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Association between obesity and risk of folate insufficiency among non-pregnant women of reproductive age in the United States

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Degree to be awarded: MPH

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Association between obesity and risk of folate insufficiency among non-pregnant women of reproductive age in the United States

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An abstract of

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Abstract

Association between obesity and risk of folate insufficiency among non-pregnant women of reproductive age in the United States

By Meryna Manandhar

Background: Folate insufficiency in the mother during the periconceptional period is associated with an increased risk of neural tube defects (NTD) in the offspring. Limited studies have examined the effect of obesity on low red blood cell (RBC) folate concentrations in non-pregnant women of reproductive-age, and the results have been inconsistent.

Objective: The purpose of this study was to examine the association between obesity, further stratified by different classes of obesity, and folate insufficiency in non-pregnant women of childbearing age (20-44 years) in the United States.

Methods: We used data from the U.S National Health and Nutrition Examination Survey (NHANES) (2007-2010). Folate insufficiency in our study was defined as RBC folate concentration (<748 nmol/l) where a woman is at risk for having NTD-affected pregnancy. High risk NTD category was defined based on RBC folate concentration of ≤585 nmol/l and elevated risk was defined by 586-747 nmol/l. We examined the association between obesity and folate insufficiency using multivariable logistic regression. We estimated crude and adjusted prevalence odds ratios (aPOR) and 95% confidence intervals accounting for the complex survey design.

Results: Overall, among the 30,878 eligible women, 32.69% were obese and 25.58% were overweight. We noted that 32.30 % of all women examined had folate insufficiency. Obesity was a significant determinant of overall RBC folate status, based on log-transformed RBC folate concentration (P = 0.0245). However, after adjusting for potential confounders, there was no association between obesity and folate insufficiency (aPOR:0. 79; 95% CI:0. 50, 1.23 for high risk NTD category and aPOR:0. 67; 95% CI:0. 45,1.00 for elevated NTD risk category). The association also did not persist when examined by obesity strata.

Conclusion: Our analyses show that obesity is not associated with folate insufficiency among women of reproductive age in the United States. Future studies should examine the association to better understand the relation between body weight and its impact on folate metabolism pathways, especially among obese women of reproductive age.

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INTRODUCTION

Obesity and overweight

Obesity is currently a serious problem in the United States and is growing in prevalence. Centers for Disease Control and Prevention (CDC) have defined obesity as body mass index (BMI) of 30 kg/m^2 (kilogram/ meters squared) or above[1]. Obesity is considered a global epidemic mainly resulting from genetic predisposition, unhealthy diet, and sedentary lifestyle factors [2, 3]. Obesity, including overweight (BMI=25 kg/m^2- <30 kg/m^2), has become one of the rising health concerns in the United States. According to the Organization for Economic Co-operation and Development (OECD) Health Statistics 2017, the United States has the highest obesity rate in the world[4]. The rising prevalence rate of obesity and overweight in the United States have increased to more than 65% in the last few years compared to other countries. Obesity itself is expected to rise to 47% of the total US population by 2030 [4]. The National Center for Health Statistics (NCHS) data brief by the (CDC) in 2017 reported that year 2015-2016 obesity prevalence among adults in the United States was 39.8%, which is about 3% more compared to their 2011-2014 estimates [5, 6]. The NCHS data brief on year 2014-2015 showed, the prevalence of obesity was higher among women (38.3 %) compared to men (34.3%)[5]. In women, the prevalence of obesity was highest among non-Hispanic Blacks (54.8%) and Hispanic women (50.6%) [6].

According to Cogswell *et al.,* about 44% of non-pregnant women of reproductive age were obese or overweight until 1994; rates of overweight and obesity were most prevalent among African-American and Mexican American women [7]. Data from the

2002 National Survey of Family Growth (NSFG) showed that in a population of women aged 20-44 years in the U.S., 24.5% were overweight and 23.0% were obese (10.3% were morbidly obese) [8]. An age-adjusted analysis performed using the nationally representative NHANES 2013-2014 survey cycle concluded that the prevalence of obesity was 40.4% and this obesity trend significantly increased in linear trend from 2005 to 2014 in women [9].

Whereas men consume more calories than women, fat storage is higher among women [10]. One possible mechanism to explain this increased fat storage among women is the female sex hormone, estrogen [9]. Estrogen increases fat body mass in women by decreasing hepatic fatty acid oxidation, leading to increased fat stores, which occurs more during early pregnancy in preparation for the pregnancy [10].

Obesity and overweight in reproductive aged women have shown to be associated with some important demographic and lifestyle factors. About two-thirds of reproductive aged women in the US have obesity and overweight issues, whereas there is also a tendency for a quick transition from overweight to obesity in reproductive aged women [11]. The study also concluded that females of childbearing age with less education and younger age tend to gain weight and transition to obesity to a greater extent [11]. Unfortunately, being obese and overweight are associated with increased difficulty in conception, complications during pregnancy, and long-term health issues [7, 8]. In addition, obese women have higher risks of delivering a child with birth defects such as neural tube defects compared to normal weight women [7].

