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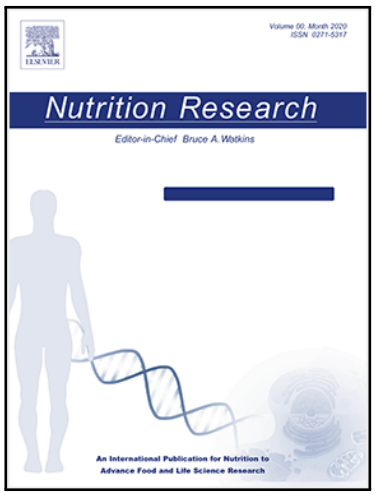

Vitamin B6 and folate intake are associated with lower risk of severe headache or migraine in adults:an analysis based on NHANES 1999-2004

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Highlights

- A high level of vitamin B6 and folate intake (vitamin B6 ≥ 2.39 mg/day, folate ≥ 502.01ug/day) were related to lower odds of migraine.
- There was a non-linear relationship between vitamin B6 and folate intake on migraine.
- Vitamin B6 and folate intake did not interact with sex, age, family income and education level.
- A high level of vitamin B6 and folate intake exhibited a synergistic interaction in lowering the risk of migraine.

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Abbreviations: AP: attributable proportion of interaction; NHANES: National Health and Nutrition Examination Survey; RERI: relative excess risk due to interaction; S: synergy index.

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ABSTRACT

Previous studies have shown that B vitamins can relieve migraine. However, the association between vitamin B6 and folate, two important B vitamins consumed in the diet, with migraine have received minimal attention. This study explored the independent relationships between dietary vitamin B6 and folate intake with migraine and the interaction effect of these two nutrients on migraine in US adults. We hypothesized that vitamin B6 and folate intake would be inversely associated with migraine. This study included cross-sectional data from participants aged 20 years and older who participated in the National Health and Nutrition Examination Survey (NHANES) from 1999 to 2004. We conducted multivariate logistic regression and restricted cubic spline (RCS) regression to explore the association between dietary vitamin B6 and folate intake on migraine. Also, relative excess risk due to interaction (RERI), attributable proportion of interaction (AP), and synergy index (S) were utilized to assess additive interactions. A total of 7017 participants were included in this study, 1350 of whom were migraineurs. We determined that vitamin B6 and folate intake revealed a negative association with severe headache or migraine [0.66 (95 % CI: 0.47-0.89, P = 0.01); 0.57 (95 % CI: 0.42-0.78, P = 0.002)], respectively. Also, a significant interaction effect between a high mass of vitamin B6 and folate intake was observed for a lower risk of migraine [RERI: 0.28(95%CI: 0.05-0.51); AP: 0.45(95%CI: 0.05- 0.86); S: 0.58 (95%CI: 0.40- 0.83)]. A high mass of vitamin B6 and folate intake (vitamin B6 intake ≥ 2.39 mg/day and folate intake ≥ 502.01ug/day)

presented a synergistic interaction with migraine, suggesting that these two nutrients might be beneficial in preventing migraine.

Keywords: Vitamin B6, Folate, NHANES, Migraine, Synergistic interaction

1.Introduction

A migraine headache is a widespread neurological disorder affecting over one billion people worldwide, of whom approximately 45.1 million live in constant pain [1]. Migraine prevalence in the United States is approximately 18.2% in women, 6.5% in men, and is most frequent in individuals under the age of 50 [2]. Previous studies have elucidated that natural compounds such as zinc and selenium are critical in human health [3-4]. Other studies have revealed that diet and nutrition are highly associated with migraine [5-6]. Therefore, exploring the effects of specific nutrients might facilitate preventing and treating migraine.

Vitamin B6 is water-soluble and exists as three closely related compounds with similar physiological effects, including pyridoxine, pyridoxal and pyridoxamine [7]. A previous study reported that vitamin B6 is a critical cofactor in the homocysteine metabolic pathway [8]. In particular, the active form of vitamin B6 is essential as a coenzyme for cystathionine synthase and cystathioninelyase, which are required to metabolize homocysteine to cysteine. Researchers suggested that disruption of the neurovascular endothelium caused by elevated homocysteine (Hcy) levels could result in migraine [9]. Moreover, a study of female Caucasian Australians suffering from migraine reported that compared to placebo, vitamin B6 supplementation significantly reduced homocysteine levels and alleviated headache severity and degree of disability

in migraineurs [10].

Folate is also a water-soluble vitamin, including endogenous dietary folate and its synthetic form, folic acid [11]. It has been reported that homocysteine metabolism depends on the presence of the cofactor folate, and that Hcy levels tend to be elevated in the presence of a folate deficiency [12]. Sadeghvand S et al. reported a positive correlation between serum Hcy levels and the frequency and characteristics of migraine attacks [13]. Dietary folate has been found to reduce serum Hcy levels [14]. A previous study revealed that 95 migraine patients supplemented with folate and pyridoxine in their diet for three months experienced a significant reduction in migraine severity and the frequency and duration of headache [15]. In addition, folate is an essential nutrient that supports mitochondrial energy metabolism linking migraines to mitochondrial function [16]. Furthermore, mitochondrial dysfunction has been observed in migraine patients [17].

However, few studies have examined the association between vitamin B6 and folate with migraine in the general population. This study explored the relation between dietary vitamin B6 and folate intake on severe headache or migraine in adults in a general population using data from the NHANES. We also identified interactions between vitamin B6 and folate intake with the risk of severe headache or migraine. Based on the dietary patterns found in this population, we hypothesized that there was an inverse relationship between dietary consumption of vitamin B6 and folate and severe headache or migraine. Furthermore, the dose–response correlation between dietary vitamin B6 and folate intake on severe headache or migraine was also

described, respectively.

