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# Association of dietary intake of theobromine with periodontitis: NHANES 2009–2014

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## Abstract

**Background** Theobromine intake usually comes from coffee, tea, and cocoa foods. Related studies have shown that theobromine is a bioactive molecule with anti-inflammatory, antithrombotic, anti-fat, and other effects. Periodontitis is a kind of oral inflammatory disease with high incidence, which is characterized by alveolar bone resorption leading to tooth loosening and loss. Therefore, this study aims to investigate whether theobromine intake correlates with periodontitis and whether it is a risk or protective factor for periodontitis. It hopes to provide a basis for theobromine-related diet or drugs to prevent and treat periodontitis.

**Methods** The study employed a cross-sectional design and utilized data from the National Health and Nutrition Examination Survey (NHANES) collected between 2009 and 2014. The exposure factor was theobromine intake, derived from two-day, 24-hour total nutrient intake data from dietary data. Periodontitis-related indicators as outcome factors were derived from the oral health component of the examination data. We used weighted multiple logistic regression, fractional Response Model, subgroup analysis, and the effect moderation test to explore the relationship between theobromine dietary intake and periodontitis severity based on weighting and adjusting for confounding factors.

**Results** After adjusting for relevant confounding factors, weighted logistic regression showed that theobromine intake was negatively correlated with periodontitis-related indicators (mean periodontal pocket depth, mean clinical attachment loss, and the percentage of sites with PD ≥ 4 mm). And theobromine intake was positively correlated with the number of teeth.

**Conclusion** This study demonstrated theobromine intake may serve as a protective factor against the development of periodontitis.

**Keywords** NHANES, Theobromine, Periodontitis, Dietary intake, Oral health

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## Introduction

Periodontitis is a microbiome-driven chronic inflammatory and destructive disease that causes irreversible damage to periodontal tissue due to attachment loss and alveolar bone resorption. With the development of periodontitis, there will be bad breath, loose teeth, and a decline in quality of life [1]. In addition, periodontitis is also associated with diabetes, systemic lupus erythematosus, rheumatoid arthritis, cardiovascular disease, Alzheimer's disease, chronic kidney disease, cancer (head and neck cancer, lung cancer, digestive tract cancer) [2] and many other systemic diseases [3]. Therefore, we must pay attention to the impact of periodontitis on systemic diseases, and explore related preventive measures as an indispensable part of the diagnosis and treatment of periodontitis.

Theobromine (3,7-dimethylxanthine) is a psychoactive stimulant commonly consumed in daily life, as it is usually found in cocoa, coffee, and tea. Its main source is the seeds of the cacao tree. Theobromine is a purine alkaloid produced by bacterial systems and caffeine catabolism in higher plants. It acts as an adenosine receptor antagonist to stimulate the central nervous system and improve reaction times on psychomotor tasks, but the stimulating effect of theobromine is about one-fifth that of caffeine [4]. Its pharmacokinetics are considered to be readily distributed through body fluids across biological membranes and metabolized in the liver, with a half-life of approximately 7.2 h and a plasma clearance of 1.20 $mL{\cdot}min^{-1}{\cdot}Kg^{-1}$ [5]. Relevant studies have shown that theobromine e has the effects of relaxing smooth muscles, stimulating the central nervous system and respiratory system, stimulating the heart, and diuresis, dilating coronary arteries, and stimulating skeletal muscles [5]. In addition, many scholars have studied methylxanthine in recent years and found that it also has antioxidant [6], anti-inflammatory [7], anti-cancer [8], and lipolysis effects [9]. Further, it is known to inhibit the Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus [10] and reverse the age-related decrease in porosity of liver sinusoidal endothelial cells [11] with many other beneficial effects.

Current research suggests that while theobromine offers various health benefits, there is a lack of animal and clinical studies specifically addressing its potential role in periodontitis. As a result, the relationship between the two has not been fully or effectively verified. In contrast, there has been ongoing debate over the role of caffeine, one of the three primary methylxanthines, in periodontitis. Its potential as either a protective or risk factor remains controversial [12, 13]. Recent studies [14, 15] have indicated that toothpaste containing theobromine and prepared theobromine solution may inhibit oral bacteria to prevent dental caries and facilitate the remineralization of tooth enamel. Furthermore, animal dosing studies and cell culture experiments [7, 16] have demonstrated that theobromine and its derivatives possess anti-inflammatory properties, enhance microcirculation in blood tissues, and decrease the adhesion and aggregation of white blood cells. Given that periodontitis is an inflammatory condition influenced by the microbiota, we hypothesize that theobromine may play a positive role in its pathogenesis. In addition, most of the above literature studies on theobromine were based on the administration of theobromine to experimental animals, and the preparation of the solution into cell culture, while there were few studies on dietary intake. To explore this, we analyzed data from the extensive NHANES database to assess the relationship between dietary theobromine intake and periodontitis, as the database does not provide data on theobromine levels in the blood.

## Materials and methods

### Data source

This cross-sectional study uses a publicly accessible NHANES dataset and requires no further ethical review board approval (https://wwwn.cdc.gov/nchs/nhanes/Default.aspx).

### Study population

This study was based on the data from the three two-year periods of 2009–2010, 2011–2012, and 2013–2014. These periods were the periods for which we had available public data on both theobromine intake and periodontal examinations. A total of 29,404 people were included in the data from 2009 to 2014, of whom 18,690 had missing periodontitis data and 1633 had missing data on theobromine intake in their diet over two days. Therefore, a total of 9081 people were included in the final statistical analysis. We made a flow chart (Fig. 1) to visually represent the data screening process. The covariates with missing values and the number of missing values also were shown in Fig. 1. In addition, the National Health and Nutrition Examination Survey (NHANES) was a stratified, multistage, and nationally representative probability sample survey of non-institutional civilians in the United States. The survey was approved by the Ethics Review Board of the National Center for Health Statistics (NCHS), and each participant signed a written informed consent.

