Original Article

Total folate, natural folate and synthetic folic acid intake associations with adult depressive symptoms

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Background and Objectives: In the United States, synthetic folic acid has become an important source of folate in the era of mandatory folic acid fortification. This study aimed to evaluate the associations between total folate, natural folate and synthetic folic acid intakes and depressive symptoms among US adults. **Methods and Study Design:** Cross-sectional data was collected from the National Health and Nutrition Examination Survey (NHANES) 2007-2016. Dietary data were obtained through two 24-h dietary recall interviews. Depressive symptoms were detected by PHQ-9 (Patient Health Questionnaire-9), participants whose depression scores over 10 points were diagnosed as depressive symptoms. Weighted logistic regressions were used to analyze the associations between different forms of folate and depressive symptoms. **Results:** Among 19244 participants included in this study, 9.2% of them met the definition of depressive symptoms. In fully adjusted models, total folate intake and natural folate intake were inversely associated with depressive symptoms respectively, while synthetic folic acid was not. The multivariate-adjusted odds ratios (ORs) with 95% confidence intervals (CIs) of depressive symptoms were 0.69 (0.54-0.89), 0.51 (0.39-0.68) for the highest versus lowest quartile of total folate and natural folate intake, respectively. We also found two L-shaped dose-response curves among total folate intake, natural folate intake with depressive symptoms, respectively. **Conclusions:** Total folate intake, natural folate intake may be inversely associated with depressive symptoms among US adults, while the association was not significant between synthetic folic acid and depressive symptoms.

Key Words: depressive symptoms, folate, NHANES, synthetic folic acid, natural folate

INTRODUCTION

Depression is a common mental disease that troubles people around the world today. It is estimated that over 264 million people worldwide suffered from depression, according to the WHO.[1] Although effective treatments could be obtained today, 8.1% of Americans aged over 20 years had depression in the United States.[2] Therefore, more attention is needed to be given to the prevention of depression.

Folate is a general term for a family of compounds. Natural folate widely exists in plant and animal foods, such as animal liver, kidney, green leafy vegetables and fruits. Folic acid is a synthetic form of folate, which is widely used in fortified foods and supplements. In 1998, the United States introduced mandatory folic acid fortification of cereal-grain products for preventing neural tube malformation.[3] Since then, synthetic folic acid has become an important source of folate for the US population. Unlike natural folate, folic acid has no coenzyme activity until it is reduced to tetrahydrofolate. Differences in the metabolism of natural folate and synthetic folic acid may lead to different effects on health. An US prospective study conducted in the post fortification era revealed that natural folate was negatively associated with rectal, but not colon cancer in women, and folic acid intake was negatively associated with colon, but not rectal cancer in men.[4]

Folate plays a vital role for the nervous system. Through one carbon cycle, folate could reduce serum homocysteine concentration,[5] which is considered as a risk factor for depression.[6,7] That suggests the folate may be associated with depression. In recent years, some clinical and epidemiological researches were carried out to explore the relationship of folate intake and depressive symptoms.[8-12] Among these studies, most observational studies were limited to the effects of total folate intake (natural folate + synthetic folic acid) on depressive symptoms. As we know, few observational studies have investigated the associations between natural folate, synthetic folic acid and depressive symptoms, respectively.

This study aimed to evaluate the associations between total folate, natural folate and synthetic folic acid intakes and depressive symptoms in US adults over 20 years old. And we also assessed the dose-response relationship between folate and depressive symptoms.

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METHODS

Population

The data used in this study were collected from National Health and Nutrition Examination Survey (NHANES). NHANES is a cross-sectional survey, which is administered by the National Center for Health Statistics (NCHS) at the Centers for Disease Control and Prevention (CDC). All participants provided written informed consent, and the Research Ethics Review Board approved the study protocol.[13] Data on health and nutrition status of the US population were collected through in-person interviews, telephone interviews, questionnaires and laboratory tests. Data from five survey cycles (2007-2008, 2009-2010, 2011-2012, 2013-2014 and 2015-2016) were analyzed in this study.

There are altogether 49296 respondents participating in the five survey cycles. Among them, we excluded 20845 participants younger than 20 years old, 470 pregnant or lactating women, 4624 participants with an unfinished depression questionnaire and 4037 participants with incomplete 24-h dietary recall data. Furthermore, participants were excluded who had extreme total energy intakes of <500 or >5000 kcal/day for women (n=54), and <500 or >8000 kcal/day for men (n=22). Finally, 19244 individuals were included in the present study (Figure 1).

