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Association between Serum Folate Levels and Depression in Reproductive Aged U.S. Women, NHANES (2011-2012)

By

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Degree to be awarded: MPH

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By

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Bachelor of Science of Biochemistry and Molecular Biology

University of Georgia

2012

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An abstract of

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology
2016

Abstract

Association between Serum Folate Levels and Depression in Reproductive Aged U.S. Women, NHANES (2011-2012)

By Brenda Nguyen

Background: Depression is the leading cause of disability and affects about a quarter of United States population. Some studies have shown that low serum folate levels are associated with an increased risk of depression, particularly pronounced among women; however, results varied by study size and design.
Objective: We examined, using latest data, the association between serum folate level and depression among non-pregnant U.S. women of reproductive age.
Methods: We used data from the 2011-2012 National Health and Nutrition Examination Survey (NHANES). We compared serum folate levels between women with and without self-reported depression based on Patient Health Questionnaire-9 (PHQ-9) scores. We examined the association between serum folate and depression by controlling for potential co-variables using multivariable linear and logistic regression analysis. Crude and adjusted odds ratios (cORs and aORs) and 95% confidence intervals (CI) were estimated. Additionally, we examined effect modification by race and ethnicity.
Results: Among 1,324 non-pregnant women (age 20-44 years), 16.7% (n=221) were screened positive for depression. There was no statistical difference in the median serum folate concentrations between participants with and without depression (17.8 ng/ml and 17.2 ng/ml, respectively; P=0.5). The unadjusted association of serum folate concentration and depression was not significant (cOR: 1.02; 95% CI: 0.98, 1.07). After adjustments for potential co-variables, serum concentration of folate was weakly and positively associated with depression (aOR: 1.10; 95% CI: 1.02, 1.19).
Conclusions: Our study found a weak association between serum folate and depression among women of reproductive age in the United States. We recommend future studies to conduct prospective analyses to confirm the association between serum folate and depression women of reproductive age to demonstrate potential benefits from regular and adequate folic acid use.

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Acknowledgements

This research project would not have been possible without the support of many people. I feel truly fortunate to have had the opportunity to work with my mentor, Dr. Vijaya Kancherla, and I thank her for her continuous guidance and encouragement. I would like to thank my thesis committee members, Dr. Hind Beydoun and Professor Paul Weiss, for all of their guidance through this process; your discussion, ideas, and feedback have been absolutely invaluable. I would especially like to thank my amazing family for the love, support, and constant encouragement I have gotten over the years.

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INTRODUCTION

Depression is the leading cause of disability and the fourth leading contributor to the global burden of disease and is recognized as an important cause of morbidity and mortality worldwide (1,2). This disorder is the most common type of mental illness, affecting more than 26% of the U.S. adult population (3) and is characterized as a mental disorder associated with a range of emotional, cognitive, and physical behavioral symptoms including loss of interest or pleasure, feelings of guilt, disturbed sleep or appetite, low energy, and poor concentration (2,4). Depressive symptoms interfere with daily life and can contribute to personal adverse health effects (4,5).

Women are twice as likely as men to report a lifetime history of major depressive episodes (1). According to the 2009-2012 National Health and Nutrition Examination Survey (NHANES), among a population of 18-39 year old, the prevalence of those displaying significant depressive symptoms was 9.3% in women compared to 5.8% in men (6). Biological processes are thought to be involved in the predisposition of women to depression, including genetically determined vulnerability and hormonal fluctuations (7). Psychosocial events, such as role-stress and sex-specific socialization, are considered to increase the vulnerability of women to depression (7). Depression among 50% of reproductive aged women is undiagnosed and untreated due to high cost, opposition to treatment, and stigma related to perceived mental illness in society (1). Women with depression are at a high risk for adverse reproductive outcomes, including lower fertility and negative pregnancy outcomes (e.g., preterm births and low birth weight infants), and impaired maternal functioning and bonding (1,8,9).

Nutrition can play a key role in the onset as well as the severity and duration of depression (10,11). Evidence has suggested that low folate levels may play an additional role in

the risk of depression. (12–14). Folate is a B-vitamin that is derived from diet or supplementation. Dietary folate is found in green leafy vegetables, legumes, beans, liver, citrus fruits, and yeast (15). Depressed patients have consistently been found to have lower serum folate concentrations, and patients with very low folate levels were associated with higher depression scores than patients with normal folate levels (12,16). Folate plays an important role in critical brain metabolic pathways (12), this vitamin is involved in the methylation processes and the synthesis of neurotransmitters in the central nervous system (e.g., serotonin), plausibly linking to depressive symptoms (12,16). In addition, genetic polymorphisms of methylenetetrahydrofolate reductase (MTHFR) enzyme, which is present in approximately 60% of the U.S. population, catalyzes the conversion of methylenetetrahydrofolate to the active form of the vitamin, making it difficult to convert folate, whether dietary or supplemental, to the active form (15).

In 1998, the U.S. government mandated the fortification of the food supply with folic acid in order to reduce neural birth defects (17). The public health recommendation is that women consume 400 µg folic acid per day (17,18). Today, clinicians diagnose serum folate level ranging from 3 to 4.5 ug/L as folate deficiency (19). However, average serum folate levels among those with depression are greater than this guideline (13,14,20). It has been proposed that the folic acid levels in fortified foods may not be high enough to significantly impact illnesses connected to low folate levels (15,18). Recent studies have shown most non-pregnant women of reproductive age in the U.S. have reported consuming less than the recommended amount of folic acid from fortified foods or supplements (or both) (21).

Serum folate reflects recent folate intake and low levels are an early indicator of inadequate folate status (18). Evidence has shown that low levels of serum folate may be causally

linked to depressive symptoms (17). A study using a NHANES (2005-2006) found the odds of elevated depressive symptoms was 69% lower among women with higher serum folate compared to women with lower serum folate (13). Due to folic acid fortification, the median serum folate levels of reproductive aged women increased from 4.8 ng/ml in 1988 to 13.0 ng/ml in 2000 (18).

In addition, the proportion of women who consumed at least 400 μg folic acid daily varied significantly by race-ethnicity, ranging from 19.1% in non-Hispanic black women to 21.0% in Hispanic women and 40.5% in non-Hispanic white women (21). This has also been reflected in median serum folate levels among women of reproductive age by race and ethnicity. It has been shown that among women aged 15-44 years, non-Hispanic blacks and Hispanics have lower median serum folate levels than non-Hispanic whites (10.2 ng/ml, 11.4 ng/ml, and 13.8 ng/ml, respectively) (22). Moreover, there is evidence there are racial or ethnic differences in specific genes important in the production or function of folate (23–25). For example, genetic polymorphisms of Methylenetetrahydrofolate reductase (MTHFR), an enzyme important in converting folate to its active form, is more prevalent among African Americans than in Caucasians (24).