Maternal pregnancy-related body weight and neural tube defects in the offspring

Neural tube defects (NTD) are one of the common birth defects in the United States and globally. NTDs have a multifactorial origin, and are associated with both genetic and environmental factors [12, 13]. NTDs occur due to the failure of the neural tube closure in an embryo during the third and fourth weeks of gestation [13]. The proximal portion of the neural tube develops into a brain and the distal portion into a spinal cord. Anencephaly, defined by CDC as a baby born without parts of brain and skull results from failure of the proximal neural tube closure whereas the distal results in spina bifida [13, 14]. Every year about 300,000 babies are born with NTDs in the world, the prevalence rate per 10,000 live births for NTDs in the US is 5.3 (95% CI: 5.1, 5.4) [15]. NTDs are one of the important causes of morbidity and mortality among neonates and infants [16]. This is linked to a high economical healthcare and care workers economical burden, Grosse *et al.* did a retrospective cost calculation in 2014 of US$791,900 for each infant with a spina bifida in a lifetime including the cost of caregivers [17]. In addition, caregivers are also impacted by the psychological stress associated with caring for children with NTDs, which calls for enhanced public health awareness to prevent NTDs [18]. Globally, the health and economic burden associated with NTDs is often obscured to policy makers as most of these cases end up in elective termination of pregnancy for fetal impairment (ETOPFA) and stillbirths [16].

Several population-based research studies in the US have revealed maternal pre-pregnancy obesity to be one of the independent risk factors for NTDs [19-22]. A case-control study by Waller *et al.* (1994) showed increased risk of delivering babies with NTD in extremely obese women compared to normal weight women (OR: 1.8, 95% CI

1.1-3.0) [20]. Watkins *et al.* also suggested similar findings in addition to the dose-response association of pre-pregnancy BMI of the mother and the risk of NTD pregnancies [21]. A study involving hospitals in 55 counties of California concluded that obese women with BMI of > 38 kg/m^2 had the highest risk of suffering from NTD-affected pregnancy (OR: 2.6, 95%CI: 0.92,7.7), although this was not statistically significant [22].

Two meta-analyses done in 2008 and 2017 showed that obesity in mothers is an important risk factor for NTD in infants [23, 24]. One of these studies by Rasmussen *et al.* (2008) reported a pooled estimate of 1.7 (95% CI: 1.34, 2.15) showing the association of maternal obesity with NTD when compared to a normal weight mother [23, 24]. Huang *et al.* (2017) also reported a pooled adjusted OR of 1.68 (95% CI: 1.51, 1.87) when maternal obesity was compared to normal weight mothers [24]. In addition, they found a dose-response relationship between maternal BMI and risk of NTDs in their offspring (P<0.00001), and that for every 1 kg/m^2 of increase in maternal BMI, the odds of NTD increased by 1.027 times [24]. A case-control study conducted in China showed that mothers with pre-pregnancy obesity had higher odds of NTDs compared to normal weight mothers with aOR of 2.45 (95% CI: 1.04, 5.74) (P < 0.05), controlling for age, education, income, folic acid use, and occupational status [25]. Another case-control study by McMahon *et al.* (2013) in South Carolina, reported obese mothers to have twice the odds of having a fetus with NTDs (OR=2.06; 95% CI: 1.12, 3.81) compared to normal weight women [26].

Folic acid and NTDs

NTDs are widely preventable birth defects through primary prevention with maternal folic acid supplementation before and during early pregnancy [27-29]. A randomized, double-blinded study showed that women who received folic acid supplementation prevented 72% of recurrent NTDs [30]. With the initiation of a mandatory folic acid fortification period (2009-2011) in the United States, prevalence of spina bifida and anencephaly dropped by 28% compared to the pre-fortification period, which was 6.5 per 10,000 live births [27, 31]. The current prevalence of NTDs (spina bifida and anencephaly) in the US with prenatal ascertainment program is 7, with estimated annual cases of 2,851. Conversely, in states without the prenatal ascertainment program, the prevalence is 5.3 with estimated annual cases of 2,182 in 1999-2011[31].

The risk of folate sensitive NTD in a pregnancy can be studied using maternal RBC folate concentration as evidenced by Crider *et al*. paper published in 2014. This study found women with the highest level of RBC folate had very low risk of NTD affected pregnancy compared to lower levels of RBC [32].