2. Methods and materials

2.1. Study design

The data analyzed in this cross-sectional study were obtained from the NHANES, administered by the Centers for Disease Control and Prevention, USA. The NHANES data include a series of cross-sectional, stratified, multi-stage probability surveys of the non-institutional population in the United States [18]. The NHANES collected relevant examinations, interviews and questionnaires through home visits and mobile examination center (MEC) to assess the health and nutritional history of the US population. All NHANES study protocols were approved by the ethical review committee of the National Centre for Health Statistics (Protocol #98-12). In addition, NHANES obtained written informed consent from all participants. No additional institutional ethics review board authorization is required for secondary analysis [19]. Methodological details and the survey design of the NHANES can be found at https://www.cdc.gov/nchs/nhanes/index.htm.

Statistical power calculations were not performed before the study, and the data were obtained from the available NHANES data. We conducted a cross-sectional study of American adults in the 1999-2004 NHANES survey, as this was the only cycle that included adult headache questionnaires. These data were combined in our analysis, which resulted in 31126 participants. Our study was limited to adults 20 years of age or older. We excluded pregnant women and participants lacking critical information, such as severe headache or migraine and other missing data. The

present study included a final number of 7017 participants (Figure 1).

2.2. Migraine classification

Severe headache or migraine were determined by self-reporting as a component of the NHANES questionnaire. We categorized participants who answered "yes" into a group characterized as experiencing severe headache or migraines: "In the past 3 months, have you had severe headache or migraine?" This assessment method is valid, and other researchers in the field have made similar determinations [20].

2.3. Dietary vitamin B6 and folate assessment

Data on dietary vitamin B6 and folate intake were collected through a 24-hour recall survey. The survey utilized a retrospective dietary assessment method that provided detailed dietary and beverage consumption information within 24 hours. The 24-hour dietary recall interview information was obtained by verifying the computer-assisted dietary interview system from 1999 to 2002 and the United States Department of Agriculture (USDA) Automated Multiple-Pass Method (AMPM) from 2003 to 2004. Detailed dietary interview methods were available in the NHANES Dietary Interview Procedure Manual [21]. The dietary recall interviews were conducted in a private room of a Mobile Examination Center (MEC) by well-trained dietary interviewers fluent in Spanish and English. This study included all participants in the first 24-hour dietary review from 1999 to 2004 [22]. However, we could not utilize data on dietary supplements to assess vitamin B6 and folate intake.

2.4. Covariates assessment

Various covariates were evaluated based on previous literature [22-24]. According to

an established association or reasonable biological relationship, several covariates were selected and tested, including demographic covariates (age, sex, race, education level, marital status, and family income), lifestyle [smoking status, history of alcohol consumption], self-reported comorbidities (hypertension, diabetes, coronary heart disease and stroke) and dietary-related covariates (energy, protein, carbohydrate, and fat). Marital status was divided into married or living with a partner and living alone. The education level was classified as less than high school, completed high school or GED, and education beyond high school. According to a report by the US government, the poverty income ratio (PIR) divided family income into three categories: low (PIR≤1.3), medium (PIR>1.3 ~ 3.5), and high (PIR >3.5) [24]. Physical activity (PA) was defined as the product of metabolic equivalent value (MET) [25]. We calculated the level of physical activity based on the following formula: PA (MET-h/wk) = MET × frequency per week × duration of each physical activity [26].

2.5. Statistical analyses

We conducted statistical analyses using R software (version 4.2.1). All statistical analyses (including summary and inferential statistics) were conducted using all of the two-year MEC exam weights based on the NHANES analysis guidelines [27]. Continuous variables were described by sample-weighted means (standard error, SE) or medians (interquartile range, IQR). Categorical variables were reported as sample-weighted percentages and frequencies. To compare differences between individuals with migraine and controls, independent samples t-tests (normal distribution), the Wilcoxon Mann Whitney test (skewed distribution) and the Chi

square test (categorical variables) were performed. We divided participants into four quintiles based on dietary vitamin B6 and folate intake. Multifactorial logistic regression was used to investigate the relationship between dietary vitamin B6 and folate consumption and the occurrence of migraine. The correlation results were expressed as OR (95% CI). We tested the linear trend by inputting the median value of each type of vitamin B6 and folate consumption as a continuous variable in the model. Then, potential modifications of the association between dietary vitamin B6 and folate on migraine were estimated for the following variables: sex, age, family income, and education level. The synergistic interaction between high levels of vitamin B6 and folate intake associated with migraine was assessed by determining whether the evaluated joint effect of the two factors was greater than the sum of the independent effect of low levels of vitamin B6 and folate intake. RERI, AP and S were used to evaluate synergy. Additionally, RCS regression was used to explore the nonlinear relationship between dietary vitamin B6 and folate on migraine. Dietary vitamin B6 and folate consumption were used as continuous variables to participate in the model. Two-sided P values <0.05 indicated statistically significant difference.

3. Results

3.1. Baseline characteristic

The basic characteristics of the included and excluded individuals are provided in the Supplementary Materials (Table S1). As shown in Table 1, this study included 1350 adults (age ≥20 years) who experienced severe headache or migraine in the past three months. Compared to the participants without migraine, migraineurs tended to be

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younger (40.70 vs. 45.86, P < 0.0001), and female (61.56% vs 44.39%, P < 0.0001). Participants who experienced migraine exhibited a lower education level, lower family income, lower protein consumption were more likely to drink, and were current smokers. The average daily intake of vitamin B6 and folate in migraineurs was significantly lower than the control group (P<0.0001).