The status of the association of serum folate levels and depression among women of reproductive age is unknown. The purpose of this study is to assess the association between serum folate levels and depression, among non-pregnant U.S. women of reproductive age. We hypothesize that among these women, there is an inverse association between the level of serum folate and the severity depression, i.e., as folate levels increase, the reported severity of depression decreases among non-pregnant women of reproductive age. We also hypothesize that race and ethnicity interact with the association between folate status and the risk of depression.

REVIEW OF THE LITERATURE

The following literature review will provide necessary context to the aims and objectives of the research. There is a growing body of literature documenting the relationship between folate and depression risk factors. Research has demonstrated that various psychosocial domains, including socioeconomic status, race, gender, and occupation, can often moderate these relationships. Yet research examining the compounded effects of these demographic domains is relatively scarce. In order to further investigate the implications of gender and racial status on the relationship between folate and depression, the literature will be surveyed and reported with particular attention given to research focusing on racial and/or gender differences. The review will elucidate the knowledge gap in the research and explain why we must explore the role of gender and race/ethnicity in the association between folate and depression.

FOLATE

Folate and its physiology

Folate is a B-vitamin (vitamin B9) that cannot be synthesized de novo; it must be derived from diet or supplementation. Dietary folate is found in green leafy vegetables, legumes, beans, liver, citrus fruits, and yeast (15). Dietary folate must be converted to become the metabolically active, tissue useable form. The synthetic molecule is called folic acid which is highly absorbed (85-95%) compared to the dietary form (50%). Methylenetetrahydrofolate reductase (MTHFR) is an enzyme important in catalyzing the conversion of methylenetetrahydrofolate (MTHF) to the active form of the vitamin- 5- methylenetetrahydrofolate (5-MTHF or L-methylfolate). Genetic polymorphisms of MTHFR has been shown to be present in approximately 60 percent of the U.S. population, affecting the conversion of folate, whether dietary of supplemental, to its active form (15).

Folate is essential in numerous biochemical pathways because of its donation of a methyl group, including pathways of neurotransmitter synthesis, DNA biosynthesis, regulation of gene expression, amino acid synthesis and metabolism, and myelin synthesis and repair (15). Folate's involvement in the methylation processes and the synthesis of neurotransmitters in the central nervous system (e.g. serotonin), is plausibly linked to its effect on mood and cognition (12,15,16).

Genetic factors of folate levels

Evidence exists for the presence of genetic differences in MTHFR between races. A preliminary study investigating the allele frequencies in genetic polymorphisms of MTHFR among patients with rheumatoid arthritis compared to those without arthritis demonstrated significant differences in single-nucleotide polymorphisms (SNP) alleles between African Americans and Caucasians, regardless of disease status. African Americans had more common SNPs of the MTHFR than in Caucasians. This study highlighted important racial or ethnic differences in frequencies of common genetic polymorphism of MTHFR (24). These polymorphisms can affect the conversion of folate and serum folate levels. In addition, Qit et. al. have identified and characterized a human folate transporter located in the small intestine called PCFT/HCP1 (25). This characterized PCFT/HCP1 functions as an electrogenic, proton-coupled, high affinity transporter for folates and antifolate compounds, at optimal low pH (5.5-6.5). Moreover, they found that participants who suffer from hereditary familial folate malabsorption were homozygous for a mutation in this particular PCFT/HCP1 gene (25). Therefore, genetic differences can influence the function of folate transportation and uptake.

Fortification of food supply with folic acid in the United States

In 1998, the U.S. Food and Drug Administration (FDA) mandated the fortification of the food supply with folic acid in order to reduce neural tube defects, a serious birth defect of the spine and the brain that occur during pregnancy. The U.S. Public Health Service (USPHS) recommended women consume 400 µg folic acid per day (17,18). Following the institution of this mandate, reported prevalence of NTD-affected pregnancies and NTD-affected live births decreased by 27% and 26%, respectively, in the following year (26). Today, clinicians use serum folate level ranging from 3 to 4.5 µg/L as a guideline to diagnose folate deficiency (19).

Gender and race effects on folate levels

According to a study using the 1999- 2000 National Health and Nutrition Examination Survey (NHANES) to quantify the impact of the 1998 FDA folic acid fortification policy by estimating folate intake (from food and supplements) of a nationally representative population showed that the proportion of women aged 15-44 years who consumed more than 400 µg/day increased since fortification, but has not reached the FDA's target goal. Furthermore, total folate intake varied by race/ethnicity and gender. Among 15-44 year old women, non-Hispanic whites had the highest median folate per day intake of 380 µg/day, whereas non-Hispanic blacks and Mexican Americans trailed behind with 295 and 329 µg/day, respectively. This same trend was similar among 15-44 year old men; however, their daily median total folate intake was higher compared to women; non-Hispanic white with 469 µg/day, non-Hispanic blacks with 340 µg/day, and Mexican Americans with 432 µg/day (27). The proportion of women who consumed at least 400 µg folic acid daily varied significantly by race-ethnicity, ranging from 19.1% in non-Hispanic black women to 21.0% in Hispanic women and 40.5% in non-Hispanic white women (21).

In terms of serum folate, a study using a NHANES (2005-2006) found the median serum folate levels of women of reproductive age also varied by race and ethnicity. It has been shown that among women aged 15-44 years, non-Hispanic blacks and Hispanics have lower median serum folate levels than non-Hispanic whites (10.2 ng/ml, 11.4 ng/ml, and 13.8 ng/ml, respectively) (22).

DEPRESSION

Depression is the leading cause of disability and the fourth leading contributor of the global burden of disease, and is recognized as an important cause of morbidity and mortality worldwide (1,2). It is the most common type of mental illness, affecting more than 26% of the U.S. adult population (3). Depression is characterized as a mental disorder associated with a range of emotional, cognitive, physical behavioral symptoms including loss of interest or pleasure, feelings of guilt, disturbed sleep or appetite, low energy, and poor concentration (2,4). The diagnostic criteria by the American Psychiatric Association dictates depression as a period of at least two weeks during which there is a prominent and relatively persistent dysphoric, sad, depressed mood, or the loss of interest or pleasure in nearly all activities . This is associated with at least four of the following symptoms: change in appetite or weight; sleep and psychomotor activity disturbances; fatigue; difficulty concentrating, planning or making decisions; feelings of worthlessness, remorse, inappropriate guilt, and recent thoughts of death or suicide (28,29). Depressive symptoms interfere with daily life and can contribute to personal adverse health effects (4,5).

