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# Correlation between dietary vitamin B2 intake and helicobacter pylori infection in US adults: a cross-sectional study



### Abstract

**Objectives** Helicobacter pylori (H. pylori) infection is a widely recognized factor in causing inflammation in the stomach and significantly increases the likelihood of developing gastric cancer. The relation between intake of vitamin B2 in the diet and testing positive for Helicobacter pylori is still unclear.

**Methods** This cross-sectional study used data from the NHANES conducted in the United States in 1999–2000. Vitamin B2 consumption was measured from one 24-hour recall interview. H. pylori seropositivity was confirmed through an ELISA test measuring serum IgG protein levels. Multivariable logistic regression models, subgroup analysis and sensitivity analysis were performed to evaluate the possible association between dietary vitamin B2 consumption and H. pylori seropositivity.

**Results** Out of the total 2,859 participants, 1,257 had H. pylori seropositivity, with males making up 47.5% and the mean age was 49.7 years old. There was a significant inverse association between vitamin B2 intake and H. pylori [odds ratio (OR): 0.88, 95% confidence interval (CI) (0.78–0.99), p = 0.031], after full adjustment for covariates. When dietary vitamin B2 analyzed as a categorical variable, the risk of H. pylori seropositivity in the highest quartile Q4 group decreased by 39% compared to lowest quartile Q1 (OR 0.61, 95% CI 0.44–0.86, p = 0.004, Model 3). The findings of subgroup analysis and sensitivity analysis were constant and dependable.

**Conclusion** This study implies that increased dietary vitamin B2 consumption may be related with a decreased incidence of Helicobacter pylori seropositivity.

Keywords Helicobacter pylori, Seropositivity, Vitamin B2, Riboflavin, NHANES

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#### Introduction

The gram-negative bacterium Helicobacter pylori (H. pylori), is known to cause infections in the human stomach and is closely linked to a number of digestive disorders, such as peptic ulcer disease, gastritis, and gastric tumor [1–4]. With an estimated 50% global incidence, it is most common in underdeveloped nations and some regions, such Africa and Latin America [5, 6]. The H. pylori prevalence is even higher than 80% in Jordan, Guatemala, Colombia, Nicaragua, Ecuador [7]. Exploring the changeable factors associated with H. pylori is crucial in lowering the chances of H. pylori infection. Recently, there has been an increasing focus on investigating the relationship between H. pylori seropositivity and micronutrient consumption [8, 9].

Vitamin B2, also known as riboflavin, which is water soluble, is a critical vitamin in mitochondrial energy metabolism. Vitamin B2 is essential for maintaining human health. Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) are the main coenzyme forms of dietary vitamin B2 found in food [10]. Exposure to UV light can trigger the activation of vitamin B2, leading to the targeted breakdown of pathogen DNA and RNA, as well as the prevention of virus, bacteria, and protozoa replication in blood products. Low dietary intake of vitamin B2 is a public health concern [11, 12]. Currently, vitamin B2 deficiency can lead to gastrointestinal problems [13], cognitive abnormalities [14], skin diseases [15], and metabolic diseases [14, 16-18]. It is crucial to include vitamin B2 in one's diet to avoid ariboflavinosis, a condition that can cause cheilitis, tongue sores, and a scaly rash in the genital area.

Despite interest in micronutrients and risk of H. pylori [8, 19], the association between vitamin B2 intake and H. pylori seropositivity remains understudied. To fill this gap, we performed the study using NHANES data from the 1999–2000 cycle to study the relationship between the consumption of vitamin B2 in the diet and the presence of H. pylori seropositivity. We hypothesize that vitamin B2 intake is associated with H. pylori seropositivity.

#### **Materials and methods**

#### Study design and participants

Every two years, the NHANES study employs a sophisticated approach to choose a sample that accurately represents the U.S. population [20]. The primary objective is to assess the well-being and dietary condition of people in the United States. Approval for the survey protocol has been granted by The National Center for Health Statistics Institutional Review Board in order to uphold ethical standards. Furthermore, prior to being included in the study, all participants gave written informed consent. NHANES gathers a broad spectrum of information, such as demographics, dietary habits, medical examination outcomes, laboratory results, and questionnaire responses [21]. The information used in this research is accessible on the NHANES site, and does not require any ethical evaluation.