3.2. Association between dietary vitamin B6 and folate consumption and migraine

The results of the multi-factor logistic regression models are shown in Table 2 and Table 3. There was a negative association between vitamin B6 intake and migraine (Table 2). In model 3, the adjusted OR values for dietary vitamin B6 intake and migraine in Q2 (1.14-1.65mg/day), Q3 (1.66-2.38mg/day) and Q4 (≥2.39mg/day), compared to individuals with lower vitamin B6 intake Q1 (≤1.13mg/day), were 0.85 (95% CI: 0.69-1.05, P= 0.12), 0.84(95% CI: 0.65-1.09, P=0.18) and 0.66 (95% CI: 0.47-0.89, P=0.01), respectively (p for trend <0.015). Given that the P values for Q4 were statistically significant, we combined Q1-Q3 as a reference group. The OR values for vitamin B6 intake and migraine in Q4 (≥2.39mg/day), compared to individuals in Q1-Q3 (<2.38 mg/day), was 0.72 (95% CI: 0.59,0.93, P= 0.02).

Dietary folate intake was inversely related to migraine history (Table 3). In model 3, The ORs for the association between quintiles of dietary folate and migraine were 0.86 (95% CI: 0.66-1.12, P = 0.23), 0.94 (95% CI: 0.76-1.18, P = 0.58) and 0.57(95% CI: 0.42-0.78, P = 0.002), compared the Q2 (240.01-347ug/day), Q3 (347.01-502ug/day) and Q4 (≥502.01ug/ day) quintiles of folate with the lowest

quintile (≤240 ug /day), respectively (p for trend <0.001). Similarly, we combined Q1-Q3 as a reference group. The OR values for folate intake and migraine in Q4 (≥502.01ug/ day), compared to individuals in Q1-Q3 (<502ug/day), was 0.62 (95% CI: 0.49-0.80, P< 0.001).

In the RCS (Figure 2A), we observed a non-linear relationship between dietary vitamin B6 consumption and migraine (P = 0.03), using a reference point of 2.38 mg/day. The OR value for the relationship between vitamin B6 and migraine decreased with increasing dietary vitamin B6 intake. When the vitamin B6 consumption was higher than 2.38mg/day, the OR value was significantly lower than 1.00. Results seen in Figure 2B indicate a non-linear relationship between dietary folate consumption and migraine (P = 0.002), using a reference point of 502 ug/day. Moreover, the prevalence of migraine declined with increasing dietary folate intake until it reached 853.2 ug/day, after which the risk of migraine reached a plateau.

3.3. Stratified analysis

Stratified analyses were performed in several subgroups to assess possible modifications on the effect of the relationship between dietary vitamin B6 and folate on migraine. After stratification by sex, age, family income and education level, no significant interactions were observed (Figure 3, Figure 4).

3.4. Interaction between vitamin B6 and folate intake on migraine

Results shown in Table 4 indicated that the interaction indicator RERI was 0.28 (95%CI: 0.05-0.51), AP was 0.45 (95%CI: 0.05-0.86), and S was 0.58 (95%CI: 0.40-0.83), revealing that the interaction of high levels of vitamin B6 and folate intake

on migraine was statistically significant and the effects were synergistic. Based on these results, after adjusting for the potential variables included in this study, the AP was 0.45, indicating that the interaction between high mass of vitamin B6 and folate intake could prevent 45% of migraines.

4. Discussion

In the present study, we utilized NHANES 1999–2004 data that included 7017 people to study the associations of vitamin B6 and folate intake with migraine and their interactions on migraine. Examination of dietary vitamin B6 and folate intake revealed a negative association with severe headache or migraine. We also observed a non-linear relationship between dietary consumption of vitamin B6 and folate on migraine, using reference points of 2.38 mg/day and 502 ug/day, respectively. Moreover, we observed a synergistic interaction between high mass of vitamin B6 and folate intake on a lower risk of severe headache or migraine.

There is considerable interest in using dietary interventions to prevent migraine and severe headache [28]. For example, vitamin B2 (riboflavin) and coenzyme Q10 have been widely investigated, and supplementation with these nutrients has been shown to benefit migraine sufferers [29]. In this study, we found that vitamin B6 intake was negatively associated with severe headache or migraine, after controlling for confounding factors. Bahrampour N et al. concluded that dietary vitamin B6 intake was inversely related to migraine after analyzing data from 266 migraine patients in Iran and adjusting for confounding factors, which was consistent with the results observed in this study [30]. Another study reported that vitamin B6

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supplementation reduced the prevalence of migraine disability and alleviated headache frequency and pain severity [31]. In addition, when using a dietary vitamin B6 consumption of 2.38 mg/day as a reference point, we found that the OR in the RCS was significantly lower than 1.00. Therefore, we recommend that adults consume more foods rich in vitamin B6, including liver, whole grain cereals, peanuts, and bananas, which might prevent migraines [8].

There is limited evidence for the association between folate intake and migraine in adults. In our study, we observed that dietary folate intake was significantly inversely associated with severe headache or migraine, after adjusting for confounding factors. A study of 141 adult Caucasians revealed that dietary folate intake was significantly negatively associated with migraine disability and frequency [32]. In 1969, the effect of folate supplementation on migraine was studied by Kopjas TL et al [33]. Among 31 adult migraineurs, 60% had complete relief of their headache after the first folate supplementation, and the remaining individuals required a second supplementation. Thus, the beneficial effects of folate intake on migraine were influential in proposing strategies to prevent migraines in adults. Furthermore, we observed that migraine prevalence declined with increasing dietary folate in the RCS, using a reference point of 502 ug/day. Therefore, we recommend that adults should consume more foods rich in folate, such as pulses, yeast, fruit, green leafy vegetables, liver and kidney [34].