A body of literature has shown that depression is a causal risk factor for a myriad of chronic disorders, including arthritis, asthma, cancer, cardiovascular disease, diabetes, hypertension, chronic respiratory disorders (3,30–33). In addition, poor health behaviors

correlated to depression may explain the association between depression and chronic disorders, including elevated rates of smoking, alcohol consumption, physical inactivity, obesity, and sleep disturbances (29,34).

The prevalence of depression may increase in which studies showed earlier onset of depression, especially in adolescents, and overall risk of suffering depressive symptoms is increasing (35–37). Furthermore, the majority of people who suffer from depression do not get treatment. According to a study using the 2009- 2012 NHANES, 7.6% of the U.S. population aged 12 and over had moderate or severe depression in the past two weeks. Among those people who reported severe depressive symptoms, many serious difficulties in work, home and social activities were reported; less than half reported having contacting mental health counseling in the past year (6).

Gender effects on depression

The prevalence of depression varies by sex. Women are twice as likely as men to report lifetime history of major depressive episodes (1). The higher rates of depression in women may be due to artefactual factors, including women are being likely to report depressive symptoms than males, the lack of temporal stability in reporting mental disorders, women being more likely to seek medical help, and the course of depression differing based on gender. However, it has been concluded that there is a higher order biological factor that principally contributes to the gender differentiation in some expression of depression. The effect of adverse events experiences during childhood is greater among females, including sexual abuse (38,39). Psychosocial events such as limitations in social roles and cultural norms are considered to increase the vulnerability of women to depression (7,39).

According to the 2009-2012 NHANES, among a population of 18-39 year old, the prevalence of those displaying significant depressive symptoms was 9.3% in women compared to 5.8% in men (6). Biological processes are thought to be involved in the predisposition of women to depression, including genetically determined vulnerability and hormonal fluctuations specific to women (7). Depression among 50% of reproductive aged women is undiagnosed and untreated due to high cost, opposition to treatment, and stigma related to perceived mental health in society (1). Women with depression are at a high risk for adverse reproductive outcomes, including lower fertility and negative pregnancy outcomes (e.g., preterm births and low birthweight infants); and impaired maternal functioning and bonding (1,8,9).

Race and ethnic differences in rates of depression

The epidemiology of depression in the U.S. between ethnic and racial groups varies in prevalence rates, age of onset, severity, disability, and treatment use. Major depression chronicity was higher among Mexican Americans, Puerto Ricans and African Americans compared to Whites. In addition, among those in this population diagnosed with depression, Vietnamese, Mexican and African Americans were the least likely to receive therapy. These findings suggest that the depression chronicity found largely in Mexican Americans and African Americans might relate to their lack of access to depression therapy (40). In recent study in 2005, prevalence of depression was significantly higher in whites than in African Americans and Mexican Americans, according to NHANES III. In this same study, they found poverty and lack of education to be main contributors to this pattern. Hispanics had a significantly higher rate of employment compared with whites and African Americans. African Americans had significantly more years of education than Hispanics, and whites had significantly more years of education

than both of these groups. Furthermore, whites has an earlier age of first major depressive episode compared to African Americans (41).

SERUM FOLATE LEVELS AND DEPRESSION

While many studies have focused on folate deficiency, studies of folate within the normal range are of even more interest, as depression is a symptom of many nutrient deficient states. The nature of the association between folate levels within the normal range and depression has been studied. Studies conducted after folic acid fortification have shown that a significant inverse relationship between serum folate and depression remain true among a population who are not folate deficient, which ranges from approximately 3 to 4.5 μg/L (11–13,16,42). Therefore, this suggests that the folic acid levels in fortified foods may not be high enough to significantly impact the diseases connected to low folate levels (15,18). A recent randomized controlled trial in a clinic in Turkey assessed nutritional status and biochemical parameters, including folate, of patients diagnosed with depression based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV-TR). This study found a statistically significant lower serum folate among the depressed patients compared to non-depressed patients; the median folate among the depressed was 5.12 ng/mL compared to 8.68 ng/mL in the control group ($P<0.001$). In addition, they found that folate consumption was lower among the depression group compared to the control group, 193.5 and 261.8 μg, respectively (11).

Depressed patients have been consistently found to have lower serum folate concentrations, and patients with very low folate levels were highly associated to have higher depression scores than do patients with normal folate (12,16). Several studies have shown that this inverse association between serum folate and depressive symptoms was mostly found among women, whereas the association was not significant among men (13,42–44). A study using a

NHANES (2005-2006) found the odds of elevated depressive symptoms was 69% lower among women with higher serum folate compared to women with lower serum folate (13).

In comparison, one case-control study has shown that there is no association between depression and serum folate. A study in Hong Kong among newly admitted in-patients with depression displayed no association between folate levels and depression based on the Hamilton Depression Rating Scale, the Beck Depression Inventory and Global Assessment Scale scores (45).

In addition, folate's central involvement in the methylation processes and the synthesis of neurotransmitters in the central nervous system (e.g. serotonin), is plausibly linked to its effect on mood and cognition (12,15,16). A 2007 meta-analysis of case-controls and cross-sectional studies showed there was an increased risk of depression among people with MTHFR polymorphism (Odd ratio=1.36, 95% CI:1.11, 1.67), with no discernable statistical between-study heterogeneity (23).

Impact of supplementation with folic acid and its derivatives on depression

Low folate has been demonstrated in depressive populations and in poor responders to antidepressants (14). The effectiveness of folate in the treatment of depression has been debated in the past years. Depressed patients with low or normal folate levels may benefit from augmenting a primary antidepressant medication with folate either initially, at the onset of treatment, or later after no response to primary treatment. In addition, low folate levels in depressed patients have been associated with increased levels of antidepressant treatment resistance (14). In a recent systematic review and meta-analysis of randomized controlled trials of folic acid supplementation for depression, evidence showed that the short-term use (days to a few weeks) of folic acid supplementation does not contribute to improve depressive symptoms in

adults with major depression treated with antidepressants; however, more prolonged consumption (a few weeks to years) decreased the risk of relapse and the onset of clinically significant symptoms of depression in people at risk (46).

Supplementation with folic acid differed between men and women. A randomized controlled trial used 500 μg of folic acid or placebo adjuvant with 20 mg fluoxetine in 127 subjects with depression based on the Hamilton Depression Rating Scale (HDRS) of greater than 20. They found that after 10 weeks, there was a statistically differential reduction after 10 weeks on HDRS among women, however there was no effect in men. Among women, the scale score on completion was 6.8 ± 4.1 in the fluoxetine plus folate group, as compared to 11.7 ± 6.7 in the fluoxetine plus placebo group (47).