Recently in 2015 a study by Tinker *et al.* showed 22.8% of US non-pregnant reproductive aged women (12-49 years) had RBC folate levels related to higher occurrence of NTD [33]. The adjusted OR of having prevalence of ≥ 9 NTD cases / 10,000 live births related to RBC folate concentration was found to be 0.68 (95% CI :0. 51, 0.91) in the obese group [33]. On the contrary, a small population based cross-sectional study with non-pregnant reproductive aged women in Alabama reported higher odds of protective association of RBC folate level in obese women for NTDs (OR: 1.74, 95%CI: 1.07,2.84) compared to BMI of < 30 kg/m^2 [34]. Some other studies have

identified that even after the fortification period, increased maternal weight was moderately associated with the risk of NTDs [35, 36].

Folates and Folic Acid

Sources of Folate and Folic Acid in the United States

Dietary Intake

Dietary intake is one of the primary sources of folate and folic acid in the United States. Folate is a water-soluble vitamin B complex (vitamin B9) naturally found in food such as meat, green vegetables, egg yolk, and citrus fruits [37]. Folic acid is the synthetic oxidized form of vitamin B used in the vitamin supplement and fortified foods such as bread, cereal, and flour, and is widely available in our body [38].

Folic acid supplementation

Intake of folate in the form of multivitamins, minerals or supplements is another common source in addition to dietary intake. The US Public Health Service recommended all women of childbearing age to take 400 μg of folic acid daily to prevent the occurrence of NTDs [39]. Women with health concerns such as diabetes, BMI > 35 kg/m^2, and family history of NTD are recommended to start folic acid supplementation with 500 μg daily at least 3 months before pregnancy [40]. NHANES III data showed that women of childbearing age with higher BMI were taking less nutritional supplement compared to lower BMI women [41]. Obese women were also found to have less folic acid supplementation prior to pregnancy; this was thought to be due to unplanned pregnancies largely resulting from birth control pill failures [42].

Mandatory fortification of staple foods

In 1998, the FDA mandated fortification of cereals with folic acid with approximately 140 μg of folic acid added per 100 g of enriched cereals [43]. It was also reported that the total folate consumption in women of childbearing age differed considerably by age and race/ethnicity after the fortification period in the United States, showing < 400 μg of folic acid intake in Hispanic and Non-Hispanic Black women [44]. The CDC conducted a study comparing the pre- and post-fortification period; the results showed an increase in the level of folate post-fortification by 2-3 folds in Mexican-Hispanics and non-Hispanic Whites. In contrast, the increase was insignificant in non-Hispanic Blacks [39]. The CDC also published a report that showed that the number of NTDs was reduced from 4000 to 3000 from 1999 - 2000 after post-fortification of 1998 [39, 45]. Despite the mandatory fortification program and efforts to increase folic acid awareness, there are approximately 2/3rd of reproductive aged women in the United States who are consuming less than 400 microgram of folic acid during pregnancy [44].

Folate metabolism in the human body

Folic acid is more bioavailable than folate in the human body, indicating that folic acid is readily absorbed. Methylene tetrahydrofolate reductase (*MTHFR*) is an enzyme which converts both forms of folate vitamin to L-methylfolate, which is the active form of vitamin [37]. Folate donates a methyl group necessary for many biochemical processes such as regulation of gene expression, neuronal synthesis, amino acid synthesis, and cellular metabolism [37, 46]. It plays a vital role in cell development during a methylation cycle for making homocysteine; it is also necessary for RNA and DNA biosynthesis [47]. Inadequate folate levels in the human body gives rise to certain types

of chronic diseases such as vascular diseases, megaloblastic anemia, cancers, depression, and birth defects [37, 48]. In the US population, approximately 60% of individuals contain a genetic modification to *MTHFR* that impacts the folate conversion process [46]. Many researchers have found the maternal *MTHFR C677* genotype to be the strongest predictor and a risk factor for NTDs [49].

Association of blood folate with BMI and NTDs

Multiple studies have examined the relationship between maternal peri-conceptional nutrition, vitamins, folate and folic acid with NTDs in children [50]. Folate insufficiency among mothers has been linked to many congenital abnormalities such as NTDs, cleft lip/palate, and heart defects in the fetuses [51, 52]. Pregnant women require a greater amount of folate than usual because of the additional burden of increasing cellular growth and development of the fetus, therefore, a higher dose of folate (200 μg in addition) is recommended [48]. Folate can be measured in serum and RBC levels which are established to have a differential association with pregnant women's BMI [53]. Specifically, serum folate represents the level of recent folate intake and the RBC folate indicates intracellular long-term tissue folate stores in a person [49, 54].

A folate pharmacokinetics study performed in women of reproductive age documented at baseline obese women had 29% lower serum folate compared to normal-weight women, whereas the RBC folate was 14% more in obese women. After 10 hours of folic acid intake, there was a differential cellular uptake and low plasma folate in obese women compared to normal weight, and the study concluded that obesity does not alter the folate absorption but can affect the redistribution of the folate in the tissue [55]. A recent study in 2019 showed that obese and overweight people have lower serum folate,

which could be affected by many factors such as blood dilution, excretion of urine, metabolic changes, and intestinal folate absorption may be influenced by adipose tissue [56, 57]. The decrease in serum folate level in obese women might trigger up-regulation of folate levels (folic acid absorption in intestinal cells may increase) in RBC [57, 58]. Bird *et al*. also found a positive correlation of RBC folate with fasting plasma glucose level and triglyceride, which could also explain the high level of RBC folate in obese people[57]. RBC folate reflects excess folic acid storage during erythropoiesis which could be described by the high activity of cytochrome P450 in obese people[59].

