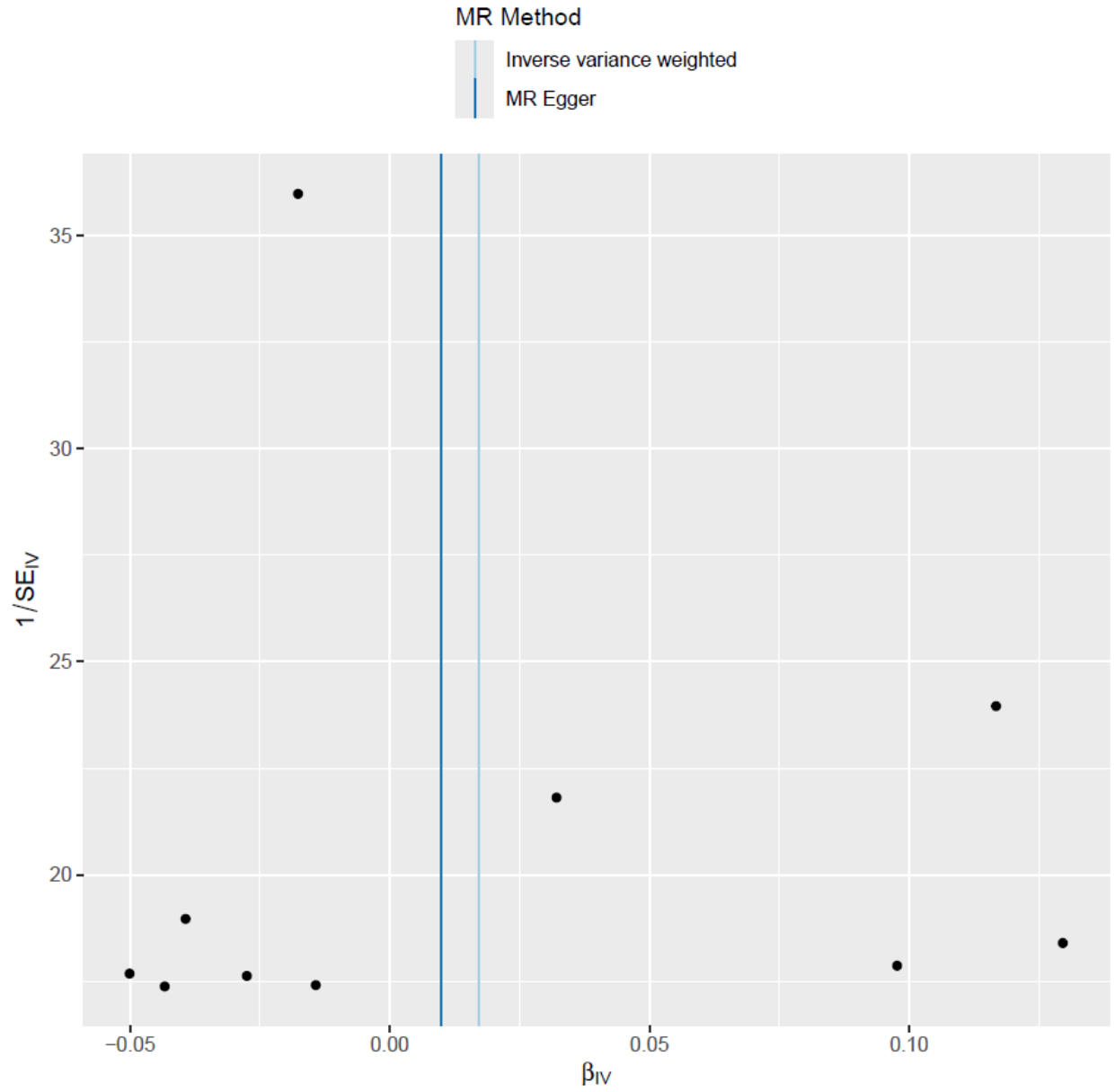
Supplementary Fig. 3 The scatter plot of MR analysis of causal associations between each circulating levels of copper SNPs and *H. pylori* infection (threshold: $P < 1\times10^{-5}$)



Supplementary Fig. 4 Leave-one-out method for MR analysis (threshold: $P < 1\times10^{-5}$)