### Theobromine

This study used total nutrient intake data based on NHANES dietary data. Dietary data were gathered utilizing the United States Department of Agriculture's (USDA) dietary data collection tool, the Automated Multiple Pass Method (AMPM) (http://www.ars.usda.gov/ba/bhnrc/fsrg). The intake of energy, nutrients, and other food components in these foods and beverages

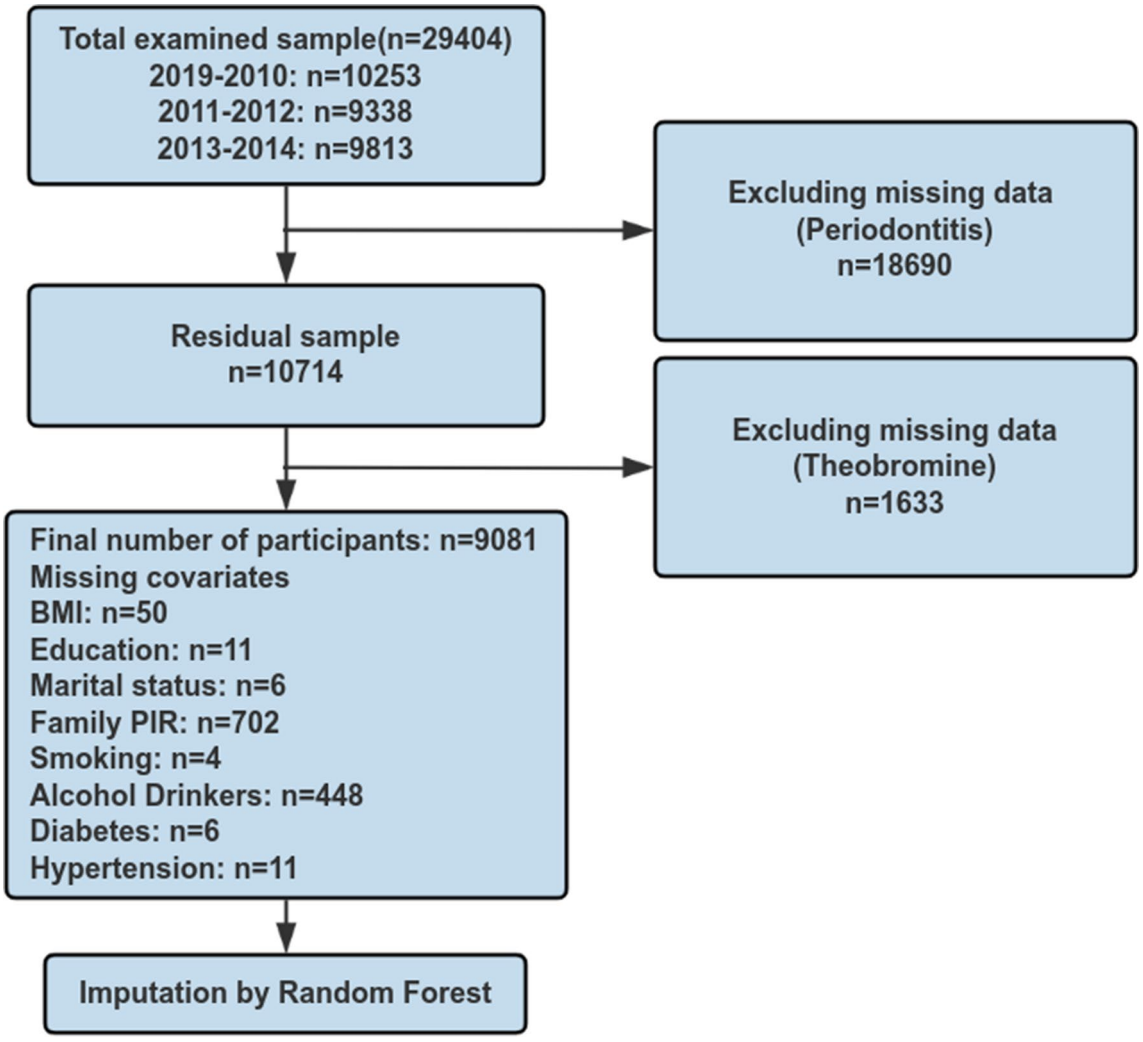


**Fig. 1** Flow chart of data filtering

was calculated based on the type and number of foods and beverages consumed (including all types of water) in the 24 h prior to the interview. The average of two days of dietary theobromine intake (mg) was used as the theobromine intake for the final analysis. The initial dietary recall interview was conducted in person at the Mobile Examination Center (MEC), followed by a second interview via telephone 3 to 10 days later. A comprehensive set of measuring tools, including glasses, bowls, cups, bottles, household spoons, measuring cups and spoons, rulers, thick sticks, bean bags, and circles, was provided in the MEC dietary interview room to help participants accurately report food quantities (https://www.cdc.gov/nchs/nhanes/measuring_guides_dri/measuringguides.htm). After completing the in-person interview, participants received measuring cups, spoons, rulers, and a food model booklet with two-dimensional drawings of the available measuring guides to assist in reporting food amounts during the telephone interview.

#### Periodontitis

The data on periodontitis was obtained from the oral health periodontal section of the NHANES database, which included participants aged 30 years and older with a minimum of one natural tooth. Examiners were trained and calibrated, and they were visited 2–3 times a year for repeated exams. The dental examiner measured pocket depth (PD) and Clinical attachment loss (AL) at 6 sites on each tooth in four quadrants. The final measurements were recorded with the assistance of a health technician who inputted the observations from all examiners directly into the computerized data collection system. According to the Centers for Disease Control (CDC) / American Academy of Periodontology (AAP) definition, participants were classified as having no, mild, moderate, or severe periodontitis. The specific definitions are as follows: (1) Mild periodontitis: two or more interproximal sites with AL ≥ 3 mm and two or more interproximal sites with PD ≥ 4 mm (not on the same tooth) or one site

with a PD ≥ 5 mm; (2) Moderate periodontitis: two or more interproximal sites with clinical AL ≥ 4 mm (not on the same tooth) or two or more interproximal sites with PD ≥ 5 mm (not on the same tooth); (3) Severe periodontitis: two or more interproximal sites with AL values ≥ 6 mm (not for the same teeth) and ≥ 1 interproximal site with PD ≥ 5 mm; (4) If the participant does not fall into any of the above categories of periodontitis, they are classified as without periodontitis [17]. In addition, we counted the mean periodontal pocket depth of the entire dentition (mean PD), the mean clinical attachment loss of the entire dentition (mean AL), the percentage of sites with PD ≥ 4 mm, and the number of teeth (except for the third molar, dental implants, and residual roots) to help explore the relationship between theobromine intake and periodontitis.