The mechanism underlying our finding that there was a synergistic interaction between high mass of vitamin B6 and folate intake on migraine may be supported by

studies of homocysteine metabolism or mitochondria. Hcy is a highly reactive amino acid that causes endothelial damage by inhibiting the release of nitric oxide (NO) [35]. NO super-sensitivity may be associated with pain transmission, nociceptive hyperalgesia, chronic pain, inflammation, and central sensitization, it is primarily associated with the cyclic guanosine monophosphate (cGMP)-dependent pathway [36]. It is well known that migraine pain is mediated through activation of the trigeminovascular system (TVS). Endothelial dysfunction caused by hyperhomocysteinemia can lead to vascular and coagulation dysfunction [37]. These events might contribute to changes in cerebral blood flow and TVS activation. Furthermore, it has been reported that the firing rate of trigeminal neurons in response to pain increases with the application of D, L - homocysteic acid, a substance mimickings the action of homocysteine [38]. Moreover, vitamin B6 and folate are required for homocysteine metabolism (re-methylation to methionine requires folate and trans-sulfuration to cystathionine requires pyridoxine) [39]. Recently, related applications of phosphorus nuclear magnetic resonance spectroscopy have revealed changes in energy metabolism in the brains of migraine patients [40], indicating that an imbalance between brain energy requirements and ATP production exerts a key role in migraine. Because glycolysis is the primary form of energy production in the brain, mitochondrial energy production is closely associated with pathogenesis of migraine [41]. Several recent studies have elucidated that vitamin B6 and folate are involved in mitochondrial homeostasis and energy production [42-43]. Furthermore, several clinical studies have found consistent positive therapeutic effects of vitamin

B6 and folate in preventing and treating migraine in adults [15,44,45]. For instance, Askari G et al found that most migraine patients had better results in relieving headache symptoms after supplementation with folate and vitamin B6 in their study. In summary, moderate dietary vitamin B6 and folate intake might afford increased synergistic effects on migraine prevention.

There are some limitations that must be taken into account in this study. At first, dietary data was obtained based on 24-hour recall, which has inherent limitations in the reliability and effectiveness of nutritional assessment. However, Prentice RL et al. reported that 24-hour recall might provide more detail about food types and quantities than food frequency surveys [46]. Also, we did not utilize data on dietary supplements to assess vitamin B6 and folate intake, because reliable and consistent information on dietary supplements was unavailable from the three NHANES cycles. Moreover, since the introduction of the mandatory folic acid food fortification in 1998, in the US food chain are present two different forms of folate, natural food folate and folic acid, which have different bioavailability. However, the dietary folate intake in this study has been presented as total folate rather than as dietary folate equivalents which takes into account that folic acid has 1.7 times higher bioavailability than natural food folate and by that way the current study might underestimates the actual folate intake. Secondly, the identification of migraine was limited to a single self-report questionnaire of migraine or severe headache. In addition, there were no data on other characteristics of the participants' migraine, such as severity or the presence of aura and other symptoms. Nonetheless, the results of this migraine assessment were

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consistent with the findings of the American Migraine Prevalence and Prevention (AMPP) study. That study indicated that 17.4% of participants reported “severe headache”, of which 11.8% met the International Headache Disorder Type II (ICHDII) criteria for migraine, 4.6% met the criteria for “possible migraine” and only 1% were identified as “other severe headache” [47]. Thus, these data provide meaningful insight into the otherwise lack of epidemiologic data linking diet and migraine. Third, this study was conducted among American adults and excluded special groups such as minors. Therefore, we were unable to analyze special populations or other races, which resulted in insufficient extrapolation power for this study. Therefore, additional evidence is needed to validate the generalizability of these results. Also, we could not remove the effect of nonrandom missing data on the results due to baseline differences between included and excluded individuals. Finally, our study was cross-sectional, meaning causal inferences could not be made.

This study presented several advantages. First, this study provided epidemiological evidence of the significant association between dietary vitamin B6 and folate intake on migraine in a representative general population across the United States. Second, we provided reliable correlations by adjusting for multiple confounders in our statistical analyses. Stratified analyses were performed in several subgroups to identify existing differences. Furthermore, we evaluated the dose-response effect of dietary vitamin B6 and folate on migraine and provided practical recommendations. Third, no previous studies on the interaction between vitamin B6 and folate intake on migraine have been published. This study indicated a

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possible synergistic effect between a high level of vitamin B6 and folate intake.

5. Conclusion

This study indicated that high vitamin B6 and folate intakes (vitamin B6 intake ≥ 2.39 mg/day and folate intake ≥ 502.01ug/day) were significantly related to lower odds of severe headache or migraine using NHANES data from 1999-2004. Moreover, we found that high vitamin B6 and folate intakes exhibited a synergistic interaction in lowering the risk of migraine. Therefore, adults could appropriately increase vitamin B6 and folate intake to prevent migraine. Although adjusting dietary habits requires considerable motivation, such efforts could be effective and worthwhile in preventing migraine.

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CRediT authorship contribution statement

Sheng Tian: Conceptualization, Methodology, Formal analysis, Writing – original draft, Visualization. Xin-Ping Yu: Conceptualization, Software, Writing – review & editing. Lan-Xiang Wu: Validation, Data curation. He-Qing Zheng: Software,

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Visualization. Xian-Hui Zhong: Formal analysis, Software. Yong-Gang Xie: Validation. Wei Wu: Writing – review & editing, Supervision, Project administration, Funding acquisition.

Author Declarations

None.