Problem Statement

While evidence has shown that gender and race are critical aspects in depression risk factors as well as folate levels, the effect of these variables has received little empirical attention in assessing the relationship between folate and depression. Studies have shown that fortification has helped women of reproductive age to increase their folic acid intake and prevent occurrence of birth defects in their infants. However, with the present level of fortification**,** two-thirds of non-pregnant American women of reproductive age are not consuming the recommended amount of folic acid. Furthermore, low consumption of folic acid differs across race-ethnicity groups, which has been seen among Hispanic and non-Hispanic black women than among non-Hispanic white women (21). Although the link between depression and serum folate has been fairly established, with much of the research focused on the United States, limited research has explored the link between serum folate levels and depression among women of reproductive age and across race-ethnicity groups. This gap in knowledge prevents a compelling assessment if

race/ethnicity plays a role in association between folic acid intake and depression outcomes among women of reproductive age. More complete assessment will provide empirical evidence for determining which populations to mainly target, as well as to hypothesize any additional causal factors that could play a role within racial groups. We can use this evidence to inform improved folic acid intake among certain racial groups of women. The research done in this project will also help identify new areas of interest for interventions for women of reproductive age at risk of depression. Understanding of specific associations may allow determination of focal areas within each racial group that can be used to guide interventions and help programs to allocate their resources effectively.

MATERIALS AND METHODS

Study Population

We used data from the 2011-2012 The National Health and Nutrition Examination Survey (NHANES) (n=13,431), conducted by the National Center of Health Statistics (NCHS) at the Centers for Disease Control and Prevention (CDC). The overall response rate for this cycle was 70%. NHANES is a cross-sectional study employing stratified multistage probability design. The study collects survey data on health and nutritional status of non-institutionalized civilians of the U.S. population through a series of interviews, examinations, and laboratory measurements. Information on demographic characteristics is collected during the personal interview, and serum folate levels are measured during the physician examination (48). A total of 1,820 adult women age 20-44 years old completed both household interviews and medical examinations (Figure 1). For this evaluation, women who were pregnant (n=137) were excluded because pregnancy may modify the effects of serum folate levels on depression (12). Pregnancy status in NHANES is determined by self-report, supplemented by urine human chorionic

gonadotropin testing for most women. Additionally, women with missing serum folate levels, or did not complete the Patient Health Questionnaire (PHQ-9) were also excluded (n=359). Further information on NHANES is available at http://www.cdc.gov/nchs/nhanes.htm.

Serum Folate Measures

Serum and whole blood samples were sent to the National Center for Environmental Health at the CDC for analysis by use of a microbiologic assay method. Detailed descriptions of the folate analysis methodology and quality assurance is described in the NHANES laboratory procedure manuals (49,50).

Depression Measures

The 9-item Patient Health Questionnaire (PHQ-9) depression scale was used to assess the frequency of symptoms experienced and reported by participants during the past 2 weeks. The PHQ-9 scores, ranging from 0 to 27, indicate the presence and severity of depression, with scores of 5, 10, 15, and 20 being the cut-points for mild, moderate, moderately severe, and severe depression, respectively. In this evaluation, we defined those with depression with a PHQ-9 score of $\geq$ 10 (5,51).

Covariates

The demographic variables in our analyses included respondents' age, race/ethnicity (Non-Hispanic White, Non-Hispanic Black, Mexican American, Hispanic, or other), education (<high school, high school, or >high school), employment (employed or other), marital status (married, divorced/separated/widowed, single, or living with partner), household income ($\leq$\$24,999; \$25,000-54,999; \$55,000-74,999; $\geq$\$75,000) body mass index (BMI, self-reported weight divided by height in square) and health insurance status (private, public, or none). Smoking status was categorized as current smokers (participants who had smoked cigarettes,

cigars or tobacco at the time of the interview), former smokers (those who were not current smokers, but smoked ≥100 cigarettes in their entire lifetime), and never smokers (those who had never smoked or smoked < 100 cigarettes during their lifetime). Physical activity was assessed by asking respondents whether, during the previous month, they had participated in any moderate or vigorous physical activities or exercise other than their regular job (measured and assessed by metabolic equivalent (MET) multiplied by duration of the activity and frequency converted to per week unit). Participants who did not elicit any activity were considered sedentary and given a MET of zero. Alcohol consumption was assessed during the alcohol and drug assessment component of the medical examination. Persons who reported no alcohol drinking in their entire lives were considered to be 'never drinkers'. Historical drinkers were defined as persons who reported drinking at least 12 drinks of alcohol in entire life, but none in the past 12 months. Current drinkers were defined as persons who reported at least 12 drinks of alcohol in the past 12 months. The amount of current alcohol consumption was determined from self-reported number of days with alcohol drinking and the average number of drinks per day when they drank alcohol in the past 12 months. As defined by NHANES, one drink which was equivalent to 10 g ethanol represented 12-oz of beer, 4-oz of wine or 1-oz of liquor. For current drinkers, number of drinks per day, on average, was calculated as ([#drinks on a drinking day × # of drinking days over the past 12 monthsx10g] / 365.25). The use of antidepressant(s) was also taken into consideration in the analyses.

Statistical Analysis

Statistical analyses were conducted using SAS, version 9.4 (SAS Institute Inc.), and SUDAAN, version 11.0.1 (Research Triangle Institute), with NHANES-provided sample

weights and accounting for the survey's complex sampling design. We used the SUDAAN SUBPOPN statement to subset the NHANES data to match our inclusion/exclusion criteria.

Previous epidemiological research observing serum folate and depressive disorders typically categorized continuous serum folate into tertiles to illustrate the relationship between folate and depressive disorders due to non-linearity of the relationship (13,42,43). Accordingly, we divided serum folate into tertiles based on the control group (non-depressed population meeting criteria), with the first tertile being the lowest level of serum folate and the third tertile being the highest (<12.8 ng/ml, 12.8-23.5 ng/ml, and ≥23.6 ng/ml). Characteristics of the study population were evaluated with descriptive statistics, presenting mean values for continuous variables and percentages for categorical variables. To test the statistical difference between the groups, we used χ^2 test for categorical variables and two-sided *t*-tests for continuous variables. A p value of <0.05 was considered significant. Additionally, we quantified the relative effect size among variables between the groups. Categorical variables were evaluated by using simple logistic regression (effect size expressed by Somer's D). Somer's D demonstrates how many more concordant than discordant pairs exist divided by the total number of pairs. Values range from -1 (all pairs disagree) to 1 (all pairs agree). Larger values indicate that the model has better predictive ability (52). In contrast, for covariates that were continuous, the 2-tailed independent samples *t*-test (effect size expressed by Cohen's D) was used. Cohen's D is defined as the difference between the means divided by the pooled standard deviation, defined as, but not limited to, small (0.2), medium (0.5) and large (0.8) (53).