Lower RBC folate levels in early pregnancy increases the risk of NTDs (25.4 NTDs per 10,000 live births at 500 nmol/L) but there is less risk with RBC concentrations around 1000 nmol/L of RBC folate level [49, 50, 60]. The World Health Organization recommends RBC folate level to be above 400 ng/ml (906 nmol/L) to reduce the NTD occurrence in women of reproductive age but there is no recommended level for serum folate level [49]. Some randomized studies showed that the serum folate in non-pregnant women of childbearing age responded to dietary folate, folate supplementation, and fortification, whereas the RBC folate levels only responded to folate supplement and fortified food [49]. Shen *et al*. observed that pregnant women serum folate was negatively associated with BMI, whereas RBC folate had a positive relation [53].

There have been few studies examining the folate levels in obese and overweight women of reproductive age, and the results have been inconsistent. In 2017, Maffoni *et al.* published a paper that reviewed studies from 2005-2015 evaluating folate levels in non-pregnant obese reproductive age women. The authors concluded that some

researchers had found a positive association between BMI and RBC folate levels, some have found inverse relation of BMI with plasma folate, while others have not found any association between BMI and folate levels [61]. Tinker *et al.* study showed the association of suboptimal RBC folate level (having prevalence of ≥ 9 NTD cases / 10,000 live births) and obese women with an adjusted OR of 0.68 (95% CI :0. 51, 0.91) [33]. A higher BMI in women of childbearing age is associated with lower levels of serum folate, even after controlling for some variables such as age, race and folate intake shown in a paper by Mojtabai (used NHANES II and NHANES 1999-2000) [41]. The paper also documented a 0.92 ng/ml decrease in serum folate with every 10 kg/m^2 increase of BMI [41]. The pharmacokinetic study by da Silva *et al.* also showed that the RBC folate level was higher in obese women compared to normal weight [55].

Inconsistencies in these previously published studies could be due to differences in methodology, eligibility criteria, and covariables considered while studying the association [61]. Women with pre-pregnancy obesity and overweight have lower levels of vitamin B12, iron, and folate levels resulting in 2-3 times the odds of nutrient deficiency compared to normal weight women. These findings are hypothesized to result from diet restriction or poor choice of the diet [26, 62, 63]

Pregnant women have increased physiological demands for nutrients supply because of the growing fetus. There is a difference in the folate metabolism and distribution in the tissue of a pregnant women depending on the BMI and the endocrine function[64]. Women with higher BMI have lower folate levels [64]. Reduced level of serum folate in obese women could also be attributed to the redistribution of folate in the maternal tissue limiting the supply of folate to the growing fetus [65]. Therefore,

researchers have recommended obese women of childbearing age to take folate supplement and increase dietary intake of folate. In addition, some researchers believe obese women should take a higher dose of folic acid regardless of pregnancy [66].

Other risk factors

Previous papers examining the relationship of obesity and folate levels in women of childbearing age have also evaluated other important demographic, socioeconomic, and lifestyle factors. In a California study with controlled folate intake in young women, African-American women were found to have lower levels of serum and RBC folate compared to Mexican-American and Caucasian women during repletion and depletion of folate intake [67]. Racial and ethnic disparities in the reproductive aged women have been linked with decreased intake of folate supplementation in the U.S. [39, 68, 69]. A study found that the odds of not using folic acid supplements are significantly higher in Hispanic and non-Hispanic Black compared to non-Hispanic White women before conception [69]. When it comes to age, younger women compared to older aged women tend to take the folic acid supplement less frequently [65, 70]. Women's educational level also plays a significant role in considering folic acid supplementation before and during pregnancy [71]. In a study conducted in Canada, education status below high school was a risk factor for not taking folic acid supplementation with a prevalence ratio of 0.73 (95% CI: 0.61, 0.87) [70]. This same study also identified risk factors for lack of folic acid supplementation in a childbearing age women such as obesity, unemployment, low income, language barrier, and smoking [70].

Lifestyle factors that can influence folate levels include smoking, alcohol use, birth control pills, fortified cereal, folic acid supplementation and physical activity [61,

65]. Smokers have higher folate requirements because of increased erythropoiesis induced by smoking, hence the serum and folate RBC are lower in smokers than non-smokers [41]. Previous studies had reported childbearing aged women using birth control pills had lower RBC folate levels than the nonusers and it could be because of reduced folate metabolism [72].