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Legends to Figures

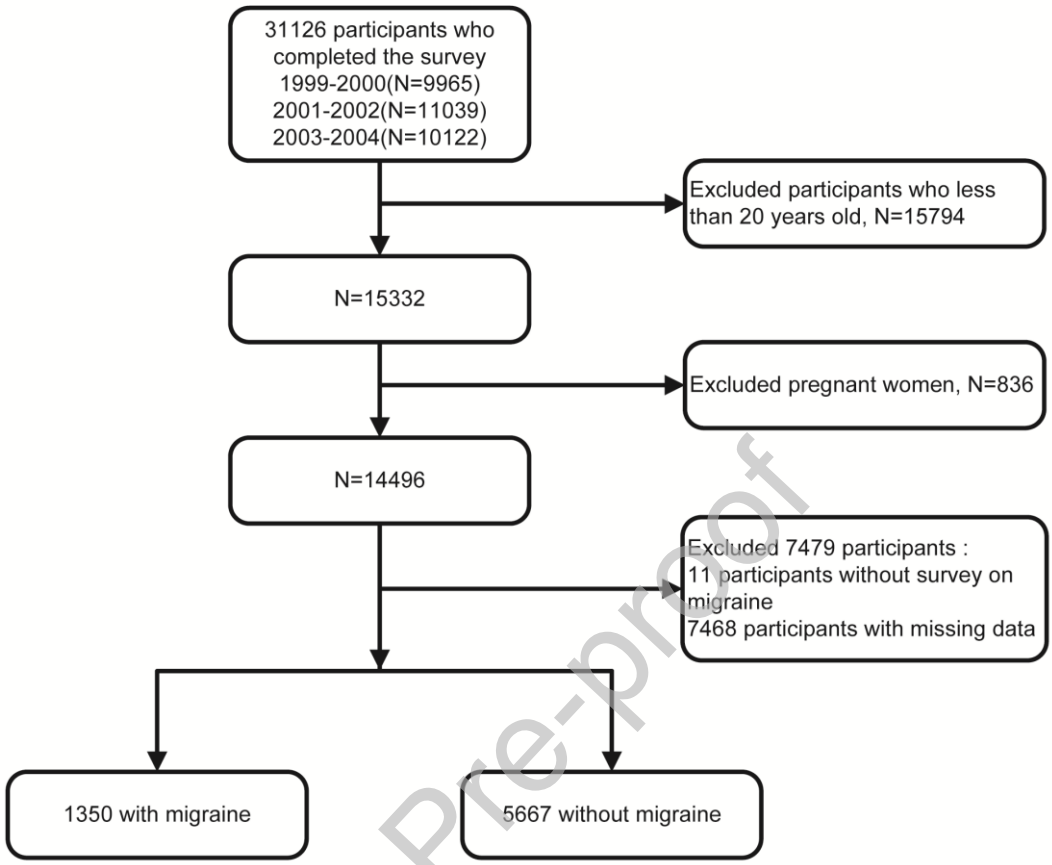

Fig. 1 Inclusion and exclusion flow chart.

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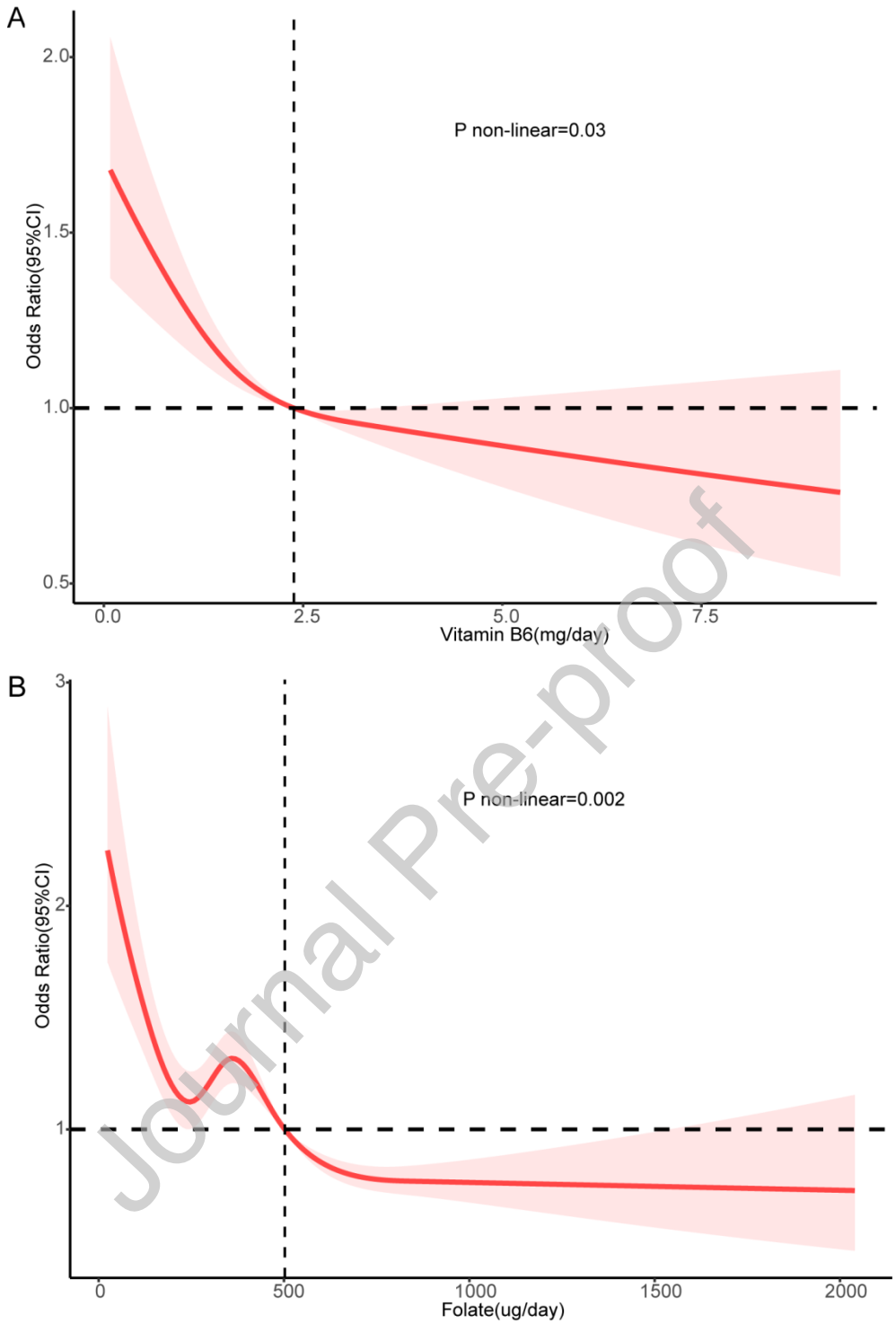

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Fig. 2 Restricted cubic spline analysis for the associations between dietary vitamin B6(A) and folate(B) intake on migraine odds ratio in the NHANES 1999–2004. Vitamin B6 and folate was treated as a continuous type variable for restricted cubic spline analysis. A nonlinear trend was found between vitamin B6 and folate intake on

migraine. Covariates included age, sex, marital status, race, education level, family income, smoking status, drinking status, hypertension, diabetes, stroke, coronary heart disease, energy consumption, protein consumption, carbohydrate consumption, fat consumption, MET. Solid line, OR; Shade, 95% CI. MET, metabolic equivalent value.