Dietary assessment

Dietary intake data was assessed by two 24-h dietary recall interviews. The first dietary recall interview was collected in-person in the Mobile Examination Center (MEC) and the second interview was collected by telephone 3 to 10 days later. We used the average dietary intake data of two interviews. Participants who did not complete both two dietary surveys were excluded. Dietary folate or folic acid intakes from foods were calculated using USDA's Food and Nutrient Database for Dietary Studies.[14] NHANES investigated total amount of supplements used in the past 30 days through household interview. The folic acid supplements were divided by 30 as the daily folic acid supplement intake.

This study included 3 folate measurements: natural folate intake, synthetic folic acid intake and total folate intake. Natural folate refers to all natural folate derived from foods. Synthetic folic acid was the sum of food-source synthetic folic acid and daily folic acid supplement intake. We used dietary folate equivalent (DFE) to reflect total folate intake, which considered natural folate intake, synthetic folic acid from foods and folic acid supplements.[15]

DFE (μg) = natural folate (μg) + 1.7 × (food folic acid + folic acid supplements) (μg)

Natural folate, total folic acid, and total folate intakes were categorized based on quartiles, respectively. According to intake level, all participants were divided into quartiles (quartile 1: <25th percentile, quartile 2: ≥25 to 50th percentile, quartile 3: ≥50 to 75th percentile, quartile 4: ≥75th percentile), respectively.

Depressive symptoms

NHANES used the PHQ-9 depression screening scale to detect depressive symptoms. PHQ-9 consists of nine items, all based on the description of depression in DSM-

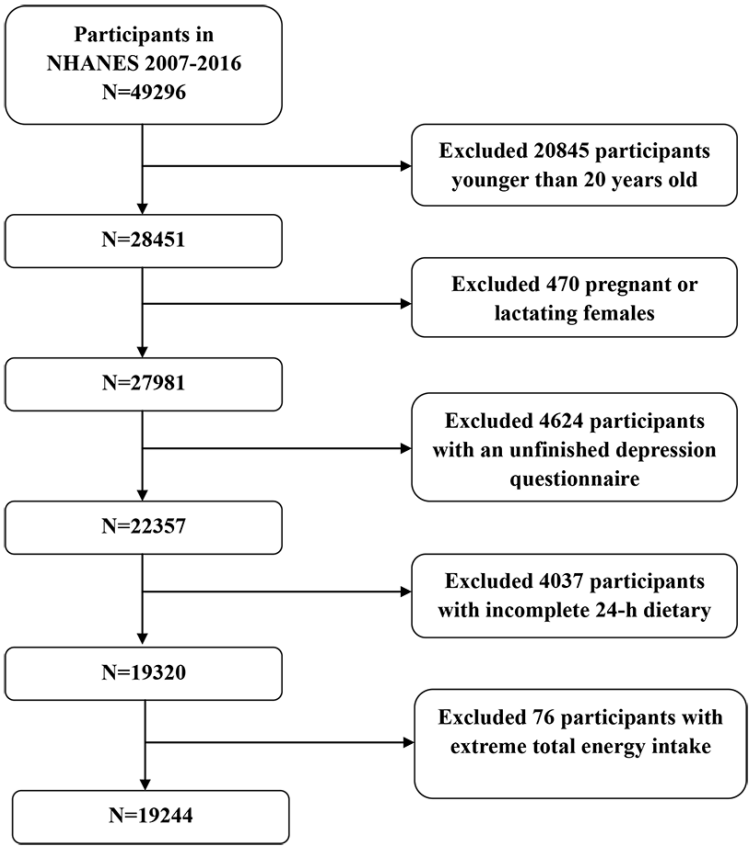

Figure 1. Flow chart for the selection of the study participants.

IV. Each item has four options: "No" (0 points), "Several days" (1 point), "More than half of the time" (2 points), "Almost every day" (3 points). The total scores are the sum of the scores of nine items, ranging from 0 to 27. Participants whose total scores ≥10 were classified as depressive symptoms. This criterion has been confirmed to have good specificity and sensitivity.[16,17]

Covariates

For reducing the confounding, we adjusted some covariates in multivariate models, referred to our published literature.[18-20] Demographic factors included gender, age (20~44 years, 45~59 years, ≥60 years), race (Mexican-American, other Hispanic, non-Hispanic white, non-Hispanic black, other races), marital status (married/living with partner, divorced/separated/widowed), educational level (below high school, high school, over high school), annual family income (below $20000, $20000 and over). Energy and dietary vitamin B-12 intake were gained from two 24-h dietary recalls. Body mass index (BMI) was categorized as underweight (<18.5 kg/m^2), normal weight (18.5 kg/m^2 to <25 kg/m^2), pre-obesity (25 kg/m^2 to <30 kg/m^2) and obesity (≥30 kg/m^2). Physical activities were acquired through questionnaires. Individuals were identified as smokers if they smoked more than 100 cigarettes in life.[18,19] And, drinkers were defined if they had at least 12 alcohol drinks per year.[19] Additionally, participants' using antidepressants were obtained from the prescription medication questionnaire.