In the following analyses, we used serum folate as a continuous variables to avoid the assumption of homogeneity of risk within groups, as well as to avoid loss of power and inaccurate estimations. We first assessed various predictors of low serum folate by conducting

multivariate linear regression with serum folate as our outcome and predictors included socioeconomic and health style characteristics. Then we conducted multivariable logistic regression (MLR) to control for *a priori* selected potential confounders for determining the independent contribution of serum folate to depression. Variables selected as possible confounders in the multivariate analysis were age, race/ethnicity, BMI, education level, cigarette smoking, alcohol use, physical activity, health insurance, household income, and antidepressant use. We analyzed how each potential confounder changed the odds ratio of having depression in order to confirm that serum folate was associated with depression and not due to confounders. For interaction analysis, we performed stratified analysis to test for interaction. For interaction variable, a multivariate logistic regression model was constructed for each subgroup. Unadjusted and adjusted odds ratios (cORs and aORs) and associated 95% confidence intervals (CI) were calculated. All statistical tests were performed at the alpha of 0.05.

RESULTS

Overall, 1,324 women met the eligibility for the study after exclusion of pregnant women and those missing serum folate levels and PHQ-9 data. Of the eligible women, 16.7% were depressed and 83.3% were not depressed. The distribution of sample characteristics of the study population is shown in Table 1 by depression symptoms status based on PHQ-9 score. Depressed and non-depressed groups did not differ significantly on age, race/ethnicity, antidepressant use, and physical activity. Participants with depression were significantly more likely to be less educated, be unemployed, and have lower income than their non-depressed counterparts ($P < 0.05$). Women with depression were more likely to have public health insurance, consume more alcohol, and smoke at the time of the interview compared to women without depression (Table 1). Among women with depression, a majority were non-Hispanic blacks (66.8%), followed by

non-Hispanic whites (12.6%), Mexican American (8.6%), other Hispanic (7.4%), and Other (3.2%). There were no statistical difference in median serum folate between women with and without depression (17.8 ng/ml and 17.2 ng/ml, respectively; effect size=0.146; p=0.5). The unadjusted odds ratio of serum folate level on depression was 1.02 (95% CI: 0.98, 1.07) for women with depression as compared to women without depression. In contrast, serum folate tertiles also did not show any differences between the two groups (effect size=0.028, *P*=0.9587). The unadjusted odds ratios for the tertiles of serum folate on depression were also statistically not significant.

Association between serum folate with socioeconomic, lifestyle and health related factors: multivariate linear regression

Multivariate linear regression was conducted with outcome variable serum folate as predicted by various socioeconomic, lifestyle, and health related factors (Table 2). Among women with depression, other Hispanics had lower serum folate levels by approximately 82 ng/ml, as compared to non-Hispanic whites ($P<0.05$), whereas the other race/ethnicity groups had higher folate status. Mexican Americans had higher serum folate level by 20 ng/ml ($P<0.05$) and Other had a higher serum folate level by 17 ng/ml ($P<0.05$). Lower folate status within the depression group was related to participants who were unmarried, had public or no health insurance compared to having private insurance, had lower income, were unemployed, were less educated, or were underweight or overweight. Within the non-depressed group, lower serum folate levels were observed to be among non-Hispanic blacks ($\beta= -3.03$, $P<0.05$) compared to non-Hispanic whites, and those who consumed more alcohol ($\beta= -5.41$, $P <0.05$) (Table 2).

Associations of depression with serum folate: logistic multiple regression analysis

The association between serum folate and depression based on the PHQ-9 score are presented in Table 3. Association between serum folate and depression remained non-significant after single adjustments for socioeconomic variables (age, marital status, income, education, and race/ethnicity) and health and lifestyle factors (BMI, alcohol consumption, health insurance, employment, antidepressant use, and smoking status). This suggests potential confounders were not observed to explain the higher risk of depression related to serum folate levels. In the fully adjusted model with all covariates, the risk of depression was weakly and positively associated with serum folate (aOR 1.10; 95% CI: 1.02-1.19), with a 7.8% increase in risk from the unadjusted association (cOR 1.02; 95% CI: 0.98-1.07) (Table 3). The logistic regression analysis for the estimated effects of serum folate level on depression is presented in Table 4. An adjusted model was created which included race/ethnicity, age, education, employment, household income, marital status, smoking status, alcohol consumption, body mass index, insurance coverage, and antidepressant use. Interaction variables were assessed with race/ethnicity and found no significant interactions (data not shown). In the multivariate model, depression was associated with marital status of women who were divorced, separated, widowed, or living with a partner (aOR: 12.28; 95% CI: 2.59, 58.15); and obesity (aOR: 7.26, 95% CI: 1.08, 48.88).

DISCUSSION

Our results from this national cohort of U.S. women of reproductive age (aged 20–44 years) using data from NHANES (2011-2012) suggest that serum folate was associated with depression. The serum folate level's effect on depression was however relatively weak in our cohort. Furthermore, race and ethnicity did not interact with the association between folate status and the risk of depression in our study.

Previous studies exploring the association between serum folate and depression have examined the association among general populations, or among the elderly or youth (11–14,46). To our knowledge, our study is the first observational study to examine the association between serum folate and depression among adult women of reproductive age. A number of studies that have closely examined the relationship between serum folate and depression in other populations showed a significant association between the two variables. One case-control study involving adults aged 20-85 from NHANES (2005-2006) reported an inverse and significant association between serum folate and depression in both men and women (13). A second study involving 213 adult outpatients with major depressive disorder (MDD) found low folate levels to be associated with the presence of melancholic depression (20). In contrast, a third case-control study in which 59 adults aged 18-60 in a mental health center in Turkey demonstrated that serum folate was lower in depression group (11). There are also previous studies that did not find an association between serum folate and depression. One case control study conducted on newly admitted in-patients with depression in Hong Kong showed that that folate levels were not related to patients' duration of illness, depression screenings, and prior psychotropic drug usage (45). This suggests that although Western and British studies have revealed an association between folate and depression, culturally patterned health beliefs and dietary practices can influence the relationship between folate status and depression in different societies.