Problem Statement

Our survey of the literature found that only a few population–based studies have examined the relation between body weight and blood folate concentration in non-pregnant, reproductive-aged women in the United States. The results from these studies have been inconsistent. Some studies found RBC folate level to be positively associated with obesity i.e. as body weight increases RBC folate level increases, whereas serum folate level decreased with increasing obesity, and some did not show any association [57, 60]. About two thirds of women of childbearing age are obese or overweight in the US [11]. Multiple studies have linked obesity as one of the risk factors for women of childbearing age for delivering a child with NTD. Women with higher BMI have an increased probability of attaining sufficient folate levels to prevent NTDs in their offspring compared to lower BMI women regardless of folic acid supplement use [34]. This was significant only for RBC folate level and not for serum folate level [34]. Hence the study suggested obese women were not required to use higher dose of folic acid to achieve preventative level of folate for NTDs [34] .The association between obesity and RBC folate cut-off levels in terms of NTD risk category has not been examined in a larger population of women living in the United States with publicly available national data. Hence, there is a need to evaluate in a large population-based sample, the

association between body weight and folate insufficiency among non-pregnant women of reproductive age, using current available data. NHANES 2007-2010 data are the most recent data and have not been explored previously to study this association.

Purpose Statement

The purpose of this study is to examine the association between body weight and folate insufficiency (NTD risk determined using RBC folate concentrations) in non-pregnant women of childbearing age in the United States [32]. We used NHANES database (2007-2010) cycle aiming to gather current and updated information about the folate levels in childbearing age women nationally. Establishing and understanding the association between maternal body weight and NTD is essential during the current and growing obesity epidemic in the United States. This study will provide additional and detailed information on the association of folate insufficiency with sub-classes of obesity compared to normal weight women. Moreover, it is critical to educate and spread awareness about significant factors contributing to lower folate levels in obese women. Early intervention and attention should be given to study the prevention of neural tube defects in obese women planning to become pregnant.

MATERIALS AND METHODS

Data Source

We used pre-collected, de-identified secondary data from the US National Health and Nutrition Examination Survey (NHANES). NHANES is a continuous survey on health and nutrition since the early 1960s conducted by the National Center for Health

Statistics (NCHS), Division of Health and Nutrition Examination Surveys (DHNES), a part of the Centers for Disease Control and Prevention (CDC). It is a law-authorized exclusive survey because it includes interviews of non-institutionalized civilian residents of the United States in their homes, complete health examinations and laboratory sample collection in a mobile examination center (MEC). NHANES representatives interview household members of all ages randomly selected by a computer algorithm. This study is a secondary data analysis conducted under good clinical practice (GCP) guidelines and was deemed to be eligible for IRB exemption by the Emory University Institutional Review Board.

For our analysis, we used NHANES data from two consecutive cycles, first cycle in 2007-2008 and the second cycle in 2009-2010. These are the latest available datasets in NHANES where folate levels were measured using the same microbiological assay, a standard method used by the Centers for Disease Control and Prevention (CDC). Since 2007, there is an oversampling of all Hispanics including Mexican Americans, leading to a decrease in sample sizes of Hispanics, low-income White, and other racial and ethnic groups. We utilized demographic, dietary, laboratory, examination, and questionnaire datasets. This information about the NHANES dataset was collected and can be found under NHANES Survey Methods and Analytic Guidelines in the CDC website https://wwwn.cdc.gov/Nchs/Nhanes/AnalyticGuidelines.aspx.

Only women of non-pregnant reproductive age were included for our analysis. Pregnancy status was only available for women aged (20-44) years. Due to disclosure risks, other women including those who were pregnant were excluded from this study. Therefore, our sample consisted of non-pregnant women 20 to 44 years of age.

Pregnancy status was confirmed either by urine pregnancy test or was self-reported by the women at the MEC.

We excluded women who were underweight (BMI < 18.5 kg/m^2). In this study, women with missing serum folate values, RBC folate values, and BMI status were also excluded from the analysis.

Measurement of Serum and RBC folate

Blood specimens were collected in MEC and sent to the Division of Laboratory Sciences, National Center for Environmental Health at CDC. The serum and RBC folate levels were determined by microbiological assays and were abstracted from the NHANES laboratory dataset. The RBC and serum folate levels were not normally distributed in our dataset; therefore, we performed a log-transformation for both serum and RBC folate levels. Serum folate and RBC folate levels were divided into quartiles based on the median range of folate levels obtained from univariate analysis of the normal weight group of women. The lowest quartile was categorized as the low folate level, the second and third quartile were combined to form a medium level, and the fourth or the highest quartile represented high folate level for both the serum and RBC folate. As a result, low serum folate was defined as <3.19 nmol/l, medium as 3.19-3.88 nmol/l, and high quartile as >3.88 nmol/l. RBC folate concentrations were also divided into the low (< 6.63 nmol/l), medium (6.63-7.17 nmol/l), and high (>7.17 nmol/l) concentrations based on the quartile cut-off levels.