Some chronic health conditions were also considered. The history of hypertension was based on the question "Have you ever been told by a doctor or other health professional that you had hypertension, also called high blood pressure?" Similarly, the history of diabetes was also based on self-reported diagnosis.

Statistical analysis

Stata15.0 was used for all data analysis. Account for the complex survey, non-response, we used the sample weights in our analyses to construct a nationally representative sample. When combining 5 cycle surveys, new sample weights were conducted by taking one-fifth of the original weights, according to the official guidance of NHANES.

Kolmogorov-Smirnov test was used to evaluate whether continuous variables had a normal distribution. Intakes of energy, vitamin B-12, total folate, natural folate, synthetic folic acid had non-normal distributions according to normality test, so median value and interquartile range were used to describe the averages and variations, respectively. Mann-Whitney U test was used to compare the medians of continuous variables between the depressive symptoms group and non-depressive symptoms group. Chi square test was used to compare the distributions of categorical variables between two groups. The multiple adjusted logistic regression model was used to calculate the odds ratios (ORs) and 95% confidence intervals (95% CIs) for depressive symptoms according total folate intake, natural folate, synthetic folic acid intake, with the quartile 1 as reference group. Model 1 adjusted for age and gender. Model 2 additionally adjusted for race, educational level, annual family income, BMI, hypertension, diabetes, taking antidepressants, smoking, drinking, physical activity, energy intake and dietary vitamin B-12 intake. In addition, we conducted stratified analyses to examine whether these associations differed by age and gender. In sensitive analysis, individuals with self-reported antidepressants using were excluded for testing the stability of the results.

We also assessed the dose-response relationship by restricted cubic spline with knots at the 5th, 25th, 50th, 75th and 95th percentiles of the exposure distribution, adjusted for all covariates. All p values were two-sides with a statistical significance level of 0.05.

RESULTS

Table 1 described the demographic and behavioral characteristics of 19244 participants included in this analysis. Among them, 9.2% of individuals met the definition of depressive symptoms. Women had a significantly higher prevalence (11.6%) of depressive symptoms than men (6.7%). Compared with individuals whose PHQ score <10, depressed participants were more likely to be middle-aged and obese. Depressed participants tended to have lower family income and education level, and they have a higher risk of self-reported hypertension and diabetes. Smokers were more likely to obtain depressive symptoms. Intakes of total energy, vitamin B-12, total folate, natural folate, synthetic folic acid of depressed subjects were significantly lower than those without depressive symptoms.

The associations between folate intakes and depressive symptoms were shown in Table 2. Without adjustment, the crude ORs with 95% CIs of depressive symptoms suggested that total folate intake, natural folate intake, synthetic folic acid intake were negatively associated with the prevalence of depressive symptoms. Model 1 adjusted age and gender, showing the same results as the unadjusted model. In fully-adjusted models, the associations between total folate intake, natural folate intake and depressive symptoms were attenuated but remained significant, the OR (95% CI) of total folate intake, natural folate intake and depressive symptoms were 0.69 (0.54-0.89) and 0.51 (0.39-0.68) for the highest versus lowest quartile, respectively. However, the association of synthetic folic acid intake and depressive symptoms was no longer statistically significant in fully-adjusted model (p for linearity=0.39).

Figure 2 and figure 3 presented the dose-response relationships between total folate intake, natural folate intake and depressive symptoms, respectively. The figures showed two L-shaped does--response curves. The does-response relationship between synthetic folic acid intake and depressive symptoms was not assessed as result of the non-significant association between the two in multivariate-adjusted model.

The results of subgroup analysis stratified by age and gender were shown in Table 3 and Table 4, respectively. Among men, the ORs (95% CIs) of depressive symptoms were 0.56 (0.36-0.89), and 0.47 (0.30-0.73) in multivariate-adjusted models for total folate and natural folate intake, respectively. In women, natural folate was still negatively associated with depressive symptoms, but the negative association of total folate intake was no longer

Table 1. Descriptive characteristics of the study participants. (n=19244)