#### Covariates

Based on clinical consensus and previous studies [18–20], this study included the following factors as covariates: sex (Male, Female), age (Years), race/ethnicity (Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, Other Race Including-Multi Racial), education (Less than 9th grade, 9-11th grade, High school graduate/GED or equivalent, Some college or AA degree, College graduate or above), marital status (Widowed, Divorced, Separated, Never married, Living with a partner), family poverty income ratio (Family PIR: < 1.3, 1.3–3.5, ≥ 3.5), body mass index (BMI: < 25 kg/$m^2$, 25–30 kg/$m^2$, ≥ 30 kg/$m^2$), smoking (Never smoker, Former smoker, Current smoker), diabetes (Yes or No), alcohol drinkers (Yes or No). Hypertension results were based on participants' self-reports of being diagnosed with high blood pressure (HBP) by a professional doctor (Yes or No).

#### Statistical analysis

Weights were used in the following population descriptions and statistical analyses. For the description of basic population characteristics, we employed mean, standard error, median, and quartiles for continuous variables, while statistical frequency and percentages were used for categorical variables. Furthermore, stratified analyses were performed based on the severity of periodontitis. The p-values for continuous variables were derived from weighted linear regression, whereas the p-values for categorical variables were obtained through the weighted chi-square test. Given the skewness of theobromine as a continuous variable, we applied the Box-Cox transformation prior to performing statistical analysis. This allowed us to examine the relationships between the variables and enhance the accuracy of the results. In addition to describing the basic demographic characteristics of the original data, we used the random forest method [21] (MissForest package of R) to interpolate the missing covariates (race, education, marital status, family PIR, BMI, smoking, alcohol drinkers, diabetes, hypertension) and described the basic demographic characteristics after interpolation. Following random forest interpolation, we calculated the out-of-bag error (OOBError). The normalized root mean square error (NRMSE) was less than 0.001, which indicates high predictive accuracy, while the proportion of falsely classified instances (PFC) was 0.158. Based on commonly accepted thresholds in this field, where an NRMSE below 0.001 and a PFC under 0.2 are considered acceptable, the interpolation results were deemed satisfactory. Due to the failure of the proportional odds assumption in the ordinal logistic regression analysis ($P < 0.05$), we employed a multivariate logistic regression model to investigate the relationship between periodontitis and theobromine intake. We also used the same method to explore the relationship between mean PD, mean AL, number of teeth and theobromine intake. And because the percentage of sites with PD ≥ 4 mm was a percentage variable, we used a weighted fractional response model for statistical analysis. Crude Model did not adjust for any covariates, Model I adjusted for age, sex, and race, and Model II adjusted for age, sex, race, hypertension, diabetes, smoking, body mass index, family poverty income ratio, alcohol drinkers, education, and marital status. Variance inflation factor (VIF) was used to screen collinearity for all independent variables, and variables with VIF < 5 were eliminated.

Additionally, we used two statistical forms for theobromine intake — Boxcox-transformed theobromine intake (BC-Theobromine) and Tertiles of theobromine intake for subgroup and effect modification analysis on all significant covariates. The effect moderation test was conducted using a model that adjusted for all covariates except the effect modifier (Model II), with the likelihood ratio test as the method. Since the fractional response model for the percentage of sites with PD ≥ 4 mm adopted robust standard errors, the effect moderation test was based on the Wald test. For age, smooth curve fitting combined with threshold analysis results was used to group the subjects. Subgroup analyses were weighted and adjusted for variables other than those used for stratification. The results of the subgroup analysis, along with the forest plots, are shown for variables that had significant effect moderation effects. The statistical analysis software utilized in this study included R version 4.2.1, EmpowerStats version 2.0, and Stata version 17.0. In addition, 95% confidence intervals were used for all analysis results, with $P < 0.05$. And we provided an abbreviation list of the abbreviations used in the article (Supplementary material 4).

## Results

### Basic demographic characteristics

Ultimately, a total of 9081 participants were successfully included in the study analysis, with Table 1 illustrating the basic demographic characteristics of the overall group and after stratification based on periodontitis grade. In addition, we also analyzed the basic population characteristics data after random forest interpolation of missing values of covariates (Supplementary material 1). The study population consisted of individuals aged 30 and older, with an average age of 51.2 ± 0.3. Approximately 48% of participants were male and 52% were female. All covariates, except for alcohol drinkers, were significantly associated with periodontitis ($P < 0.001$). In comparison to individuals with no or mild periodontitis, those with moderate to severe periodontitis exhibited a higher prevalence of male sex, smoking habits, unmarried status, lower educational attainment, lower family poverty index, and comorbidities such as diabetes and hypertension. Furthermore, because the first 32.3% of theobromine dietary intake was 0 (no intake) and smooth curve fitting indicated that there were no clear demarcation effect points, tertiles were utilized as a method of categorizing theobromine intake into low, medium, and high levels, as opposed to quartiles or threshold groupings. The mean theobromine intake levels and their respective Standard deviation were 0.007 ± 0.057 mg/day in the low-intake group, 12.156 ± 8.145 mg/day in the medium-intake group, and 88.779 ± 70.755 mg/day in the high-intake group. From Table 1, we found that the theobromine intake of individuals with moderate to severe periodontitis was relatively low.

### Effects of theobromine intake on periodontitis

All variables passed the collinearity screening. The results of the weighted logistic regression of theobromine intake and periodontitis-related indicators were shown in Tables 2 and 3. The results showed that boxcox-transformed theobromine intake and tertile of theobromine were significantly negatively correlated with periodontitis-related indicators (periodontitis classification, mean PD, mean AL, percentage of sites with PD ≥ 4 mm) in all models (OR < 1/Coefficient < 0, $P < 0.05$). The number of remaining teeth was positively correlated with theobromine intake (OR > 1, $P < 0.05$). Trend tests for tertiles of theobromine were significant in all three models ($P < 0.05$).