In the NHANES 1999-2000 cycle, 9,965 individuals participated in the study. Figure 1 displays the 2,859 participants who were 20 years old or above in the study sample. A total of 7,106 subjects were excluded from the study: 5,085 due to being under the age of 20, 735 due to incomplete H. pylori data, and 626 due to incomplete dietary vitamin B2 data. Additionally, 1,141 subjects were excluded due to missing data for covariates, including marital status (n = 438), poverty-income ratio (PIR; n = 537), smoking status (n = 8), educational level (n = 13), alcohol use (n = 137), hypertension (n = 42), diabetes (n = 50), and body mass index (BMI; n = 37). Ultimately, 2,859 participants were included in the final analysis. All protocols adhered to the principles outlined in the Declaration of Helsinki and followed the guidelines of the STROBE initiative for reporting observational studies in epidemiology.

#### **Dietary vitamin B2 intake**

Nutritional intake assessments were carried out during face-to-face interviews at the NHANES mobile examination centers. Participants underwent two 24-hour dietary recalls to gather dietary intake data. Interviewers who were trained conducted the first recall interview in person at the Mobile Examination Center (MEC). The follow-up interview occurred either over the phone or through mail within a timeframe of 3 to 10 days. Dietary evaluations were conducted by averaging the information from these two recalls (26). Dietary vitamin B2 intake on the first day was analyzed in accordance with NHANES analytic guidelines [22]. Vitamin B2 intake was analyzed by considering it in both continuous and categorical forms (Q1-Q4). Vitamin B2 intake quartiles (Q1-Q4) were established by dividing the distribution of vitamin B2 intake into four sections, representing varying levels of intake from low to high. The quartile ranges are as follows: Q1 (<1.14), Q2 (1.14-1.64), Q3 (1.65-2.33), and Q4 (>2.33).

#### H. pylori antibody measurement

Blood samples were taken from individuals and frozen at -80 °C before being analyzed at the University of Washington following the NHANES guidelines. H. pylori IgG antibodies were tested with an ELISA kit from Wampole Laboratories in Cranbury, NJ to measure the level of antibodies [23]. Participants were categorized into two groups based on their H. pylori seropositivity using the standard ELISA cut-off value: seropositive (OD value  $\geq 0.9$ ) and seronegative (OD value < 0.9) [24, 25].



Fig. 1 Flowchart of the study

#### Covariates

The study considered several potential confounding factors by referencing prior research and utilizing clinical judgment. Included in these considerations were age, sex, marital status, poverty to income ratio (PIR), level of education, body mass index (BMI), smoking status, alcohol intake, diabetes, high blood pressure, and C-reactive protein (CRP). Age was displayed as a continuous factor, whereas gender was evaluated as a categorical variable with options for male and female. Marital status was classified as either being married or living with a partner, or living alone. The PIR (a ratio of family income to poverty threshold) was utilized to assess socioeconomic status, categorizing individuals into low (PIR < 1.3) and high  $(PIR \ge 1.3)$  groups according to their PIR value compared to 1.3. Educational level was classified into three groups: less than 9 years, 9 to 12 years, and more than 12 years. BMI was classified into three categories: normal weight (BMI less than 25 kg/m2), overweight (BMI between 25 and 30 kg/m2), and obesity (BMI greater than 30 kg/ m2). Smoking status was classified as either non-smoking or smoking according to the response to the SMQ 020

(Smoked at least 100 cigarettes in life). Likewise, alcohol consumption was divided into non-alcohol consumption and alcohol consumption based on the answer to the questionnaire (ALQ100: Had at least 12 alcohol drinks/1 year? ). Diabetes diagnosis in the study was based on the Diabetes Questionnaire (DIQ) guestion DIQ010, which asked participants if they had ever been informed by a doctor or health professional that they have diabetes or sugar diabetes. Individuals who answered yes to the DIQ010 inquiry were categorized as having diabetes. In this research, hypertension was identified using question BPQ020 from the Blood Pressure & Cholesterol Questionnaire (BPQ), which asked participants if they had been diagnosed with hypertension by a healthcare professional. Participants responding affirmatively to the BPQ020 question were categorized as having hypertension. Laboratory measures included C-reactive protein (CRP).