	Without depressive symptoms (PHQ <10)	With depressive symptoms (PHQ ≥10)	*p* value
Number of subjects	17479 (90.8)	1765 (9.2)	
Categorical variable†			
Age (%)			<0.001
20~44 years	6970 (39.8)	686 (38.9)	
45~59 years	4313 (24.7)	567 (32.1)	
≥60 years	6196 (45.5)	512 (29.0)	
Gender (%)			<0.001
Men	8820 (50.5)	634 (35.9)	
Women	8659 (49.5)	1131 (64.1)	
Race (%)			<0.001
Mexican American	2508 (14.3)	251 (14.2)	
Other Hispanic	1745 (10.0)	248 (14.1)	
Non-Hispanic white	7800 (44.6)	763 (43.2)	
Non-Hispanic black	3670 (20.1)	385 (21.8)	
Other race	1756 (10.0)	118 (6.7)	
Marital status (%)			<0.001
Married/Living with partner	10628 (60.8)	789 (44.7)	
Widowed/Divorced/Separated/Never married	6843 (39.2)	975 (55.3)	
Educational level (%)			<0.001
<high school	3787 (21.7)	630 (35.7)	
high school	3954 (22.6)	416 (23.6)	
>high school	9726 (55.7)	719 (40.7)	
Annual family income (%)			<0.001
Under $20000	3756 (22.4)	744 (44.3)	
$20000 and over	13013 (77.6)	934 (55.7)	
BMI (%)			<0.001
<18.5 kg/m^2	251 (1.4)	30 (1.7)	
18.5 to <25 kg/m^2	4672 (27.0)	377 (21.7)	
25 to <30 kg/m^2	5856 (33.8)	446 (25.6)	
≥30 kg/m^2	6551 (37.8)	887 (51.0)	
Physical activity (%)			<0.001
Vigorous activity	3265 (19.1)	311 (18.1)	
Moderate activity	3703 (21.7)	306 (17.8)	
Other	10094 (59.2)	1104 (64.1)	
Ever had hypertension (%)	6239 (35.7)	836 (47.4)	<0.001
Ever had diabetes (%)	2186 (12.5)	357 (20.2)	<0.001
Had at least 12 alcohol drinks/1 year (%)	12621 (72.2)	1282 (72.6)	0.703
Smoking at least 100 cigarettes in life (%)	7445 (42.4)	1028 (58.2)	<0.001
Currently taking antidepressants (%)	1296 (7.4)	474 (26.9)	<0.001
Using folic acid supplement or not	5416 (31.0)	447 (25.3)	
Continuous variable‡			
Total folate intake, μg DFE/day	483 (335)	420 (299)	<0.001
Natural folate intake, μg/day	202 (131)	162 (117)	<0.001
Synthetic folic acid intake, μg/day	155 (156)	136 (135)	<0.001
Vitamin B-12 intake, μg/day	4.21 (3.62)	3.79 (3.60)	<0.001
Energy intake, kcal/day	1979 (976)	1833 (1029)	<0.001

†Categorical variables are represented as number of participants (%).
‡Continuous variables are represented as median (interquartile range).

statistically significant. After age stratification, the negative association between natural folate and depressive symptoms was no longer statistically significant in middle-aged people (45≤age<60 years).

In sensitivity analysis (Table 5), after excluding the subjects with self-reported antidepressant medication using, the results were stable.

DISCUSSION

In this study, we included 19244 participants to assess the associations between folate intakes and depressive symptoms. Negative associations between total folate intake, natural folate intake and the risk of depressive symptoms persisted after adjusting for potential confounding factors, the dose-response relationship analysis showed two L shaped curves. Meanwhile, null association was found between synthetic folic acid intake and depressive symptoms.

So far, some previous studies have reported the association between folate intake and depressive symptoms.[10,21-23] This study revealed that total folate intake was negatively related to depressive symptoms, which is consistent with the previous meta-analysis.[24] In coherence with our results, a Finnish population-based study showed lower dietary folate consumption was associated with a higher risk of melancholic depressive symptoms.[25] In another Japanese study of early adolescence, higher folate intake was independently linked to lower incidence of depressive symptoms.[9] However, a survey conducted in Quebec, where introduced folic acid fortification policy, did not

Table 2. Weighted odds ratios (95% confidence intervals) between depressive symptoms and different forms of folate