For the age variable, the smooth curve fitting indicated a breakpoint near the age of 60 years, and the threshold analysis identified a breakpoint at 56 years ($P < 0.05$), thereby establishing 56 years as the threshold (refer to Supplementary Material 2 and Supplementary Material 3 for details). Subsequent effect moderation results indicated that Family PIR did not significantly modify the negative correlation between theobromine intake and all periodontitis indicators ($P > 0.05$). Stratified analysis showed the same effect direction (periodontitis classification, mean PD, mean AL: OR < 1, percentage of sites with PD ≥ 4 mm: Coefficient < 0, number of teeth: OR > 1). For variables with effect moderation test $P < 0.05$, we showed the subgroup analysis results and forest plots in Figs. 2 and 3.

Subgroup and effect modification analysis revealed several significant interactions. When the outcome variable was the number of teeth (Theobromine) or mean AL (Tertiles of theobromine), gender acted as an effect modifier, with greater benefits observed in men. In age-stratified analyses, for both mean AL and number of teeth (Theobromine), age appeared to moderate the effect, with individuals over 56 years old showing more pronounced benefits. Race was found to modify the effect when the outcome was the number of teeth (Theobromine) or percentage of sites with PD ≥ 4 mm (Tertiles of theobromine), suggesting that Non-Hispanic White individuals had more favorable outcomes. Smoking also moderated the effects on mean AL, mean PD, and number of teeth. Former smokers experienced greater benefits with mean AL and number of teeth, while current smokers showed better outcomes for mean PD. Education was an effect modifier for all indicators of periodontitis, with High School graduates/GED holders or equivalents potentially experiencing greater benefits. When the outcome was AL and theobromine was treated as a continuous variable, marriage appeared to have an effect modification, with married individuals benefiting more, though this effect was not significant for other periodontal indicators. For the outcome of the number of teeth (BC-Theobromine), BMI and diabetes acted as effect modifiers. Individuals with BMI ≥ 30 kg/m² showed greater benefits, while borderline diabetes showed an inverse trend (OR (95% Cl) = 0.329 (0.083, 1.306), $P = 0.115$), although this was not statistically significant. These factors had no significant modifying effects on other periodontal indicators and may have limited clinical relevance. Finally, hypertension appeared to modify the effect when the outcome was mean AL and number of teeth, with hypertensive individuals benefiting more. However, due to sample size limitations and biological plausibility in subgroup analysis, these conclusions may not be clinically significant.

## Discussion

Based on the results above, theobromine dietary intake was negatively correlated with periodontitis, indicating that theobromine may be protective. There was almost no literature on the correlation between theobromine and periodontitis, but there were many studies on the correlation between coffee and periodontitis. As reported in related studies, coffee intake was found to be a risk

**Table 1** Basic demographic characteristics ($N=9081$)

| Characteristics | Total($N=9081$) | Periodontitis | | | | *P*-value |
|---|---|---|---|---|---|---|
| | | No ($N=4543$) | Mild ($N=441$) | Moderate ($N=3153$) | Severe ($N=944$) | |
| **Age** (years) | 51.2 ± 0.3 | 48.6 ± 0.3 | 47.8 ± 0.7 | 56.0 ± 0.4 | 55.0 ± 0.5 | < 0.001 |
| | 50.0 | 47.0 | 46.0 | 56.0 | 54.0 | |
| | (40.0, 61.0) | (38.0, 58.0) | (38.0, 57.0) | (45.0, 66.0) | (46.0, 62.0) | |
| **Sex** N (%) | | | | | | < 0.001 |
| Male | 4382 (48.1) | 1790 (41.7) | 232 (55.0) | 1711 (54.1) | 649 (70.1) | |
| Female | 4699 (51.9) | 2753 (58.3) | 209 (45.0) | 1442 (45.9) | 295 (30.0) | |
| **Race** N (%) | | | | | | < 0.001 |
| Mexican American | 1269 (7.7) | 467 (5.3) | 91 (12.2) | 510 (10.3) | 201 (13.3) | |
| Other Hispanic | 892 (5.2) | 438 (4.6) | 46 (7.3) | 333 (6.0) | 75 (5.1) | |
| Non-Hispanic White | 4086 (70.3) | 2340 (75.8) | 177 (61.1) | 1275 (64.2) | 294 (56.2) | |
| Non-Hispanic Black | 1861 (10.3) | 746 (7.8) | 96 (14.1) | 733 (12.4) | 286 (18.9) | |
| Multiracial and Other Races | 973 (6.6) | 552 (6.4) | 31 (5.3) | 302 (7.0) | 88 (6.6) | |
| **BMI** (kg/m$^2$), N (%) | | | | | | < 0.001 |
| <25 | 2333 (26.4) | 1241 (27.9) | 84 (17.4) | 780 (25.2) | 228 (24.9) | |
| [25, 30) | 3114 (35.4) | 1552 (35.7) | 151 (37.3) | 1068 (34.4) | 343 (35.8) | |
| ≥ 30 | 3584 (38.2) | 1729 (36.4) | 206 (45.4) | 1282 (40.4) | 367 (39.3) | |
| **Diabetes** N (%) | | | | | | < 0.001 |
| No diabetes | 7699 (87.9) | 4042 (91.0) | 387 (88.7) | 2517 (82.6) | 753 (83.9) | |
| Prediabetes | 250 (2.5) | 115 (2.3) | 10 (1.4) | 101 (3.2) | 24 (2.1) | |
| Diabetes | 1126 (9.6) | 383 (6.7) | 44 (9.9) | 533 (14.1) | 166 (14.1) | |
| **Smoking** N (%) | | | | | | < 0.001 |
| Never smoker | 5130 (56.9) | 2957 (64.9) | 272 (61.5) | 1544 (46.3) | 357 (34.3) | |
| Former smoker | 2338 (26.8) | 1053 (24.7) | 84 (21.8) | 944 (31.3) | 257 (28.1) | |
| Current smoker | 1609 (16.3) | 533 (10.4) | 85 (16.7) | 661 (22.4) | 330 (37.6) | |
| **Alcohol Drinkers** N (%) | | | | | | 0.108 |
| No | 2264 (20.6) | 1122 (20.2) | 102 (20.9) | 843 (22.4) | 197 (17.0) | |
| Yes | 6369 (79.4) | 3192 (79.8) | 307 (79.1) | 2170 (77.6) | 700 (83.0) | |
| **Education** N (%) | | | | | | < 0.001 |
| Less than 9th grade | 794 (4.6) | 225 (2.5) | 42 (5.6) | 374 (7.2) | 153 (10.6) | |
| 9-11th grade (Includes 12th grade with no diploma) | 1168 (9.5) | 396 (6.2) | 61 (9.2) | 514 (13.7) | 197 (19.0) | |
| High school graduate/ GED or equivalent | 1953 (20.9) | 806 (17.0) | 109 (27.074) | 777 (25.4) | 261 (30.5) | |
| Some college or AA degree | 2614 (30.1) | 1399 (30.4) | 137 (31.7) | 860 (29.8) | 218 (27.6) | |
| College graduate or above | 2541 (34.9) | 1713 (44.0) | 92 (26.4) | 621 (23.8) | 115 (12.3) | |
| **Marital status** N (%) | | | | | | < 0.001 |
| Married | 5358 (64.3) | 2858 (69.1) | 246 (59.4) | 1751 (58.5) | 503 (53.6) | |
| Widowed | 646 (5.3) | 245 (4.0) | 21 (3.7) | 301 (8.0) | 79 (6.2) | |
| Divorced | 1139 (12.2) | 527 (10.8) | 52 (13.1) | 428 (14.1) | 132 (16.0) | |
| Separated | 333 (2.4) | 119 (1.7) | 19 (2.4) | 133 (3.1) | 62 (5.2) | |
| Never married | 1020 (9.8) | 537 (9.5) | 66 (14.0) | 324 (9.8) | 93 (10.2) | |
| Living with partner | 579 (5.9) | 255 (5.0) | 37 (7.4) | 212 (6.6) | 75 (8.8) | |
| **Family PIR** N (%) | | | | | | < 0.001 |
| <1.3 | 2378 (17.7) | 930 (12.8) | 110 (18.4) | 983 (23.8) | 355 (32.1) | |
| [1.3,3.5) | 3033 (34.8) | 1381 (30.2) | 169 (42.7) | 1155 (41.4) | 328 (39.6 | |
| ≥ 3.5 | 2968 (47.6) | 1921 (57.0) | 113 (38.9) | 764 (34.8) | 170 (28.4) | |
| **Hypertension** N (%) | | | | | | < 0.001 |
| No | 5635 (65.6) | 3074 (69.8) | 300 (67.6) | 1740 (59.0) | 521 (57.7) | |