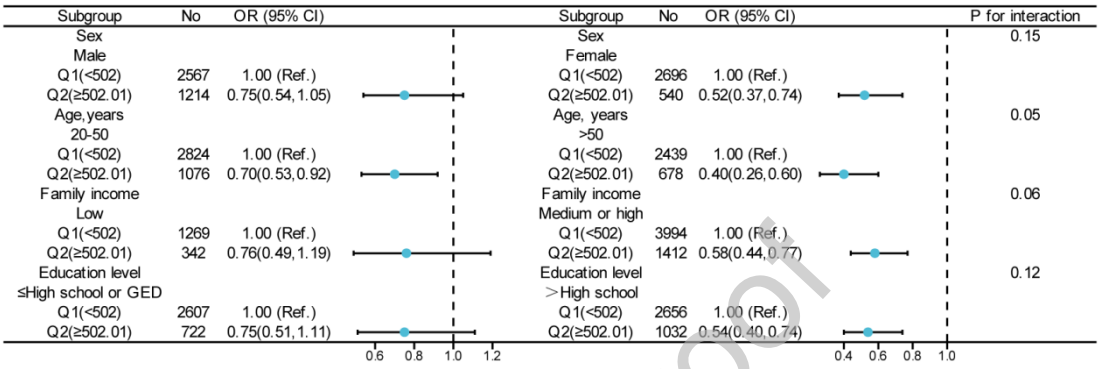

Fig. 3 Interaction effects between folate intake and different subgroup (sex, age, family income, education level) for migraine in the NHANES 1999–2004. Folate intake did not interact with any other subgroup. Except the stratification variables themselves, each stratification factor was adjusted for all other variables (age, sex, marital status, race, education level, family income, smoking status, drinking status, hypertension, diabetes, stroke, coronary heart disease, energy consumption, protein consumption, carbohydrate consumption, fat consumption, MET). MET, metabolic equivalent value.

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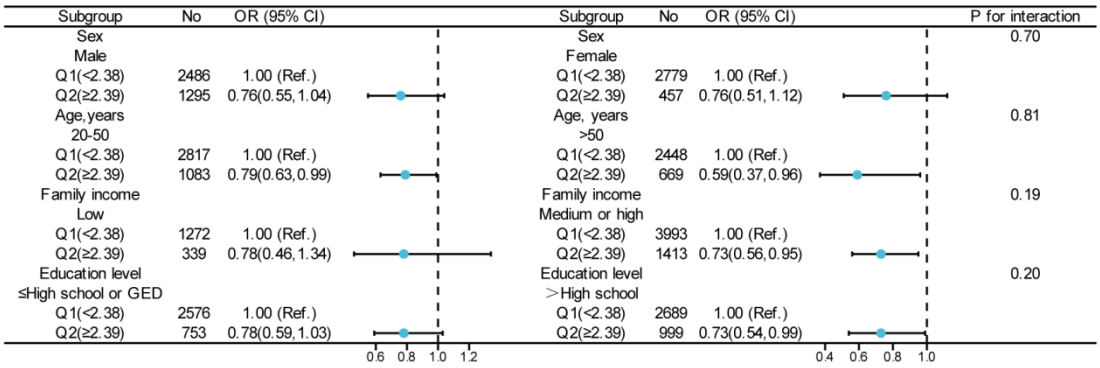

Fig. 4 Interaction effects between vitamin B6 intake and different subgroup (sex, age, family income, education level) for migraine in the NHANES 1999–2004. Vitamin B6 intake did not interact with any other subgroup. Except the stratification variables themselves, each stratification factor was adjusted for all other variables (age, sex, marital status, race, education level, family income, smoking status, drinking status, hypertension, diabetes, stroke, coronary heart disease, energy consumption, protein consumption, carbohydrate consumption, fat consumption, MET). MET, metabolic equivalent value.

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Table 1. Baseline characteristics of participants in the NHANES 1999–2004.

	Total	**Migraine**	**Control**	
	***n* = 7017**	***n* = 1350**	***n* = 5667**	
Characteristic	**Mean (SE) or n (%) [a]**	**Mean (SE) or *n* (%) [a]**	**Mean (SE) or *n* (%) [a]**	***P*[b] value**
Age, years, mean	44.79(0.34)	40.70(0.41)	45.86(0.39)	< 0.0001
Sex				< 0.0001
Male	3781(52.06)	519(38.44)	3262(55.61)	
Female	3236(47.94)	831(61.56)	2405(44.39)	
Race				0.23
Non-Hispanic white	4062(77.34)	726(74.89)	3336(77.98)	
Non-Hispanic black	1141(8.27)	234(9.04)	907(8.06)	