	Cases/Participants	Intake cutoff	Crude	Model 1[†]	Model 2[‡]
Total folate intake, μg DFE/day					
Quartile 1	559/4801	<325.5	Ref	Ref	Ref
Quartile 2	464/4819	325.5 to <458.5	0.75 (0.62-0.90)**	0.78 (0.65-0.94)*	0.86 (0.68-1.10)
Quartile 3	370/4814	458.5 to <647.3	0.56 (0.47-0.66)**	0.61 (0.51-0.72)**	0.69 (0.55-0.85)**
Quartile 4	362/4810	≥647.3	0.52 (0.44-0.62)**	0.60 (0.50-0.71)**	0.69 (0.54-0.89)**
p[§]			<0.001	<0.001	0.004
Natural folate intake, μg/day					
Quartile 1	610/4790	<136.5	Ref	Ref	Ref
Quartile 2	480/4819	136.5 to <191.5	0.69 (0.57-0.83)**	0.72 (0.59-0.87)**	0.82 (0.67-1.02)
Quartile 3	379/4810	191.5 to <267.0	0.45 (0.38-0.53)**	0.49 (0.41-0.58)**	0.59 (0.47-0.73)**
Quartile 4	296/4825	≥267.0	0.38 (0.32-0.46)**	0.43 (0.35-0.52)**	0.51 (0.39-0.68)**
p[§]			<0.001	<0.001	<0.001
Synthetic folic acid intake, μg/day					
Quartile 1	486/4818	<89.1	Ref	Ref	Ref
Quartile 2	476/4802	89.1 to <147.5	0.91 (0.76-1.09)	0.95 (0.79-1.14)	0.91 (0.74-1.14)
Quartile 3	419/4813	147.5 to <333.4	0.81 (0.68-0.96)*	0.86 (0.72-1.03)	0.91 (0.74-1.14)
Quartile 4	384/4811	≥333.4	0.70 (0.58-0.84)**	0.80 (0.66-0.96)	0.89 (0.72-1.11)
p[§]			0.001	0.01	0.39

[†]Model 1 was adjusted for age and gender.
[‡]Model 2 was adjusted for age, gender, race, marital status, educational level, annual family income, marital status, body mass index, smoking, hypertension, diabetes, physical activity, taking antidepressants, drinking, energy intake, and dietary vitamin B-12 intake.
[§]*p* for linearity was calculated by using the median value of each quartile as a continuous variable in each model.
*$p<0.05$; **$p<0.01$.

Table 3. Weighted odds ratios (95% confidence intervals) for depressive symptoms according to quartiles of folate intakes, stratified by age

	20 ≤ Age < 45 Years			45 ≤ Age < 60 Years			Age ≥ 60 Years		
	Crude	Model 1†	Model 2‡	Crude	Model 1†	Model 2‡	Crude	Model 1†	Model 2‡
Total folate intake, μg DFE/day									
<325.5	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
325.5 to <458.5	0.71 (0.54-0.93)*	0.75 (0.59-0.87)**	0.82 (0.60-1.15)	0.86 (0.63-1.17)	0.92 (0.67-1.26)	1.03 (0.68-1.60)	0.65 (0.47-0.90)*	0.67 (0.49-0.94)**	0.72 (0.48-1.09)
458.5 to <647.3	0.46 (0.35-0.61)**	0.53 (0.39-0.70)**	0.60 (0.43-0.83)**	0.68 (0.48-0.97)*	0.76 (0.53-1.09)**	0.92 (0.59-1.43)	0.51 (0.34-0.75)**	0.55 (0.37-0.82)**	0.60 (0.38-0.95)*
≥647.3	0.49 (0.37-0.64)**	0.59 (0.45-0.78)**	0.68 (0.47-0.99)*	0.53 (0.37-0.76)*	0.64 (0.44-0.92)*	0.82 (0.53-1.28)	0.51 (0.37-0.70)**	0.58 (0.41-0.82)**	0.61 (0.34-1.10)
p§	<0.001	0.001	0.06	0.001	0.01	0.24	<0.001	0.005	0.12
Natural folate intake, μg/day									
<136.5	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
136.5 to <191.5	0.71 (0.53-0.95)*	0.74 (0.56-0.99)*	0.74 (0.56-1.02)*	0.66 (0.49-0.90)**	0.70 (0.51-0.96)*	0.94 (0.64-1.38)	0.68 (0.44-1.06)	0.71 (0.45-1.10)**	0.74 (0.44-1.26)
191.5 to <267.0	0.44 (0.33-0.59)**	0.49 (0.36-0.66)**	0.59 (0.47-0.73)**	0.43 (0.32-0.57)**	0.46 (0.43-0.63)**	0.72 (0.46-1.15)	0.49 (0.35-0.69)**	0.52 (0.37-0.74)**	0.56 (0.33-0.97)*
≥267.0	0.36 (0.28-0.47)**	0.44 (0.32-0.59)**	0.51 (0.39-0.68)**	0.37 (0.26-0.53)**	0.43 (0.30-0.62)**	0.65 (0.38-1.11)	0.37 (0.24-0.58)**	0.41 (0.26-0.66)**	0.42 (0.24-0.75)**
p§	<0.001	<0.001	<0.001	<0.001	<0.001	0.09	<0.001	<0.001	0.003
Synthetic folic acid intake, μg/day									
<89.1	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
89.1 to <147.5	0.97 (0.72-1.31)	1.01 (0.75-1.36)	0.96 (0.68-1.39)	0.91 (0.64-1.30)	0.96 (0.67-1.37)	0.94 (0.59-1.50)	0.80 (0.58-1.11)	0.84 (0.60-1.18)	0.84 (0.57-1.24)
147.5 to <333.4	0.69 (0.52-0.93)*	0.76 (0.57-1.02)	0.81 (0.58-1.14)	0.90 (0.62-1.29)	0.97 (0.66-1.42)	1.07 (0.69-1.65)	0.84 (0.61-1.17)	0.89 (0.64-1.25)	0.98 (0.68-1.42)
≥333.4	0.70 (0.51-0.96)*	0.83 (0.60-1.13)	0.93 (0.64-1.36)	0.70 (0.50-0.99)	0.82 (0.58-1.17)	0.96 (0.61-1.49)	0.63 (0.44-0.90)*	0.70 (0.48-1.03)	0.77 (0.47-1.27)
p§	0.01	0.15	0.71	0.93	0.04	0.25	0.02	0.10	0.40