Supplementary Fig. 5 Forest plot of MR analysis (threshold: $P < 1\times10^{-5}$)



Supplementary Fig. 6 Funnel plot of MR analysis (threshold: $P < 1\times10^{-5}$)

Table legends:

Tables

Table 1 Baseline characteristics of participants with different *H. pylori* infection status in NHANES 1999-2000 analyses (n = 3198)

Characteristic	Overall, n = 3198 (100%)	*H. pylori* Infection Status		*P*
		Hp Negative, n = 1834 (71%)	*Hp* Positive, n = 1364 (29%)	
Age (years)	42 (17)	43 (16)	49 (17)	**< 0.001**
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**
Energy (kcal/day)	2215 (1056)	2287 (1037)	2042 (1084)	**< 0.001**
Triglycerides (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; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; CDAI, composite dietary antioxidant index.

Table 2 Associations between intake of antioxidant levels and *H. pylori* infection

Variable	***H. pylori* Infection OR (95% Cl)**						
	Continuous Variable		**Categorical variable**				
	CDAI	***P***	**Q1**	**Q2**	**Q3**	**Q4**	***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 (0.34, 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, education

Model 3 was adjusted for sex, age, race, education, energy, alcohol consumption, smoking status

Table 3 Threshold effect analysis of CDAI on *H. pylori* infection

Cut-off point	OR (95% Cl)	*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		

OR, odds ratio; Cl, confidence interval; logLR, Log-likelihood ratio

Table 4 Association of seven components of composite dietary antioxidant index and *H. pylori* infection.

	Model 1		Model 2		Model 3	
Components	**OR (95% Cl)**	***P***	**OR (95% Cl)**	***P***	**OR (95% Cl)**	***P***
Vitamins A	0.999981 (0.999965, 0.999997)	**0.021**	0.999984 (0.999971, 0.999996)	**0.021**	0.999986 (0.999970, 1.000002)	0.073
Vitamins C	0.999290 (0.998295, 1.000286)	0.148	0.999153 (0.997917, 1.000391)	0.145	0.999544 (0.997764, 1.001328)	0.475
Vitamins E	0.96 (0.94, 0.98)	**0.001**	0.978607 (0.952969, 1.004935)	0.093	0.98 (0.96, 1.01)	0.169
Carotene	0.999850 (0.999685, 1.000016)	0.072	0.999868 (0.999733, 1.000002)	0.053	0.999888 (0.999716, 1.000059)	0.128
Zinc	0.97 (0.95, 0.99)	**0.005**	0.984532 (0.966231, 1.003181)	0.088	0.99 (0.97, 1.01)	0.166
Selenium	0.998069 (0.995981, 1.000161)	0.068	0.999274 (0.996854, 1.001701)	0.492	1.000366 (0.997004, 1.003739)	0.752
Copper	0.67 (0.53, 0.84)	**0.002**	0.77 (0.61, 0.96)	**0.029**	0.750477 (0.565112, 0.996643)	**0.049**

Model 1 was adjusted for none.

Model 2 was adjusted for sex, age, race, education.

Model 3 was adjusted for sex, age, race, education, energy, alcohol consumption, smoking status.

Supplementary Table 1 Summary data of GWAS enrolled in the MR study.

Items	GWAS ID	PMID	Sample size	nSNP	Population
copper	ieu-a-1073	23720494	2603	2543646	Europeans
Anti-*H. pylori* IgG seropositivity	ebi-a-GCST90006910	33204752	8734	9170312	Europeans

GWAS: Genome-wide association study; MR: Mendelian randomization; nSNP, number of Single nucleotide polymorphisms.

Supplementary Table 2 Effect estimates of the associations between genetic instrumental variables for copper and risk of *H. pylori* infection

SNP	Chromosome	effect allele	non-effect allele	F statistic	Exposure			Outcome		
					β	SE	*P*	β	SE	*P*
rs1175550	chr1:3691528	G	A	38.28516	0.198	0.032	5.03×10^{-10}	0.0231167	0.0082652	0.00516012
rs2769264	chr1:151344741	G	T	84.74827	0.313	0.034	2.63×10^{-20}	-0.005525	0.0087003	0.525431