#### Statistical analysis

The research separated the information into two groups: continuous and categorical variables. Continuous

variables were further categorized based on the distribution's normality. The Student's t-test was utilized for comparing continuous variables that followed a normal distribution, and were presented as mean with standard deviation. Variables that did not follow a normal distribution were presented as median with interquartile range (IQR) and compared using the Wilcoxon rank-sum test. Categorical variables expressed as percentages were compared using the chi-squared test. To evaluate the significance of variations among groups divided by quartiles of vitamin B2, either the Kruskal-Wallis test or one-way analysis of variance was employed.

Multivariate logistic regression analysis was used to assess the intake of vitamin B2 in the diet, both as a continuous and categorical factor, with results shown as odds ratios (ORs) and 95% confidence intervals (CIs). Logistic regression analysis utilized three different models. Model 1 had no factors, while model 2 considered age, gender, and ethnicity, and model 3 accounted for all potential influences from model 2 as well as BMI, tobacco use, drinking habits, diabetes, high blood pressure, and C-reactive protein levels, total calories intake, total protein and total carbohydrate intake. Trend tests were conducted by incorporating the median of every quartile as a continuous variable in the models. The models were specified as follows: Model 1: unadjusted. Model 2 was modified to account for age, gender, and ethnicity. Model 3: adjusted for all confounders. Sensitivity analysis was conducted based on different cut-off value of dietary vitamin B2.

Data analysis was conducted with R software version 4.1.1, the R survey package version 4.1.1, and Free Statistics software version 1.9.2 from R Foundation for Statistical Computing in Vienna, Austria [26]. Statistical significance was determined with a two-tailed p-value less than 0.05. This cross-sectional study was reported in accordance with the guidelines outlined in the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [27].

#### Results

#### Participant demographics at baseline

The study population's characteristics are presented in Table 1. 1,257 individuals were included in the study who tested positive for Helicobacter pylori infection based on H. pylori IgG seropositivity, with 47.5% being male. The mean age was 49.7 years. The group that tested positive for IgG to H. pylori was older, had lower levels of education, income, higher BMI, higher rates of smoking and diabetes, and lower levels of dietary vitamin B2 compared to the group that tested negative for H. pylori antibodies.

## Correlation of vitamin B2 consumption in diet with H. pylori antibodies

Using multivariate logistic regression, the research examined the correlation between consumption of vitamin B2 and presence of H. pylori antibodies. The results in Table 2 show that an increase in vitamin B2 intake was link to a reduced incidence of H. pylori seropositivity. The odds ratios for the first, second, and third models were 0.79, 0.91, and 0.88(P<0.05). Assessment of Vitamin B2 intake was done as a categorical variable, revealing that individuals in the highest quartile had a notably lower rate of H. pylori seropositivity in comparison to those in the lowest quartile. After accounting for different factors such as age, sex, marital status, PIR, educational background, BMI, smoking habits, alcohol intake, diabetes, hypertension, and serum CRP levels, the odds ratio for Q4 varied in the various models, being 0.46 ( $0.37 \sim 0.56$ ),  $0.69 (0.54 \sim 0.88)$ , and  $0.62 (0.44 \sim 0.87)$ , respectively. The trend test indicated a statistically significant (p < 0.05).

#### Subgroup analysis

Subgroup analyses were performed to examine the relationship between vitamin B2 consumption and H. pylori seropositivity, taking into account different risk factors such as age, gender, education level, marital status, smoking habits, alcohol intake, PIR, and BMI. Figure 2 summarizes the subgroup analyses and interactions, aligning with the results of the multivariable regression analysis. The subgroup analysis findings were consistent with the outcomes of the multivariable logistic regression analysis. The interaction analysis did not reveal any interactions between subgroups.

#### Sensitivity analysis

We conducted the following sensitivity analyses to further validate our findings. We divided participants into two groups based on the recommended intake level of 1.1 mg/day according to the dietary guidelines [28]. The second Cut-off Value 1.6 mg/day was based on receiver operating characteristic (ROC) analysis. These results confirm the stability of our findings, suggesting that higher vitamin B2 intake is associated with a lower likelihood of H. pylori seropositivity (Table 3).