†Model 1 was adjusted for age and gender.

‡Model 2 was adjusted for age, gender, race, marital status, educational level, annual family income, marital status, body mass index, smoking, hypertension, diabetes, physical activity, taking antidepressants, drinking, energy intake, and dietary vitamin B-12 intake.

§*p* for linearity was calculated by using the median value of each quartile as a continuous variable in each model.

*$p<0.05$; **$p<0.01$.

Table 4. Weighted odds ratios (95% confidence intervals) for depressive symptoms according to quartiles of folate intakes, stratified by gender

	Men			Women		
	Crude	Model 1†	Model 2‡	Crude	Model 1†	Model 2‡
Total folate intake, μg DFE/day						
<325.5	Ref	Ref	Ref	Ref	Ref	Ref
325.5 to <458.5	0.72 (0.53-0.98)*	0.71 (0.52-0.96)*	0.80 (0.55-1.15)	0.81 (0.63-1.02)	0.80 (0.63-1.02)	0.86 (0.64-1.15)
458.5 to <647.3	0.43 (0.31-0.60)**	0.42 (0.30-0.59)**	0.53 (0.35-0.79)**	0.73 (0.60-0.89)**	0.72 (0.59-0.87)**	0.77 (0.60-0.99)*
≥647.3	0.50 (0.35-0.71)**	0.48 (0.34-0.69)**	0.56 (0.36-0.89)*	0.69 (0.55-0.85)**	0.66 (0.53-0.83)**	0.72 (0.52-1.02)
p§	0.001	0.001	0.03	0.002	0.001	0.07
Natural folate intake, μg/day						
<136.5	Ref	Ref	Ref	Ref	Ref	Ref
136.5 to <191.5	0.63 (0.47-0.84)**	0.62 (0.46-0.83)**	0.78 (0.55-1.10)	0.76 (0.61-0.94)*	0.76 (0.61-0.95)*	0.84 (0.64-1.11)
191.5 to <267.0	0.38 (0.28-0.53)**	0.38 (0.27-0.52)**	0.49 (0.32-0.74)**	0.56 (0.45-0.68)**	0.55 (0.45-0.68)**	0.64 (0.49-0.85)**
≥267.0	0.40 (0.30-0.53)**	0.39 (0.30-0.51)**	0.47 (0.30-0.73)**	0.45 (0.34-0.59)**	0.44 (0.35-0.58)**	0.54 (0.38-0.77)**
p§	<0.001	<0.001	0.001	<0.001	<0.001	<0.001
Synthetic folic acid intake, μg/day						
<89.1	Ref	Ref	Ref	Ref	Ref	Ref
89.1 to <147.5	0.71 (0.50-1.02)	0.71 (0.50-1.02)	0.77 (0.52-1.12)	1.07 (0.88-1.32)	1.95 (0.87-1.30)	0.98 (0.77-1.24)
147.5 to <333.4	0.66 (0.46-0.94)*	0.65 (0.45-0.94)*	0.72 (0.48-1.08)	0.99 (0.82-1.20)	0.97 (0.80-1.17)	1.00 (0.80-1.26)
≥333.4	0.58 (0.40-0.82)**	0.57 (0.40-0.82)**	0.67 (0.45-1.00)*	0.97 (0.78-1.20)	0.85 (0.76-1.18)	0.99 (0.75-1.31)
p§	0.008	0.007	0.09	0.58	0.47	0.98

†Model 1 was adjusted for age and gender. ‡Model 2 was adjusted for age, gender, race, marital status, educational level, annual family income, marital status, body mass index, smoking, hypertension, diabetes, physical activity, taking antidepressants, drinking, energy intake, and dietary vitamin B-12 intake. §*p* for linearity was calculated by using the median value of each quartile as a continuous variable in each model. *$p<0.05$; **$p<0.01$.