**Table 1** (continued)

| Characteristics | Total(*N* = 9081) | Periodontitis | | | | *P*-value |
|---|---|---|---|---|---|---|
| | | No (*N* = 4543) | Mild (*N* = 441) | Moderate (*N* = 3153) | Severe (*N* = 944) | |
| Yes | 3435 (34.4) | 1466 (30.2) | 141 (32.5) | 1405 (41.0) | 423 (42.3) | |
| **Tertiles of theobromine** N (%) | | | | | | < 0.001 |
| Low | 2975 (28.7) | 1326 (25.5) | 150 (31.9) | 1130 (32.1) | 369 (38.9) | |
| Medium | 3061 (33.3) | 1577 (34.0) | 141 (34.1) | 1041 (32.8) | 302 (29.0) | |
| High | 3045 (38.0) | 1640 (40.5) | 150 (34.0) | 982 (35.1) | 273 (32.1) | |
| **Theobromine** (mg/d) | 38.397 ± 1.022 | 39.320 ± 0.852 | 31.936 ± 3.019 | 39.398 ± 2.345 | 30.938 ± 3.300 | 0.029 |
| | 16.000 | 18.500 | 11.000 | 12.500 | 6.500 | |
| | (0.000, 52.000) | (0.000, 55.500) | (0.000, 37.500) | (0.000, 49.500) | (0.000, 40.000) | |
| **Mean PD** (mm) | 1.420 ± 0.019 | 1.143 ± 0.015 | 1.683 ± 0.027 | 1.645 ± 0.017 | 2.552 ± 0.040 | < 0.001 |
| | 1.321 | 1.156 | 1.643 | 1.600 | 2.478 | |
| | (1.067, 1.654) | (0.940, 1.345) | (1.465, 1.853) | (1.333, 1.917) | (2.028, 2.937) | |
| **Mean AL** (mm) | 1.627 ± 0.029 | 1.133 ± 0.014 | 1.314 ± 0.033 | 2.141 ± 0.025 | 3.658 ± 0.079 | < 0.001 |
| | 1.344 | 1.107 | 1.250 | 1.955 | 3.323 | |
| | (1.033, 1.893) | (0.899, 1.333) | (1.073, 1.458) | (1.606, 2.393) | (2.630, 4.319) | |
| **Percentage of sites with PD ≥ 4 mm** (%) | 2.846 ± 0.154 | 0.075 ± 0.006 | 3.757 ± 0.323 | 3.421 ± 0.158 | 21.840 ± 0.879 | < 0.001 |
| | 0.000 | 0.000 | 2.000 | 1.282 | 16.000 | |
| | (0.000, 1.342) | (0.000, 0.000) | (1.235, 4.192) | (0.000, 4.167) | (7.971, 30.070) | |
| **Number of teeth** (n) | 24.098 ± 0.117 | 25.427 ± 0.089 | 25.156 ± 0.285 | 22.145 ± 0.200 | 20.786 ± 0.328 | < 0.001 |
| | 26.000 | 27.000 | 27.000 | 24.000 | 23.000 | |
| | (23.000, 28.000) | (24.000, 28.000) | (24.000, 28.000) | (20.000, 27.000) | (17.000, 26.000) | |

The demographic characteristics table was the original table without missing value interpolation. Values were expressed as N (%) for categorical variables. Quantitative variables were expressed as Mean ± Se, Median, (Q1-Q3). P values were calculated using a weighted linear regression model for quantitative variables and a weighted chi-square test for categorical variables

BMI, Body Mass Index; AA, Associate's Degree; GED, General Educational Development; Family PIR, Family Income-to-Poverty Ratio; Mean PD, Mean periodontal pocket depth of the entire dentition; Mean AL, Mean clinical attachment loss in the entire dentition

factor for periodontitis [12, 22]. However, there were also studies showing that it could be a protective factor [13, 23]. The current meta-analysis and systematic review of studies indicated that coffee composition was complex and there was no significant association between coffee intake and periodontitis [24, 25]. Theobromine, caffeine, and theophylline are the three natural methylxanthines. The difference between theobromine and caffeine is that it lacks a methyl group at the first position, and this single methyl group is enough to give theobromine different physical and chemical properties. Theobromine is far less stimulating to the nervous and respiratory systems than caffeine, but research shows that it has better performance in cardiac stimulation and coronary artery stimulation [5].