#### Discussion

This cross-sectional research, which studied participants who had health examination, revealed a negative association between vitamin B2 consumption and the prevalence of H. pylori serum IgG. Furthermore, analyses stratified by different factors and sensitivity analyses consistently supported the reliability of the association between intake of vitamin B2 and the presence of H. pylori antibodies. This study is the first to explore the relationship between vitamin B2 intake and Helicobacter

Variables	Total ( <i>n</i> = 2859)	Helicobacter pylori negative (n = 1602)	Helicobacter pylori positive ( <i>n</i> = 1257)	P value	
Sex, male, n (%)	1501 (52.5)	857 (53.5) 644 (51.2)		0.229	
Age, (years)	49.7±18.6	47.1±18.7	$53.0 \pm 18.0$	< 0.001	
Race/ ethnicity, n (%)				< 0.001	
Non-Hispanic white	733 (25.6)	238 (14.9)	495 (39.4)		
Non-Hispanic black	73 ( 2.6)	43 (2.7)	30 (2.4)		
Mexican American	1384 (48.4)	1042 (65)	342 (27.2)		
Others	669 (23.4)	279 (17.4)	390 (31)		
Poverty-income ratio, n (%)				< 0.001	
< 1.3	858 (30.0)	350 (21.8)	508 (40.4)		
≥1.3	2001 (70.0)	1252 (78.2)	749 (59.6)		
Education, years, n (%)				< 0.001	
<9	482 (16.9)	117 (7.3)	365 (29)		
9–12	1194 (41.8)	624 (39)	570 (45.3)		
>12	1183 (41.4)	861 (53.7)	322 (25.6)		
Marriage status, n (%)				0.221	
Marriage or living with partner	1809 (63.3)	998 (62.3)	811 (64.5)		
Living alone	1050 (36.7)	604 (37.7)	446 (35.5)		
Body mass index, n (%)				0.007	
Normal	892 (31.2)	538 (33.6)	354 (28.2)		
Overweight	1026 (35.9)	549 (34.3)	477 (37.9)		
Obesity	941 (32.9)	515 (32.1)	426 (33.9)		
Smoking, n (%)	1364 (47.7)	737 (46)	627 (49.9)	0.039	
Hypertension, n (%)	874 (30.6)	451 (28.2)	423 (33.7)	0.002	
Diabetes, n (%)	270 ( 9.4)	105 (6.6)	165 (13.1)	< 0.001	
Alcohol use, n (%)	1928 (67.4)	1114 (69.5)	814 (64.8)	0.007	
C-reactive protein (mg/dl)	0.3 (0.1, 0.6)	0.2 (0.1, 0.6)	0.3 (0.1, 0.7)	< 0.001	
Dietary vitamin B2 (mg/day)	1.6 (1.1, 2.3)	1.8 (1.2, 2.5)	1.5 (1.0, 2.1)	< 0.001	
Calorie consumption (kcal/d)	1894.2 (1388.8, 2579.8)	2020.9 (1484.3, 2686.3)	1759.7 (1252.6, 2398.3)	< 0.001	
Protein consumption (g/d)	71.6 (50.7, 96.6)	74.9 (53.3, 101.4)	66.7 (46.9, 92.3)	< 0.001	
Carbohydrate consumption (g/d)	238.2 (166.9, 325.9)	254.9 (178.9, 339.9)	220.6 (157.1, 306.1)	< 0.001	

#### Table 1 Characteristics of participants

**Table 2** The associations between dietary vitamin B2 and H. pylori

Variable	Model 1		Model 2		Model 3	
	OR (95%CI)	P value	OR (95%CI)	P value	OR (95%CI)	P value
Vitamin B2 continuous (mg/day)	0.78 (0.73~0.84)	< 0.001	0.90 (0.83~0.98)	0.011	0.88 (0.78~0.99)	0.031
Vitamin B2 quartile (mg/day)						
Q1 (< 1.14)	1(Ref)		1(Ref)		1(Ref)	
Q2 (1.14–1.64)	0.71 (0.57~0.87)	0.001	0.78 (0.62~0.98)	0.032	0.76 (0.59~0.97)	0.029
Q3 (1.65–2.33)	0.56 (0.45~0.69)	< 0.001	0.69 (0.54~0.87)	0.002	0.65 (0.5~0.85)	0.002
Q4 (> 2.33)	0.45 (0.36~0.56)	< 0.001	0.67 (0.53~0.86)	0.002	0.61 (0.44~0.86)	0.004
P for trend		< 0.001		0.001		0.002
Model 1: unadjusted						

Model 2: adjust for sex, age, race

Model 3: adjust for sex, age, race, education, marriage status, PIR, BMI, tobacco use, alcohol use diabetes, hypertension, and C-reactive protein levels, total calories intake, total protein and total carbohydrate intake

OR, odds ratio; Cl, confidence interval

pylori seropositivity through a cross-sectional approach, based on our current understanding.