The study has several strengths and limitations. Standardized protocols, rigorous quality control in data collection and reporting, inclusion of a large nationally representative study sample and careful data analysis are the main strengths of the study. Folate measurements were collected through a microbiological assay using *lactobacillus casei*, which allows regular monitoring and use of reference preparations to improve the accuracy of the results, especially at

low concentrations (54). However, there are limitations in this assay. There can interference from growth inhibitors, including some antibiotics that can affect the measurement of folate. In addition, folate can be lost in serum sample stored at room temperatures or at colder temperatures; thus, instability of folate can complicate the standardization and calibration of the assay and may cause random errors (55). Furthermore, serum folate concentrations were measured only once in NHANES; therefore it does not accurately represent long-term status. Another limitation is that our analyses were based on crossed-sectional data, which we cannot establish temporal causality, whether the various factors examined were anecdotes, correlates, or consequences of depression. This study did not identify whether low serum folate levels caused depression, or whether depression caused the participants to have low serum folate levels. As with any observational study, confounding due to unobserved covariates is possible. Furthermore, PHQ-9 may not accurately identify anxiety and depressive disorders among women of reproductive age. The PHQ-9 have previously been validated among patients from primary care and obstetrics, with an overall sensitivity of 84%, a specificity of 72%, and a positive likelihood ratio of 2.86 (5). However, this validity conducted in gynecology clinics may not be applicable to our study population of women of reproductive age.

In conclusion, our study suggests that there is a weak association between serum folate and the risk for depression among non-pregnant women of reproductive age in the U.S. Carefully designed prospective studies with longitudinal follow-up are needed to verify that folate concentrations are linked to depressive symptoms among women of reproductive age in order to demonstrate potential benefits from regular and adequate folic acid use. There is a public health and policy implication in studying more closely in the correlation between nutrition and depression. The identification of folate status as a plausible specific risk factor for depression

raises the possibility of increasing or decreasing folic acid supplementation, or improved diet in the prevention and treatment of depression among women.

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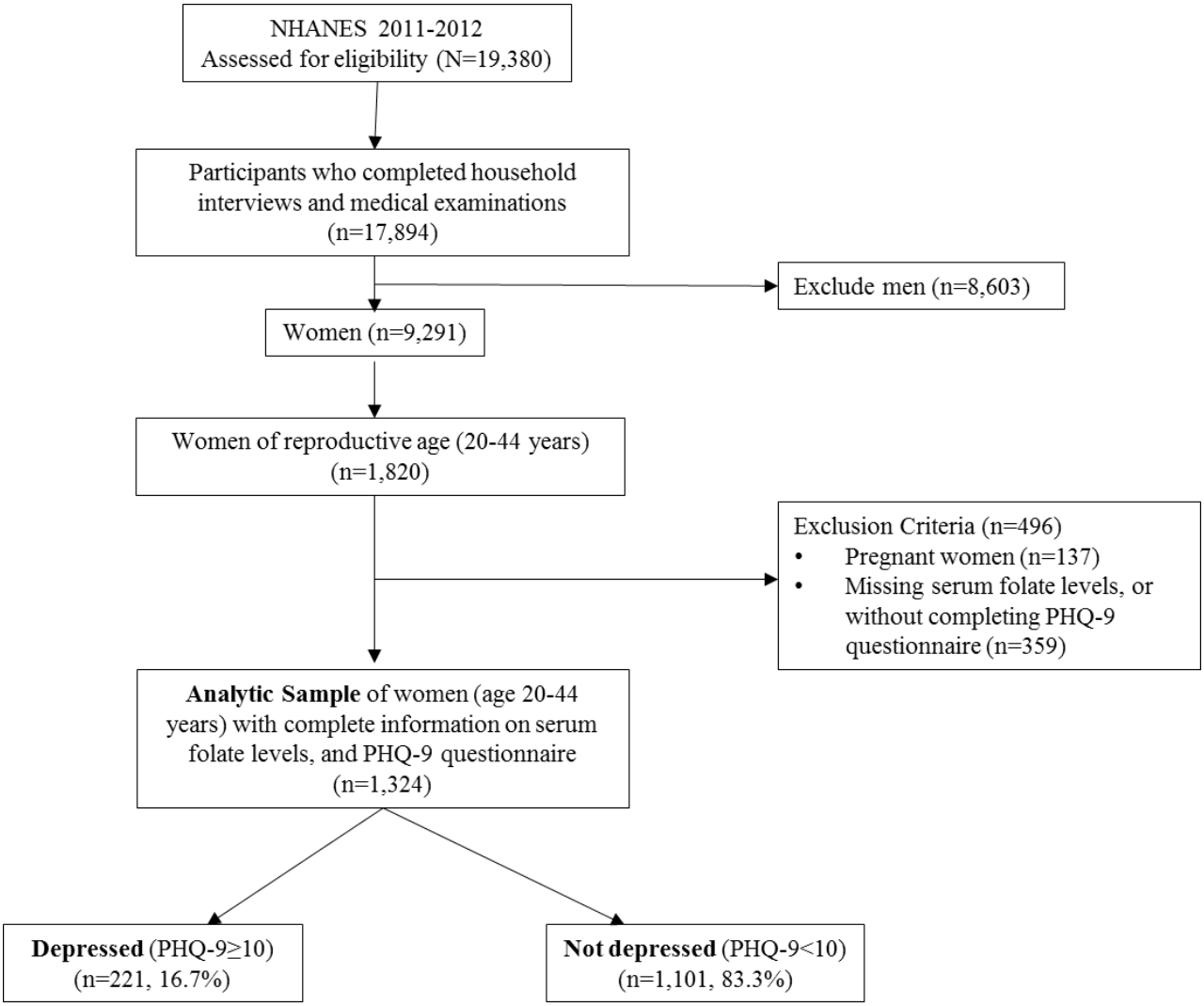
Figure 1. Study Population and Inclusion/Exclusion Criteria, NHANES, United States, 2011-2012.



[a]Abbreviations: NHANES, National Health and Nutrition Examination Survey; n, sample size; PHQ, 9–Patient Health Questionnaire-9.
[b]Percentages represented the percentages of individuals among the whole sample.