In recent years, studies by relevant scholars have shown that theobromine has anti-inflammatory effects. It can stimulate the production of nitric oxide (NO) and prostaglandin E2, increase the expression of inducible nitric oxide synthase and cyclooxygenase-2, and stimulate the phosphorylation level of macrophage Mitogen-activated protein kinase (MAPK). Acts as an immune response stimulator through NF-κB and MAPK signaling pathways [7, 26]. In addition, theobromine can increase high-density lipoprotein (HDL) cholesterol while reducing plasma low-density lipoprotein (LDL) cholesterol [27], achieving a certain degree of antithrombotic effect. In vitro studies have shown that theobromine can reduce plaque deposition and prevent dental caries [14]. In addition, pentoxifylline, another dimethylxanthine derivative prepared by cocoa bean extraction, has been reported to have the ability to reduce blood viscosity, promote microcirculation of ischemic tissue, reduce fibrinogen, stimulate the fibrinolytic system, promote the release of plasminogen activator, and reduce the adhesion and aggregation of polymorphonuclear neutrophils (PMN) [16, 28, 29]. We know that periodontitis is a common oral disease that causes alveolar bone loss, loosening, and loss of teeth due to inflammation of surrounding tissues. Recently, some studies have shown that pathological deposition of fibrin in oral mucosa can lead to serious damage of gum tissue and alveolar bone, resulting in loss and defect of dentition. Loss of fibrinolysis mediated by plasmin may be the cause of excessive immune response of oral mucosa in patients with periodontitis [30, 31]. Therefore, we hypothesized that the mechanism of theobromine's periodontal protection may lie in its antibacterial, anti-inflammatory, and anti-fibrinous effects.

Regarding the disadvantages of theobromine, people's most urgent concern about the consumption of

**Table 2** The association between theobromine intake with periodontitis-related indicators

| Exposure | Crude Model<br>OR (95%CI)<br>p-value | Model I<br>OR (95%CI)<br>p-value | Model II<br>OR (95%CI)<br>p-value |
|---|---|---|---|
| **Outcome = Periodontitis** | | | |
| **Tertiles of theobromine** | | | |
| Low | REF | REF | REF |
| Medium | 0.828 (0.783, 0.875)<br>< 0.001 | 0.894 (0.848, 0.943)<br>< 0.001 | 0.949 (0.903, 0.998)<br>0.040 |
| High | 0.790 (0.748, 0.834)<br>< 0.001 | 0.865 (0.821, 0.911)<br>< 0.001 | 0.917 (0.873, 0.963)<br>< 0.001 |
| P for trend | < 0.001 | < 0.001 | < 0.001 |
| **BC-Theobromine** | 0.856 (0.823, 0.890)<br>< 0.001 | -0.914 (0.881, 0.948)<br>< 0.001 | 0.950 (0.918, 0.984)<br>0.004 |
| **Outcome = Mean PD** | | | |
| **Tertile of theobromine** | | | |
| Low | REF | REF | REF |
| Medium | 0.889 (0.862, 0.915)<br>< 0.001 | 0.933 (0.908, 0.96)<br>< 0.001 | 0.963 (0.937, 0.989)<br>0.006 |
| High | 0.890 (0.864, 0.915)<br>< 0.001 | 0.937 (0.911, 0.963)<br>< 0.001 | 0.966 (0.941, 0.991)<br>0.009 |
| P for trend | < 0.001 | < 0.001 | 0.014 |
| **BC-Theobromine** | 0.924 (0.905, 0.943)<br>< 0.001 | 0.959 (0.94, 0.978)<br>< 0.001 | 0.978 (0.961, 0.997)<br>0.023 |
| **Outcome = Mean AL** | | | |
| **Tertile of theobromine** | | | |
| Low | REF | REF | REF |
| Medium | 0.815 (0.773, 0.860)<br>< 0.001 | 0.870 (0.829, 0.915)<br>< 0.001 | 0.930 (0.888, 0.972)<br>0.002 |
| High | 0.807 (0.767, 0.850)<br>< 0.001 | 0.869 (0.828, 0.912)<br>< 0.001 | 0.924 (0.884, 0.967)<br>< 0.001 |
| P for trend | < 0.001 | < 0.001 | < 0.001 |
| **BC-Theobromine** | 0.866 (0.835, 0.899)<br>< 0.001 | 0.913 (0.882, 0.945)<br>< 0.001 | 0.951 (0.921, 0.982)<br>0.002 |
| **Outcome = Number of teeth** | | | |
| **Tertile of theobromine** | | | |
| Low | REF | REF | REF |
| Medium | 2.326 (1.740, 3.108)<br>< 0.001 | 1.448 (1.126, 1.861)<br>< 0.001 | 2.073 (1.582, 2.716)<br>0.004 |
| High | 2.502 (1.889, 3.317)<br>< 0.001 | 1.346 (1.053, 1.719)<br>< 0.001 | 1.921 (1.476, 2.502)<br>0.018 |
| P for trend | < 0.001 | < 0.001 | 0.028 |
| **BC-Theobromine** | 1.863 (1.525, 2.277)<br>< 0.001 | 1.192 (1.001, 1.420)<br>< 0.001 | 1.519 (1.257, 1.833)<br>0.049 |

OR, Odds ratio; CI, Confidence intervals; REF, Reference

Crude Model: adjusted for no covariates; Model I: adjusted for age, sex, and race; Model II: adjusted for age, sex, race, hypertension, diabetes, smoking, body mass index, family poverty income ratio, alcohol drinkers, education, and marital status

Mean PD, Mean periodontal pocket depth of the entire dentition; Mean AL, Mean clinical attachment loss of the entire dentition; BC-Theobromine, Boxcox-transformed theobromine intake

methylxanthines such as theobromine is that it may be related to prenatal exposure and affect male fertility. Taking methylxanthine during pregnancy could cause stunted development of offspring, and it has been reported to cause testicular atrophy in animals [32, 33]. Regarding human research reports, some scholars believed that it would increase the risk of miscarriage and the incidence of malformations [34] and affect male sperm quality, but some scholars believed that it had little relationship [35, 36]. In short, the daily dietary intake of theobromine makes it difficult to reach the serious harm standard. Still, the toxicological reactions and biological activities of excessive intake or use as a drug require a large amount of research and further evaluation experiments. Attention needs to be paid to pharmacokinetics, including other drugs or effects of dietary variables,