We utilized the CDC assay used in NHANES methods for conversion from the Malloy Assay described in Tinker et al. study [33]. To define folate insufficiency RBC folate levels were created based on the two previous studies by Tinker *et al*. (2015) and

Crider *et al.* (2019) [33, 73, 74]. We have defined folate insufficiency as a RBC folate concentration where a woman is at risk for having NTD-affected pregnancy based on RBC folate categorization. The CDC microbiological assay (MBA-3) used in the NHANES 2007 to 2010 datasets to measure RBC and serum folate levels were calibrated using chloramphenicol resistant strain and 5-methyltetrahydrofolate calibrator [75]. The MBA-2 cut off levels for RBC folate concentration by WHO were not compatible to be used with NHANES data, hence MBA-3 was used [75]. The categories were as follows: 1) there is a high risk for NTD if RBC folate level is <585 nmol/L; 2) elevated risk for NTD at RBC folate level 586-747 nmol/L; 3) optimal risk for NTD at RBC folate level of 748-1215 nmol/L; 4) limited additional benefit for NTD reduction at RBC folate level ≥ 1216 nmol/L. Women having RBC folate level in high risk and elevated risk categories (<748 nmol/l) were defined as having folate insufficiency.

Obesity and overweight

According to the CDC and World Health Organization, the definition for obesity is BMI ≥30 kg/m^2, overweight is 25 -30 kg/m^2 and normal weight is 18.5-25 kg/m^2 [1, 76]. We used the BMI measures collected during the examination of the women at MEC. BMI values in our analysis were rounded to one decimal place.

Based on CDC criteria, we further classified obesity into three categories: 1) Class 1 obesity was defined as BMI of 30-35 kg/m^2; 2) Class 2 obesity as BMI of 35-40 kg/m^2; 3) Class 3 BMI as ≥40 kg/m^2[1].

Covariates

Covariates of interest were selected *a priori* based on a literature review. Age (continuous), Race/ethnicity (Non-Hispanic White, Non-Hispanic Black, Mexican–

American, Other), education (<12th grade/GED, 12th grade/GED, and > 12th grade/GED), marital status (married, single/divorced/separated/widowed/ single/living with the partner), employment (employed, unemployed), and annual household income (<$24,999, $25,000-$74,999, $≥ 75,000 were extracted from demographics dataset.

We examined selected lifestyle factors collected from the questionnaire dataset. Information on the average number of alcohol drinks per day in past 12 months, current smoking status (daily smoker, not a smoker), number of cigarettes per day in past 30 days, if they were currently diagnosed as having diabetes or no diabetes, use of birth control pills, and involvement in moderate intensity exercise were obtained from the questionnaire dataset. Moderate physical activity is described as moderate intensity activity for at least 10 minutes without taking a break and resulting in increased breathing and heart rate (NHANES 2009-2010).

We examined total folic acid diet supplement intake during a 30-day period. Two groups were created who take folic acid ≥ 400 mcg/day of folic acid diet supplement and those who take <400 mcg/day or do not take any folic acid dietary supplement at all. We examined the number of dietary supplements taken in the past 30 days, the duration of supplementation, and intake of fortified cereals. These data were taken from the dietary dataset.

Statistical analysis

Statistical analysis for this study was accomplished using SAS v 9.4 (SAS Institute Inc., Cary, NC, USA). NHANES is a nationally representative study of individuals living in the United States. Hence, sample data were weighted to measure individuals represented by the specific participants who were sampled [77]. There were

three steps involved with sample weighting which consisted of computing the base weights, adjustment for non-responders with trimming of extreme weights, and post-stratification of sample weights. These steps were completed at every survey screening, interview, and examination of the participant [77]. We analyzed selected sample gathered over 4 years from the two combined cycles of the survey (2007-2010). Therefore, the weight was calculated using one half times the 2-year MEC sampling weights (WTMEC2YR) to yield 4-year point estimates as recommended by NHANES (MEC4YR = **1**/**2** * WTMEC2YR) [77].

We compared selected characteristics between normal body weight women and overweight/ obese including various sub-groups of obese women. Descriptive statistics for continuous variables reported by weighted analyses were expressed in mean values using “proc survey means” and categorical variables were measured in weighted frequencies and weighted percentages using “proc survey logistics”. The statistical difference was tested by Rao-Scott Chi-Square test for categorical and two-sided *t*-test for continuous variables with significance level measured at P=0.05.

We used logistic regression analysis, accounting for the complex survey methods, to examine the association between body weight and folate status. Unadjusted prevalence odds ratios and 95% confidence intervals were estimated for the primary independent variable and covariables. Multiple logistic regression was used to estimate adjusted effect estimates, controlling for covariates selected from demographic, dietary, and lifestyle factors. The analyses included separate models for obesity and overweight. Potential confounders included in the multiple regression models, including age, race and ethnicity,

and education, were selected *a priori,* based on our literature review. We did not examine for interactions.