Several earlier investigations have showed that vitamin D [29] and vitamin B12 insufficiency were related with H. pylori infection [9]. However, research relevant to this

topic have been very restricted in scope. Matnuri's [30] research showed that individuals with gastric cancer and H. pylori infection have lower plasma riboflavin levels compared to those without H. pylori infection. Result of the current investigation was identical to the prior study.

Subgroup	Total	Event (%)	OR (95%CI)		P for interaction
Overall					
Crude	2859	1257 (44)	0.78 (0.73~0.84)	•	
Adjusted	2859	1257 (44)	0.88 (0.78~0.99)		
Gender					0.328
Male	1358	613 (45.1)	0.92 (0.79~1.07)		
Female	1501	644 (42.9)	0.85 (0.71~1.02)		
Age,years					0.648
<65	2115	863 (40.8)	0.83 (0.72~0.95)		
≥65	744	394 (53)	1.07 (0.85~1.35)		
Education level, years					0.34
<9	482	365 (75.7)	1.1 (0.78~1.53)	•	
9–12	1194	570 (47.7)	0.81 (0.66~0.98)		
>12	1183	322 (27.2)	0.84 (0.69~1.01)		
Alcohol ues					0.843
No	931	443 (47.6)	0.86 (0.69~1.06)		
Yes	1928	814 (42.2)	0.89 (0.78~1.03)		
Smoking status					0.75
No	1495	630 (42.1)	0.87 (0.73~1.04)		
Yes	1364	627 (46)	0.89 (0.75~1.04)		
Marital status					0.732
Married or living with partner	1809	811 (44.8)	0.88 (0.76~1.02)		
living alone	1050	446 (42.5)	0.87 (0.71~1.07)		
PIR					0.104
<1.3	858	508 (59.2)	0.88 (0.72~1.08)		
≥1.3	2001	749 (37.4)	0.88 (0.76~1.02)		
BMI					0.69
Normal	892	354 (39.7)	0.92 (0.76~1.12)		
Overweight	1026	477 (46.5)	0.91 (0.74~1.11)		
Obesity	941	426 (45.3)	0.84 (0.68~1.05)		
				0.71 1.0 1.41 Effect (95%CI)	

Fig. 2 Subgroup analyses were performed to examine the relationship between vitamin B2 consumption and H. pylori seropositivity in different groups based on age, gender, education level, marital status, smoking habits, alcohol intake, PIR, and BMI

#### Table 3 Sensitivity analysis

		Crude model		Adjusted model	
Variable n.total	n.event_%	OR (95%CI)	P value	OR (95%CI)	P value
nce Intakes for vita	min B2				
670	366 (54.6)	1(Ref)		1(Ref)	
2189	891 (40.7)	0.57 (0.48~0.68)	< 0.001	0.76 (0.6~0.95)	0.018
ike based on cut-o	ff value				
1368	692 (50.6)	1(Ref)		1(Ref)	
1491	565 (37.9)	0.6 (0.51~0.69)	< 0.001	0.78 (0.63~0.97)	0.023
	n.total ace Intakes for vita 670 2189 ke based on cut-o 1368 1491	n.total n.event_% nce Intakes for vitamin B2 670 366 (54.6) 2189 891 (40.7) ke based on cut-off value 1368 692 (50.6) 1491 565 (37.9)	n.total n.event_% OR (95%Cl) nce Intakes for vitamin B2 670 366 (54.6) 1(Ref) 2189 891 (40.7) 0.57 (0.48 ~ 0.68) ke based on cut-off value 1368 692 (50.6) 1(Ref) 1491 565 (37.9) 0.6 (0.51 ~ 0.69)	n.total         n.event_%         OR (95%Cl)         P value           nce Intakes for vitamin B2         670         366 (54.6)         1(Ref)           2189         891 (40.7)         0.57 (0.48 ~ 0.68)         < 0.001	n.total         n.event_%         OR (95%Cl)         P value         OR (95%Cl)           nce Intakes for vitamin B2         670         366 (54.6)         1(Ref)         1(Ref)           2189         891 (40.7)         0.57 (0.48 ~ 0.68)         <0.001