Table 1. Descriptive analysis of women of reproductive age (20-44 years) with and without depression, NHANES 2011-2012

Characteristics	Total (N=1324)	Depressed (n=221)	Not Depressed (n=1103)	Crude OR (95%CI)	Effect Size	*P* value
Age, mean (SD)	32.7 (0.8)	34.0 (1.6)	32.5 (0.8)	1.03 (0.96, 1.10)	0.207	0.3891
Serum folate level (ng/ml), median (25-75% quartiles)	17.3 (15.5-18.8)	17.8 (13.1-21.2)	17.2 (15.5-18.9)	1.02 (0.98, 1.07)	0.146	0.4647
1st tertile (<13.6 ng/ml)	489 (33.2)	85 (32.8)	404 (33.2)	1.00	0.028	0.9587
2nd tertile (≥13.6 and <20.9)	409 (33.4)	57 (31.0)	352 (33.8)	0.93 (.28, 3.02)		
3rd tertile (≥20.9)	426 (33.4)	79 (36.2)	347 (33.0)	0.91 (.35, 2.36)		
Race/ethnicity, n (%)					-0.067	0.2338
Non-Hispanic white	558 (65.1)	221 (12.6)	468 (66.8)	1.00		
Non-Hispanic black	359 (14.8)	468 (66.8)	275 (13.0)	2.14 (0.79, 5.81)		
Mexican American	123 (8.4)	17 (8.6)	106 (8.4)	1.13 (0.30, 4.25)		
Other Hispanic	90 (5.5)	17 (7.4)	73 (5.2)	1.51 (0.63, 3.58)		
Other	194 (6.2)	13 (3.2)	181 (6.63)	0.60 (0.18, 1.93)		
Education, n (%)					0.259	**0.0060**
< High school	207 (12.4)	66 (24.9)	141 (10.6)	1.57 (0.93, 2.66)		
High school	193 (13.9)	46 (19.7)	147 (13.0)	1.00		
>High school	924 (73.7)	109 (55.4)	815 (76.4)	**0.47 (0.27, 0.81)**		
Employment, n (%)					0.400	**0.0012**
Employed	747 (61.9)	51 (27.0)	696 (67.0)	1.00		
Unemployed	577 (38.1)	170 (73.1)	407 (33.0)	**5.87 (3.18, 10.84)**		
Household income, n (%)					0.676	**0.0134**
≤$24,999	417 (24.5)	116 (47.2)	301 (21.2)	**6.94 (1.99, 24.15)**		
$25,000-54,999	370 (31.3)	64 (29.8)	306 (31.5)	2.93 (0.67, 12.83)		
$55,000-74,999	134 (10.9)	9 (2.9)	125 (12.1)	1.00		
≥$75,000	313 (28.8)	19 (13.9)	294 (30.9)	1.30 (0.21, 8.05)		
Marital status, n (%)					0.568	**0.0073**
Married	514 (43.9)	43 (17.8)	471 (47.7)	1.00		
Divorced/separated/widowed/living with partner	331 (25.8)	102 (42.8)	229 (22.9)	**5.69 (2.01, 16.13)**		
Single	479 (30.3)	76 (36.5)	403 (29.4)	**3.57 (1.87, 6.83)**		
Health Insurance, n (%)					0.312	**0.0099**
Public	386 (21.5)	130 (47.6)	256 (17.7)	**5.02 (2.33, 10.83)**		
Private	672 (60.6)	54 (36.4)	618 (64.1)	1.00		
No	263 (18.0)	37 (16.0)	226 (18.2)	1.43 (0.60, 3.44)		

Characteristics (Continued)	Total	Depressed	Not Depressed	Crude OR (95% CI)	Effect Size	*P* value
Alcohol consumption, mean (SD)	4.3 (0.6)	9.9 (2.7)	3.5 (0.4)	**1.06 (1.02, 1.10)**	0.743	0.0238
Currently taking antidepressants, n (%)					-0.019	0.0919
Yes	85 (7.7)	21 (8.9)	64 (7.5)	1.09 (0.56, 2.11)		
No	788 (62.4)	166 (73.8)	622 (60.8)	1.00		
Smoking status, n (%)					0.300	0.0880
Never	818 (59.3)	85 (36.5)	733 (62.6)	1.00		
Current	325 (24.9)	97 (42.1)	228 (22.4)	**3.56 (1.67, 7.58)**		
Former	181 (15.8)	39 (21.5)	142 (15.0)	2.48 (0.77, 7.96)		
Physical activity (MET×hr/wk), mean (SD)	45.0 (3.2)	39.9 (8.5)	45.7 (3.2)	1.00 (0.99, 1.00)	0.073	0.5038
Measured BMI (kg/m^2), mean (SD)	29.7 (0.5)	31.7 (1.1)	29.4 (0.6)	1.03 (0.99, 1.07)	0.262	0.1211
Underweight, n (%)	38 (3.6)	2 (3.3)	36 (3.7)	2.59 (0.40, 16.97)	0.327	**0.0002**
Normal weight, n (%)	362 (29.1)	23 (10.7)	339 (31.7)	1.00		
Overweight, n (%)	309 (26.9)	38 (24.1)	271 (27.3)	2.54 (0.68, 9.42)		
Obese, n (%)	595 (39.5)	149 (58.8)	446 (36.7)	**5.38 (3.20, 9.05)**		

[a] All percentages are weighted, percentages include missing data

[b] Effect size for categorical variables are based on Somer's D, and based on Cohen's D for continuous variables

[c] Abbreviations: BMI, Body Mass Index; CI, Confidence Interval; kg, kilogram; MET, Metabolic Equivalent; n, sample size; NHANES, National Health and Nutrition Examination Survey; SD, Standard Deviation; OR, Odds Ratio

Table 2. Multivariate linear regression analysis on serum folate by depression among women of reproductive age (20-44 years), NHANES 2011-2012