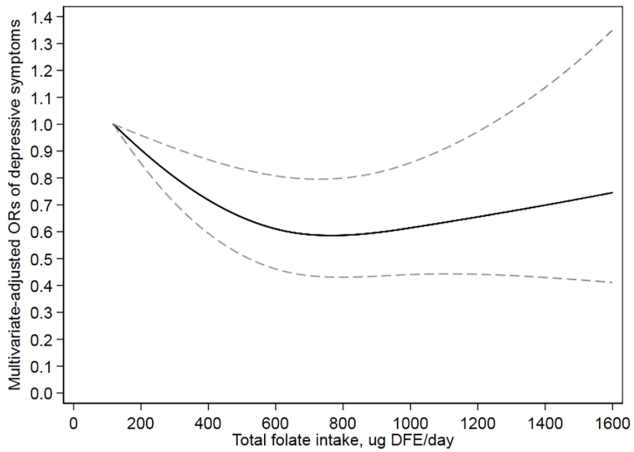

Figure 2. The dose-response relationship between total folate intake and depressive symptoms, estimated by restricted cubic spline model. The model adjusted for age, gender, race, marital status, educational level, annual family income, BMI, smoking, drinking, physical activity, hypertension, diabetes, taking antidepressants, energy and vitamin B-12 intake. The solid line and the dash line represent the estimated relative risks and their 95% CIs, respectively.

Table 5. Weighted odds ratios (95% confidence intervals) between depressive symptoms and different forms of folate (Excluding participants using antidepressant medication)

	Cases/ Participants	Intake cutoff	Crude	Model 1[†]	Model 2[‡]
Total folate intake, μg DFE/day					
Quartile 1	423/4368	<327.5	Ref	Ref	Ref
Quartile 2	337/4369	327.5 to <460.7	0.72 (0.58-0.89)**	0.75 (0.61-0.93)**	0.79 (0.60-1.02)
Quartile 3	262/4368	460.7 to <649.0	0.53 (0.43-0.65)**	0.58 (0.47-0.71)**	0.65 (0.50-0.84)**
Quartile 4	269/4369	≥649.0	0.51 (0.41-0.63)**	0.57 (0.46-0.72)**	0.63 (0.45-0.87)**
p[§]			<0.001	<0.001	0.01
Natural folate intake, μg/day					
Quartile 1	442/4358	<138.0	Ref	Ref	Ref
Quartile 2	353/4376	138.0 to <193.5	0.69 (0.56-0.86)**	0.73 (0.58-0.91)*	0.80 (0.62-1.04)
Quartile 3	280/4370	193.5 to <269.5	0.45 (0.37-0.56)**	0.49 (0.40-0.61)**	0.56 (0.42-0.74)**
Quartile 4	216/4370	≥269.5	0.39 (0.32-0.49)**	0.44 (0.35-0.55)**	0.50 (0.36-0.70)**
p[§]			<0.001	<0.001	<0.001
Synthetic folic acid intake, μg/day					
Quartile 1	349/4388	<84.0	Ref	Ref	Ref
Quartile 2	370/4363	85.0 to <142.0	1.06 (0.89-1.28)	1.10 (0.91-1.32)	1.05 (0.85-1.30)
Quartile 3	293/4348	142.0 to <229.0	0.81 (0.65-1.01)	0.86 (0.69-1.08)	0.93 (0.72-1.19)
Quartile 4	279/4375	≥229.0	0.72 (0.57-0.92)*	0.81 (0.64-1.03)	0.86 (0.64-1.15)
p[§]			0.002	0.02	0.18

[†]Model 1 was adjusted for age and gender. [‡]Model 2 was adjusted for age, gender, race, marital status, educational level, annual family income, marital status, body mass index, smoking, hypertension, diabetes, physical activity, drinking, energy intake, and dietary vitamin B-12 intake. [§]*p* for linearity was calculated by using the median value of each quartile as a continuous variable in each model. *$p<0.05$; **$p<0.01$.