RESULTS

Our analytic sample consisted of a total of 30,878 non-pregnant women of reproductive age after excluding 2,058 pregnant women, 895 underweight (BMI<18.5 kg/m^2) women, and women whose serum and RBC folate levels and BMI were missing. Among these eligible 30,878 women, 11,282 (41.72%) were of normal weight (BMI=18.5-25 kg/m^2), 8,321 (25.58%) were overweight (BMI=25-30 kg/m^2), and 11,275 (32.69%) were obese (BMI ≥30 kg/m^2). Among obese women, 45.57% were categorized as class 1 obesity (BMI=30-34 kg/m^2), 30.63% were class 2 obesity (BMI=35-39 kg/m^2), and 23.81 % were classified as class 3 obesity (BMI ≥40 kg/m^2).

Among all eligible women, 22.12% of women were in the lower concentration of serum folate level, and 21.50% of women had RBC folate levels in the lower concentration that indicated a high risk for NTD.

About 50% of the total obese and overweight women had medium serum folate levels ranging from 3.19-3.88 nmol/l and high RBC folate concentration ranging from 6.63-7.17 nmol/l, 28% of obese and 20% of overweight women had serum folate level in the lower concentration (< 3.19nmol/l) whereas, only 18% obese and 22% overweight women had RBC folate in the lower concentration (<6.63nmol/l). Similarly, 34% of obese women, 28% of overweight women had RBC folate in highest level (High > 7.17 nmol/l) shown in Table 1. There was a significant difference between normal-weight and

obese women on serum folate (P=0.0024) and RBC folate (P=0.0245) levels. Conversely, there was no significant difference observed between overweight and normal-weight women on folate levels. There was no significant difference observed in the mean RBC folate levels among obese, overweight, and normal weight women.

Descriptive analysis showed significant differences on age, race/ethnicity, and household income, moderate physical activity, and education among the normal weight, obese and overweight women (P<0.05) as shown in (Table 1).

There were significant differences for class 1, class 2, and class 3 obesity compared to normal weight women according to race, serum folate, education, annual household income, and diabetes status (P<0.05) (Table 2). About 8% of Class 3 obese women took folic acid diet supplement, which was significantly different from normal weight women, which was 20% (P<. 0001).

When considering the folate insufficiency (high risk <585 nmol/L, elevated risk 586-747 nmol/L), approximately 11% obese and overweight women and 10% normal weight women were classified with the high-risk NTD category. Among the total eligible women, 21.43 % of the women had RBC folate levels in the elevated NTD risk category, 10.87% in high risk NTD category, and 67.71% in the optimum risk category as shown in Table 1. There was no significant difference observed in the folate insufficiency (RBC folate cut-off level by NTD risk category) when obese and overweight women were compared to normal weight women. Likewise, when class 1, class 2, and class 3 obese women were compared to a normal weight woman there was no significant difference found in the folate insufficiency as shown in Table 1.

The crude prevalence odds ratio (cPOR) for the association of obesity and serum folate in non-pregnant women of reproductive age women was 0.60 (95% CI = 0.39, 0.93), which was significant, whereas cPOR for overweight and normal weight was not significant (cPOR: 1.03; 95% CI=0.67, 1.59). The unadjusted cPOR of obese and overweight women showing association with RBC folate levels were not statistically significant compared to normal weight women as shown in Table 1. Likewise, in unadjusted analyses within specific obesity categories, only class 2 obesity (cPOR: 1.77; 95% CI: 1.01, 3.09) and class 3 obesity (cPOR: 1.69; 95% CI: 1.11, 2.57) obesity was significantly associated with low level of serum folate shown in Table 2.

Association between obesity and overweight with folate insufficiency

We performed a multivariable logistic regression analysis for the association between obesity and folate insufficiency, adjusted for age, race/ethnicity, and education. In this *a priori* model for obese women vs. normal weight women, the adjusted prevalence odds ratio (aPOR) did not show any significant association between obesity and folate insufficiency (aPOR:0. 79; 95% CI: 0.50, 1.23) for high-risk NTD category and elevated risk NTD category (aPOR: 0.67; 95% CI: 0.45,1.00). Similarly in another *a priori* multivariable regression analysis, overweight was not associated with folate insufficiency (aPOR: 0.79; 95% CI: 0.48,1.32 for high risk NTD category and aPOR: 0.76; 95%CI:0. 55,1.05 for elevated risk NTD category).

Association between folate insufficiency and classes of obesity

We also performed *a priori* multivariable logistic regression analyses with sub-types of obesity as a dependent variable and folate insufficiency. There was a protective association between folate insufficiency and class 3 obesity in the *a priori* model adjusted

for the potential confounders (aPOR: 0.46; 95% CI: 0.24, 0.89) age, race/ethnicity, and education. The analyses did not show any association between other obesity classes and folate insufficiency.