**Table 3** The association between theobromine intake and the percentage of sites with PD ≥ 4 mm

| Percentage of sites with PD ≥ 4 mm | Crude Model | Model I | Model II |
|---|---|---|---|
| | Coefficient (95%CI) | Coefficient (95%CI) | Coefficient (95%CI) |
| | Robust SEs | Robust SEs | Robust SEs |
| | p-value | p-value | p-value |
| **Tertiles of theobromine** | | | |
| Low | REF | REF | REF |
| Medium | -0.480 (-0.640, -0.320) | -0.328 (-0.490, -0.166) | -0.224 (-0.382, -0.066) |
| | 0 .081 | 0 .083 | 0 .080 |
| | < 0.001 | < 0.001 | 0.005 |
| High | -0.536 (-0.696, -0.376) | -0.369 (-0.534, -0.204) | -0.262 (-0.424, -0.099) |
| | 0 .082 | 0 .084 | 0 .061 |
| | < 0.001 | < 0.001 | 0.002 |
| P for trend | < 0.001 | < 0.001 | 0.002 |
| **BC-Theobromine** | -0.407 (-0.530, -0.285) | -0.279 (-0.402, -0.155) | -0.204 (-0.324, -0.085) |
| | 0 .062 | 0 .063 | 0 .061 |
| | < 0.001 | < 0.001 | 0.001 |

Robust SEs, Robust standard errors; CI, Confidence intervals; REF, Reference

Crude Model: adjusted for no covariates; Model I: adjusted for age, sex, and race; Model II: adjusted for age, sex, race, hypertension, diabetes, smoking, body mass index, family poverty income ratio, alcohol drinkers, education, and marital status

BC-Theobromine, Boxcox-transformed theobromine intake; PD, Pocket depth

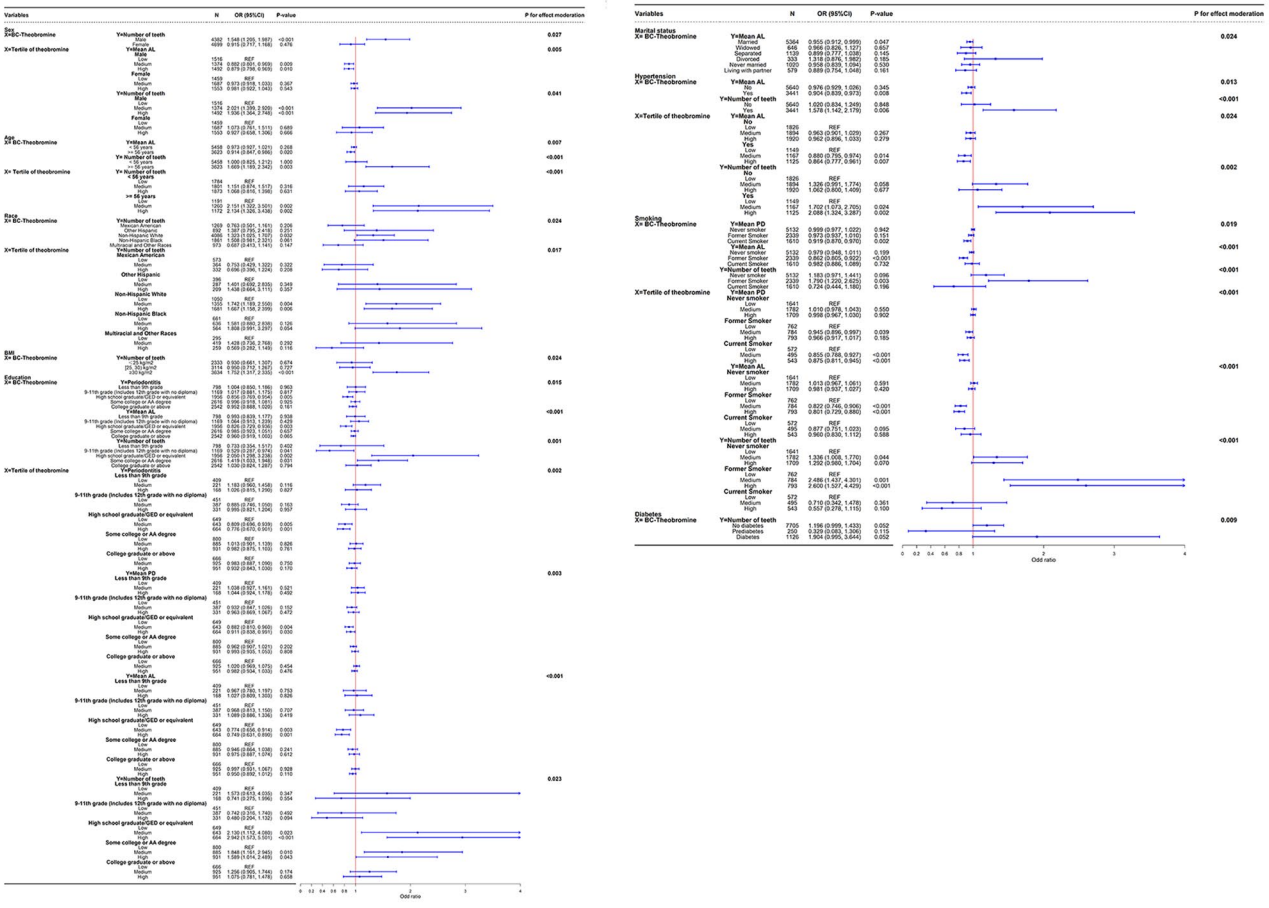

**Fig. 2** The correlation between periodontitis and theobromine intake under different covariates stratification. OR, Odds ratio; CI, Confidence intervals; N, Sample size; REF, Reference; X, Exposure; Y, Outcome; AA, Associate's Degree; GED, General Educational Development; Mean PD, Mean periodontal pocket depth of the entire dentition; Mean AL, Mean clinical attachment loss in the entire dentition; BC-Theobromine, Boxcox-transformed theobromine