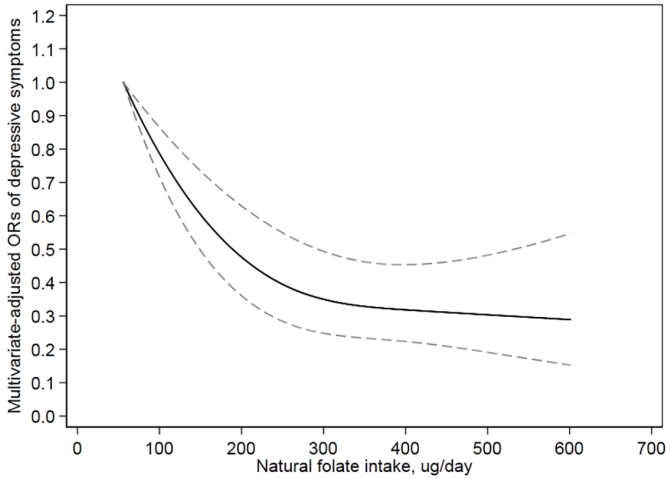

Figure 3. The dose-response relationship between natural folate intake and depressive symptoms, estimated by restricted cubic spline model. The model adjusted for age, gender, race, marital status, educational level, annual family income, BMI, smoking, drinking, physical activity, hypertension, diabetes, taking antidepressants, energy and vitamin B-12 intake. The solid line and the dash line represent the estimated relative risks and their 95% CIs, respectively.

find the association between the two in the older population.[10]

As for natural folate, few special studies have explored the effect of natural folate on depressive symptoms. This study revealed that natural folate was negatively associated with depressive symptoms. Natural folate is widely derived from various natural foods and is not synthetic. While synthetic folic acid is not associated with depressive symptoms in present research. The results suggest that food-source natural folate may be the better source of folate rather than synthetic folic acid added to foods or supplements. Similar to our results, a large-size clinical randomized controlled trial involving 4331 women showed that long-term, high-dose, daily folic acid supplementation did not reduce the likelihood of depression.[11] One possible explanation is that the metabolism of folic acid in vivo is affected by the activity of dihydrofolate reductase (DHFR). DHFR activity seems to be limited,[26,27] so that excess folic acid can not be converted into L-5-methyltetrahydrofolate with biological activity.

Moreover, this study analyzed the dose-response relationship between folate and depressive symptoms. Our results showed two L-shaped nonlinear curves in total folate intake and natural folate intake. This may be explained by a threshold effect,[22] so that excessive folate intake would not reduce the risk of depressive symptoms.

Some researchers have studied the physiological and biochemical mechanisms of folate on depressive symptoms. Researchers believe that folate plays a major role in the biosynthesis of monoamine neurotransmitters such as serotonin, dopaminedopamine, and norepinephrine. Folate participates in the synthesis of S-adenosylhomocysteine (SAM) through the re-methylation substitution of homocysteine (Hcy). SAM is indispensable for the synthesis of monoamine neurotransmitters.[28] Inadequate folate may influence the synthesis of neurotransmitters and increase the risk of depressive symptoms. In addition, insufficient folate inhibits the conversion of homocysteine to cysteine, resulting in increased serum homocysteine concentrations, which have been demonstrated to increase the risk and severity of depressive symptoms.[29] Besides dietary folate, intestinal flora is another human folate source,[30,31] which may affect the occurrence of depressive symptoms through the microbiota–gut–brain axis.[32]

This study has several advantages. At first, we assessed the associations of total folate, natural folate and synthetic folic acid intakes with depressive symptoms. As far as we know, this is the first study to explore relationships between different forms of folate and depressive symptoms. Second, we adjusted potential covariates as much as possible. Thirdly, we estimated the detail dose-response relationship between folate intake and depressive symptoms. Moreover, the sample from NHANES is large-size, and we adjusted sample weights to make the sample nationally representative. However, some limitations have to be considered. The main limitation is that this study was a cross-sectional study, which was restricted to make causal inferences. Second, the accuracy of two 24-hour dietary recall interviews is largely dependent on participants' memory. Poor memory of depressive individuals may lead to low dietary intake reporting, which exaggerated the relationship between folate intake and depressive symptoms. Thirdly, the PHQ-9 depression scale is a self-rating scale, not a clinical standard for the diagnosis of depression. Compared with non-depressed people, depressed patients are more reluctant to fill the depression scale, resulting in non-response bias.

In conclusion, this study revealed that total folate intake and natural folate intake were inversely associated with depressive symptoms, while synthetic folic acid was not. More carefully designed epidemiological studies are needed to conduct in other populations with mandatory folic acid fortification policy. In addition, biological mechanism needs to be elucidated why this strongly negative association between depressive symptoms and natural folate do not exist in synthetic folic acid.

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AUTHOR DISCLOSURES

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