Adjusted for sex, age, race, education, marriage status, PIR, BMI, tobacco use, alcohol use, diabetes, hypertension, and C-reactive protein levels, total calories intake, total protein and total carbohydrate intake. OR, odds ratio; CI, confidence interval

Our research seeks to fill a substantial gap in the existing knowledge of the connection between dietary vitamin B2 consumption and seropositivity for H. pylori. Our findings provide information on the potential connection between a lack of vitamin B2 and the likelihood of being seropositive for H. pylori, a relationship that has not been thoroughly explored previously.

The mechanism of vitamin B2 in H. pylori remains unclear, with limited clinical information on the subject. FMN and FAD are the main coenzyme forms of vitamin B2 found in food. Vitamin B2 supplementation is recommended for immunological effects in dietary supplementary and therapies for inflammatory disorders like angular cheilitis, glossitis, migraine headaches, and sepsis [13]. The mechanism involve reducing inflammatory cytokines like Interferon-gamma (IFN-γ), interleukin-1(IL-1), Nitric Oxide (NO), interleukin-6 (IL-6), and interleukin-1 $\beta$  (IL-1 $\beta$ ) [31]. Similarly, vitamin B2 reduces oxidative stress by increasing the production of inducible nitric oxide synthase (iNOS) and catalase [32]. Vitamin B2 is essential for enhancing reactive oxygen species (ROS) to combat bacterial infections, such as infections caused by Listeria monocytogenes and Staphylococcus aureus [33].

The current investigation employs an approach that provides a number of notable advantages. Firstly, it is the first investigation to evaluate the association between vitamin B2 consumption and H. pylori infection among adult adults living in the US. Secondly, this research analyzed dietary vitamin B2 consumption as categorical variables, thereby reducing confounding and enhancing the robustness of the findings. Our study has significant implications for clinical practice, research endeavors, and health policy. These results emphasize the importance for increased attention to vitamin B2 consumption in future investigations.

However, there are some limitations to consider. The NHANES's cross-sectional design prevented us from definitively linking vitamin B2 intake to H. pylori infection. Second, dietary intake of vitamin B2 was collected through one 24-hour recall interview, which may have introduced potential bias in the interviews. Thirdly, the omission of dietary supplements may have resulted in unreliable and inconsistent data. Furthermore, due to the absorption of vitamin B2 in the small intestine [34], we could not eliminate or investigate further individuals with intestinal absorption issues. However, the utilization of the IgG-based ELISA in our study is impeded by the lack of relevant tests to distinguish between recent and past H. pylori infections, despite its previous use in other research. Future studies should incorporate additional diagnostic methods to clarify infection status and further investigate the relationship between vitamin B2 intake and specific H. pylori infection types. Even after accounting for potential confounders in multivariate analyses, there could still be unmeasured variables impacting the outcomes. Hence, even with the stratification and sensitivity analyses carried out in our research, it is essential to have larger cohort studies to confirm our results.

#### Conclusion

Overall, this study found a negative relationship between the amount of vitamin B2 consumed through diet and the likelihood of having H. pylori IgG in American adults included in the NHANES database. Nevertheless, it is vital to stress that further confirmation of these findings demands the inclusion of comprehensive prospective research with lengthy follow-up periods.

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#### Author contributions

YXH is the first author of article. YXH, TA, and PZ are responsible for the concept and design of the study. TA explain the analysis. PZ, and TA are responsible for data recovery. MH draft and review the manuscript. MH is the Corresponding author. All authors contributed to the article and approved the submitted version.

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None.

#### Data availability

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://wwwn.cdc.gov/nchs/nhanes/.

#### Declarations

#### **Ethics statement**

The NCHS Research Ethics Review Committee reviewed and approved the NHANES study protocol (Protocol #98–12). The studies were conducted in compliance with the local legislation and institutional requirements. The participants supplied their written informed consent to participate in this research. Written informed permission was obtained from the individual(s) for the publication of any potentially identifiable images or data contained in this study.

#### **Consent for publication**

Not applicable.

#### Competing interests

The authors declare no competing interests.

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