	Estimated change in serum folate (ng/ml)								
Characteristics	Total (N=1,324)			Depressed (n=1,103)			Not Depressed (n=221)		
Age	0.06	(-0.22,	0.33)	-0.49	(-0.93,	-0.05)	0.06	(-0.22,	0.34)
Race/ethnicity									
Non-Hispanic white	ref			ref			ref		
Non-Hispanic black	-2.58	(-6.28,	1.13)	15.35	(-0.47,	31.18)	**-3.03[b]**	(-5.98,	-0.09)
Mexican American	1.52	(-3.79,	6.83)	**20.32[b]**	(1.52,	39.13)	2.52	(-5.25,	10.30)
Other Hispanic	4.12	(-1.69,	9.93)	**-82.57[b]**	(-110.95,	-54.19)	5.93	(-0.84,	12.69)
Other	3.20	(-3.70,	10.10)	**17.31[b]**	(5.82,	28.81)	3.26	(-4.19,	10.70)
Education									
< High school	6.29	(-5.06,	17.64)	**-23.51[b]**	(-41.13,	-5.89)	4.32	(-2.53,	11.18)
High school	ref			ref			ref		
>High school	2.78	(-2.30,	7.86)	**-13.11[b]**	(-21.71,	-4.51)	3.63	(-1.99,	9.26)
Employment									
Employed	ref			ref			ref		
Unemployed	-0.63	(-4.48,	3.22)	9.97	(-3.9,	23.85)	-1.16	(-4.07,	1.75)
Household Income									
≤$24,999	-0.37	(-9.62,	8.89)	**-40.32[b]**	(-54.52,	-26.51)	-2.16	(-10.91,	6.60)
$25,000-54,999	-3.00	(-8.60,	2.59)	**-42.69[b]**	(-59.09,	-26.28)	-2.93	(-9.00,	3.13)
$55,000-74,999	ref			ref			ref		
≥$75,000	-3.46	(-10.0,	3.10)	24.55	(-4.31,	53.42)	**-5.41[b]**	(-9.77,	-1.06)
Marital Status									
Married	ref			ref			ref		
Divorced/separated/ widowed/living with partner	-2.99	(-8.51,	2.53)	3.98	(-11.69,	19.64)	-2.69	(-6.7,	1.32)
Single (first)	-1.55	(-5.60,	2.49)	**-19.24[b]**	(-33.6,	-4.52)	0.23	(-2.86,	3.31)
Health Insurance									
Public	-1.88	(-6.55,	2.80)	**-20.08[b]**	(-27.61,	-12.56)	-2.20	(-6.09,	1.68)
Private	ref			ref			ref		
None	-4.14	(-9.60,	1.32)	**-24.29[b]**	(-33.6,	-14.98)	-2.96	(-7.44,	1.52)
Alcohol consumption	0.03	(-0.08,	0.13)	**-0.08[b]**	(-1.02,	-0.57)	-0.03	(-0.19,	0.14)
Currently taking antidepressants									
Yes	2.29	(-1.13,	5.71)	-0.84	(-2.05,	0.38)	1.74	(-1.2,	4.69)
No	ref			ref			ref		

Characteristics (Continued)	Total (N=1,324)		Depressed (n=1,103)		Not Depressed (n=221)	
Smoking status						
Never	ref		ref		ref	
Current	-1.53	(-5.10, 2.05)	**26.26[b]**	(16.68, 35.85)	-3.01	(-6.7, 0.68)
Former	2.47	(-3.34, 8.27)	-3.42	(-10.04, 3.19)	-0.45	(-3.08, 2.18)
Physical Activity (METxhr/week)	0.00	(0.01, -0.02)	0.05	(0.02, 0.07)	-0.01	(-0.03, 0.02)
Measured BMI (kg/m2)						
Underweight	0.93	(-11.35, 13.20)	**-52.65[b]**	(-81.88, -23.42)	1.72	(-11.12, 14.55)
Normal weight	ref		ref		ref	
Overweight	-0.19	(-4.75, 4.36)	-9.98	(-2.09, 22.05)	-0.31	(-4.67, 4.05)
Obese	-1.09	(-3.50, 1.33)	**11.22[b]**	(6.52, 15.91)	-3.55	(-7.56, 0.46)

[a] Values are ß (95%CI). Sampling design is taken into account in all analyses

[b] *p*-value < 0.05 for null hypothesis that β=0 based on Wald test

[c] Model was multivariate-adjusted for all variables in table

[d] Abbreviations: BMI, Body Mass Index; CI, Confidence Interval; kg, kilogram; MET, Metabolic Equivalent; n, sample size; NHANES, National Health and Nutrition Examination Survey

Table 3. Multivariable analysis associated with serum folate levels before and after adjustment, NHANES 2011-2012

Characteristics	OR (95% CI)			%Δ
Depression vs. serum folate level				
Unadjusted	1.02	(0.98,	1.07)	
Adjusted for				
Race/ethnicity	1.03	(0.98,	1.07)	1.0
Age	1.02	(0.98,	1.06)	0.0
Education	1.02	(0.98,	1.07)	0.0
Employment	1.03	(0.98,	1.07)	1.0
Household Income	1.03	(0.98,	1.07)	1.0
Marital Status	1.03	(0.98,	1.08)	1.0
Health insurance	1.02	(0.98,	1.07)	0.0
Alcohol consumption	1.02	(0.98,	1.07)	0.0
Currently taking antidepressants	1.02	(0.98,	1.07)	0.0
Smoking status	1.03	(0.98,	1.07)	1.0
Measured BMI (kg/m^2)	1.02	(0.98,	1.07)	0.0
All variables listed above	1.10	(1.02,	1.19)	7.8

[a] Abbreviations: BMI, Body Mass Index; CI, Confidence Interval; kg, kilogram; m, meter; OR, odds ratio; %Δ, Percent Change

Table 4. Logistic regression model of depression, using linear serum folate variable, among women of reproductive age (20-44 years), NHANES 2011-2012

Characteristics	Adjusted Model aOR (95% CI)		
Serum Folate level (ng/mL)	**1.10**	**(1.02,**	**1.19)**
Age	0.95	(0.84,	1.08)
Race/ethnicity			
Non-Hispanic white	1.00		
Non-Hispanic black	4.41	(0.34,	57.57)
Mexican American	0.96	(0.08,	11.58)
Other Hispanic	0.20	(0.00,	20.34)
Other	0.18	(0.00,	12.01)
Education			
< High school	2.29	(0.18,	29.47)
High school	1.00		
>High school	0.79	(0.07,	9.22)
Employment			
Employed	1.00		
Unemployed	26.34	(0.24,	7.68)
Household Income			
≤$24,999	1.86	(0.06,	62.09)
$25,000-54,999	2.77	(0.04,	188.88)
$55,000-74,999	1.00		
≥$75,000	3.79	(0.04,	391.67)
Marital Status			
Married	1.00		
Divorced/separated/ widowed/living with partner	**12.28**	**(2.59,**	**58.15)**
Single	3.91	(0.70,	21.86)
Alcohol consumption	1.02	(0.98,	1.07)
Currently taking antidepressants			
Yes	0.78	(0.38,	1.62)
No	1.00		
Smoking status			
Never	1.00		
Current	**5.82**	**(2.29,**	**17.83)**
Former	**20.70**	**(3.51,**	**121.93)**
Measured BMI (kg/m^2)			
Underweight	13.63	(0.08,	683.56)
Normal weight	1.00		
Overweight	2.56	(0.30,	21.68)
Obese	**7.26**	**(1.08,**	**48.88)**

[a] Each variable adjusted for all other variables in the model.

[b] Abbreviations: aOR, Adjusted Odds Ratio; BMI, Body Mass Index; CI, Confidence Interval; kg, kilogram; m, meter; NHANES, National Health and Nutrition Examination Survey