Mexican American	1327(5.76)	288(6.69)	1039(5.52)	
Others	487(8.63)	102(9.38)	385(8.43)	
Marital status				0.19
Living alone	2465(32.65)	503(34.29)	1962(32.22)	
Married or living with partner	4552(67.35)	847(65.71)	3705(67.78)	
Education level				< 0.001
<High school	1624(13.96)	338(17.06)	1286(13.15)	
High school or GED	1705(25.58)	354(28.44)	1351(24.84)	
>High school	3688(60.46)	658(54.50)	3030(62.02)	
Family income				< 0.0001
Low	1611(16.97)	393(23.84)	1218(15.17)	
Medium	2670(35.05)	531(37.94)	2139(34.29)	
High	2736(47.99)	426(38.22)	2310(50.54)	
Smoking				< 0.0001
Never	3519(50.57)	694(50.23)	2825(50.66)	
Current	1557(24.13)	391(30.52)	1166(22.47)	
Former	1941(25.29)	265 (19.24)	1676(26.87)	
Drinking				0.02
Never	826(10.45)	166(10.90)	660(10.34)	
Former	1273(15.02)	254(17.55)	1019(14.36)	
Mild	2483(36.16)	411(31.21)	2072(37.45)	
Moderate	1080(17.39)	228(17.86)	852(17.27)	
Heavy	1355(20.98)	291(22.50)	1064(20.58)	
Diabetes	572(5.55)	111(5.52)	461(5.56)	0.94

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Hypertension	2098(25.02)	387(25.82)	1711(24.81)	0.4
Coronary heart disease	319(3.30)	35(2.40)	284(3.54)	0.08
Stroke	167(1.66)	33(2.13)	134(1.54)	0.17
Energy(kcal/d)	2289.86(15.81)	2259.88(30.14)	2297.69(17.39)	0.26
Fat consumption (g/d)	86.36(0.69)	85.37(1.28)	86.62(0.85)	0.44
Protein consumption (g/d)	85.09(0.72)	81.35(1.31)	86.07(0.74)	< 0.001
Carbohydrate intake (g/d)	278.77(2.25)	283.13(4.58)	277.64(2.39)	0.26
Folate consumption (ug/d)	412.57(5.31)	380.01(7.55)	421.06(5.67)	< 0.0001
Vitamin B6 consumption (mg/d)	1.94(0.02)	1.78(0.04)	1.98(0.02)	< 0.0001
MET	16.23(0.66)	16.58(1.10)	16.14(0.69)	0.69

[a]Unweighted sample size was used for the number of participants in different groups for the categorical variables, whereas the percentages as well as the means and standard errors of the continuous variables are the results calculated after weighted.

[b]p value was calculated by weighted independent t-test or wilcoxon mann whitney test for continuous variable and Chi-square test for categorical variables.

Abbreviation: SE, standard error; MET, metabolic equivalent value.

Table 2. Relationship between dietary vitamin B6 intake and migraine in adult in the NHANES 1999–2004.

Quartiles		**OR (95% CI)**					
	No.[d]	**Model 1[a]**	***P* value**	**Model 2[b]**	***P* value**	**Model 3[c]**	***P* value**
Dietary vitamin B6 (mg/day)							
Q1(≤1.13)	1756	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Q2(1.14-1.65)	1753	0.78(0.66,0.93)	0.005	0.86(0.72,1.02)	0.09	0.85(0.69,1.05)	0.12

Q3(1.66-2.38)	1756	0.74(0.61,0.90)	0.003	0.85(0.69,1.06)	0.15	0.84(0.65,1.09)	0.18
Q4(≥2.39)	1752	0.54(0.45,0.65)	<0.0001	0.67(0.55,0.82)	<0.001	0.66(0.47,0.89)	0.01
P for trend	-	<0.0001	-	<0.001	-	0.015	-
Q1-Q3(<2.38)	5265	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Q4(≥2.39)	1752	0.64 (0.55,0.75)	<0.0001	0.74(0.63,0.87)	<0.001	0.72(0.59,0.93)	0.02

[a]Logistic regression using the minimum quantile as a reference. Adjusted for nothing.

[b]Logistic regression using the minimum quantile as a reference. Adjusted for age, sex.

[c]Logistic regression using the minimum quantile as a reference. Adjusted for age, sex, marital status, race, education level, family income, smoking status, drinking status, hypertension, diabetes, stroke, coronary heart disease, energy consumption, protein consumption, carbohydrate consumption, fat consumption, MET.

[d]Unweighted frequencies.

Abbreviation: OR, odds ratio; CI, confidence interval; MET, metabolic equivalent value.

Table 3. Relationship between dietary folate intake and migraine in adult in the NHANES 1999–2004.

Quartiles	**OR (95% CI)**						
	No.[d]	**Model 1[a]**	***P* value**	**Model 2[b]**	***P* value**	**Model 3[c]**	***P* value**
Dietary folate (ug/day)							
Q1(≤240)	1763	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Q2(240.01-347)	1746	0.81(0.64,1.01)	0.06	0.85(0.68,1.07)	0.17	0.86(0.66,1.12)	0.23
Q3(347.01-502)	1754	0.85(0.71,1.02)	0.07	0.96(0.80,1.16)	0.70	0.94(0.76,1.18)	0.58
Q4(≥502.01)	1754	0.54 (0.45,0.66)	<0.0001	0.63(0.51,0.77)	<0.0001	0.57(0.42,0.78)	0.002
P for trend	-	<0.0001	-	<0.0001	-	<0.001	-

Q1-Q3(<502)	5263	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Q4(≥502.01)	1754	0.61(0.51,0.74)	<0.0001	0.67(0.56,0.81)	<0.0001	0.62 (0.49,0.80)	<0.001

[a]Logistic regression using the minimum quantile as a reference. Adjusted for nothing.

[b]Logistic regression using the minimum quantile as a reference. Adjusted for age, sex.

[c]Logistic regression using the minimum quantile as a reference. Adjusted for age, sex, marital status, race, education level, family income, smoking status, drinking status, hypertension, diabetes, stroke, coronary heart disease, energy consumption, protein consumption, carbohydrate consumption, fat consumption, MET.

[d]Unweighted frequencies.

Abbreviation: OR, odds ratio; CI, confidence interval; MET, metabolic equivalent value.