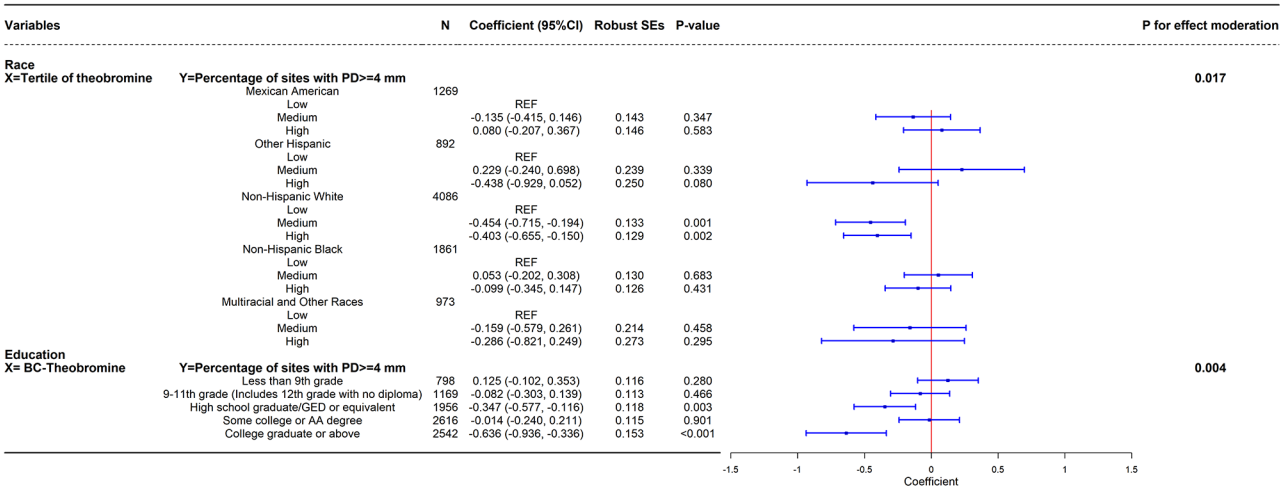


**Fig. 3** The correlation between the percentage of sites with PD ≥ 4 mm and theobromine intake under different covariates stratification. Robust SEs, Robust standard errors; CI, Confidence intervals; N, Sample size; REF, Reference; X, Exposure; Y, Outcome; AA, Associate's Degree; GED, General Educational Development; PD, pocket depth; BC-Theobromine, Boxcox-transformed theobromine

dose dependence, and their potential for teratogenicity (maternal, fetal, and neonatal metabolism).

The results of subgroup and effect modification analysis showed that gender, age, race, education, smoking, and hypertension may have effect modification effects in more than or equal to 2 periodontal-related indicators. This suggests that the mechanism of action of theobromine intake may be related to hormone levels, body metabolism, and blood circulation, and personal oral hygiene habits may have a confounding effect. Factors such as BMI, diabetes, and marriage only have statistical effect modification effects in one periodontal-related indicator, which may not have much actual clinical significance. Due to sample size limitations and biological rationality at each level, the results of subgroup analysis may not be completely clinically significant. Therefore, although the above factors show a moderating effect, further research should consider a larger sample size or other potential moderating factors to verify the robustness of these findings.

Besides, the subgroup and effect modification analysis revealed that gender, age, race, education, smoking, and hypertension may have modified the effect in at least two periodontal-related indicators. This suggests that the mechanism of theobromine intake could be related to hormone levels, body metabolism, and blood circulation, while personal oral hygiene habits may confound the results. In contrast, BMI, diabetes, and marriage only showed effect modification in one periodontal-related indicator, which may have limited clinical significance. Due to sample size limitations and biological considerations at each level, the subgroup analysis results may not all be clinically meaningful. Therefore, further research with larger sample sizes and consideration of additional potential moderating factors is needed to confirm the robustness of these findings.

The strengths of this study include the use of a large, representative NHANES population and the adjustment for many confounders through weighted analyses, enhancing the robustness of the results. However, the study also has several limitations. First, as a large cross-sectional study, it was not possible to determine a causal relationship between theobromine intake and periodontitis. Second, the assessment of theobromine intake was based on calculations from a two-day dietary survey, rather than blood or urine tests, which could introduce information bias. Although the recall period focused on the previous day's meals and was relatively brief, recall bias may still lead to omissions or inaccuracies. Additionally, respondents may be influenced by social desirability or personal emotions when reporting their responses. Third, the specific sources of theobromine intake, such as coffee, tea, or cocoa products, were not identified, which may affect the study outcomes. Fourth, self-reported data on personal oral hygiene may introduce inaccuracies in assessing hygiene levels, which were not considered a confounding factor in this study. Finally, the classification of theobromine intake into three categories lacks sufficient detail, and further investigation into the dose-response relationship is needed.

## Conclusion

In conclusion, our study found that theobromine intake was negatively correlated with periodontitis, suggesting it could serve as a protective factor. Further animal studies or clinical trials are needed to fully explore the relationship and underlying mechanisms. From a public health standpoint, promoting theobromine intake could aid in the tertiary prevention of periodontitis. Clinically,

the development of theobromine-based or derived drugs may help prevent the progression of mild periodontitis to more severe forms, offering potential therapeutic benefits in disease management.

## Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

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**Author contributions**
Y-S Z: Wrote the manuscript, found data, idea hypothesis, and data analysis. S C: Proofread the manuscript, and experimental design. S-Y L: Proofread the manuscript. M S: Data analysis. B Q: Proofread the manuscript. J-K S: Proofread the manuscript, and statistical evaluation. J-X P: Proofread the manuscript, and statistical evaluation. All authors reviewed the manuscript.

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**Data availability**
The data used in this article are publicly available here: https:/www.cdc gov/nchs/nhanes/index.ht.

## Declarations

**Ethical approval**
Data in this study were derived from the publicly available National Health and Nutrition Examination Survey (NHANES). The NCHS Research Ethics Review Committee reviewed and approved NHANES, with all participants providing written informed consent.

**Informed consent**
According to the requirements of national legislation and institutions, participation in this type of research does not require written informed consent.

**Consent for publication**
Not applicable.

**Clinical trial number**
Not applicable.

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**Competing interests**
The authors declare no competing interests.

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