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Table 4. Additive interactions analysis of vitamin B6 and folate intake on migraine in adult in the NHANES 1999–2004.

Vitamin B6 intake	**Folate intake**	**Model 1**		**Model 2**		**Model 3[a]**	
		OR(95%CI)	***P* value**	**OR(95%CI)**	***P* value**	**OR(95%CI)**	***P* value**
Low-level[b]	Low-level[c]	1.00 (reference)		1.00(reference)		1.00(reference)	
Low-level	High-level	0.60(0.46,0.78)	<0.001	0.63(0.48,0.83)	0.001	0.62(0.48,0.82)	0.002
High-level[d]	Low-level	0.66(0.52,0.84)	0.001	0.76(0.58,0.98)	0.03	0.72(0.53,0.97)	0.04
High-level	High-level[e]	0.58(0.48,0.70)	<0.0001	0.66(0.55,0.79)	<0.0001	0.60(0.44,0.83)	0.004
RERI (95%CI)		0.32(0.12,0.52)		0.28(0.04,0.51)		0.28(0.05,0.51)	
AP (95%CI)		0.55(0.19,0.91)		0.42(0.06,0.77)		0.45(0.05,0.86)	

S(95%CI)	0.57(0.42,0.77)	0.55(0.37,0.83)	0.58(0.40,0.83)

[a]Logistic regression using the minimum quantile as a reference. Each analysis adjusted for age, sex, marital status, race, education level, family income, smoking status, drinking status, hypertension, diabetes, stroke, coronary heart disease, energy consumption, protein consumption, carbohydrate consumption, fat consumption, MET.

[b]Vitamin B6 intake <2.38mg/day

[c]Folate intake<502ug/day

[d]Vitamin B6 intake≥2.39mg/day

[e]Folate intake≥502.01ug/day

Abbreviation: RERI, relative excess risk due to interaction; AP, attributable proportion of interaction; S, synergy index; MET, metabolic equivalent value.

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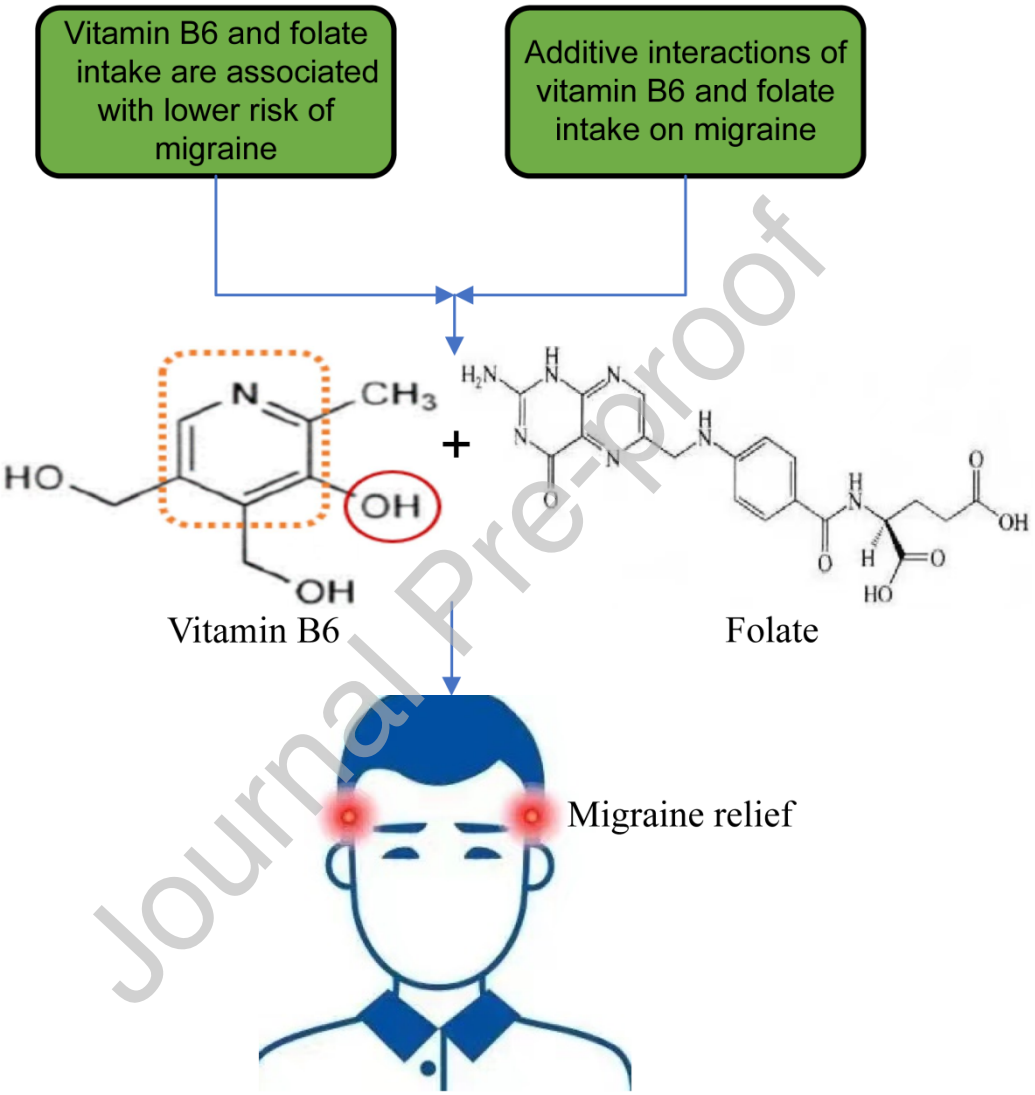
Legend to Graphical Abstract

Vitamin B6 and folate intake are associated with lower risk of severe headache or migraine in adults. Furthermore, a high level of vitamin B6 and folate intake may present a synergistic interaction in relieving migraine.



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