DISCUSSION

In this large population-based cross-sectional study using NHANES 2007-2010 data, we found no association between body weight and folate insufficiency among non-pregnant women of childbearing age in the United States. The one significant association we found for class 3 obesity and folate insufficiency could have been by chance and error from the multiple hypothesis testing we did.

To our knowledge, this is the first study analyzing the CDC-assay based measure of folate insufficiency and its association with body weight among non-pregnant women of reproductive age. We could not find any published studies examining the association between RBC folate cut off level by NTD risk category and the body weight in non-pregnant women of reproductive age. The CDC published a paper with information on the RBC folate cut off level by NTD risk category recently in 2015[33]. Therefore, our comparisons are limited. As our study is the first study applying this new evidence on folate insufficiency, it is difficult to compare our findings with previous studies using different criteria, and RBC folate cut points to establish the relation of RBC folate insufficiency in obese women.

Tinker *et al.* in 2015 used NHANES dataset to study if the reproductive age women in the United States with suboptimal RBC folate level (< 748nmol/L) have a risk

of NTD pregnancy based on RBC folate level[33]. They analyzed 4783 women who had information on folic acid dietary supplement combining the NHANES survey cycles from 2007 to 2012 [33]. One of their findings showed the odds of having suboptimal RBC folate level associated with NTD prevalence in obese women were 32% lower than the normal weight non-pregnant women of reproductive age when controlling for various factors [33]. Our analyses also found that there was 33% reduction in the odds of folate insufficiency among obese women, with a borderline significance (aPOR:0. 67,95% CI:0. 45, 1.00). Similarly, our analyses showed that women with class 3 obesity have a 54% reduction in the odds of having NTD high-risk category per CDC RBC folate cut off level.

As mentioned previously, we utilized two NHANES data cycles (2007-2010) to increase our sample size. NHANES is highly reputable national survey system, which is a program of CDC, developed to generate vital and national standard health statistics for the U.S. NHANES trained staffs select, interview, and examine the eligible sample. The samples are weighted for analysis, which eliminates biases from non-respondents, balances inadequate sampling, and generates estimates representing whole sample population [77].

There are several strengths and limitations in this study. Among the strengths, NHANES data is a complex survey involving a huge US population that is conducted by skilled professionals. NHANES has been collecting folate levels since 1974 from U.S residents, indicating an extensive and rich dataset. With regard to assay sensitivity, a roundtable summary about NHANES folate biomarkers and its measurement methods took place in the U.S. in 2010. The folates measured from (2007-2010) by the

microbiologic assay were higher and more accurate than the ones measured from (1999-20006) by Bio-Rad radioassay [78, 79]. The Division of Laboratory Sciences, National Center for Environmental Health, and CDC assure high data quality of folate analysis [80]. Our study has an analytical sample of 30,878, which is the highest sample size ever studied with NHANES data examining the association (other than our hypothesis) of folate, NTD, and obesity in women of reproductive age. We compared the proportions of our normal weight, overweight and obese women with the distribution of weights in pre-pregnant women in 2011-2015 published by CDC in 2018. The study comprised of 45% normal weights, overweight=25.8%, and obese 25.6%, which was very similar to our proportions of normal weights, overweight, and obese women (41.72%, 25.59%, and 32.69%)[81]. When compared to NCHS data brief (utilizing NHANES survey cycle 2015-2016) published in 2017, obese women aged 20-39 years were 36.5% whereas we had 32.69 % obese women aged 20-44 years of age[6].

There were some major limitations in this analysis. Since NHANES is a cross-sectional study, we could not establish a temporal relationship between obesity and folate insufficiency. Regarding the data availability, the latest data cycles with serum and RBC folate levels were 2007-2008 and 2009-2010 data cycle. We could not use recent datasets because folate levels were not available. In addition, both data cycles did not have information on the serum vitamin B12 and homocysteine levels. Hence, we could not explore the association of vitamin B12 and homocysteine levels with obesity. Finally, we considered the combination food to be the variable chosen to characterize the fortified cereal, which might not completely represent the covariate.

In conclusion, we did not find evidence for the association between obesity and folate insufficiency in non-pregnant women of reproductive age. Future studies should examine this association in a study measuring the RBC folate levels in the context of a weight loss intervention program in obese/overweight women. Also, it is recommended to understand the relationship between body weight and its impact on folate metabolism pathways, especially among obese women. The association between obesity and NTD is well documented in previous studies, but some studies including ours have inconsistent findings with RBC folate levels and obesity. As a result, we also recommend studying the causal pathway between maternal weight and folate insufficiency (NTD risk in the offspring) using the most current data so that early intervention should be given to study the prevention of neural tube defects in obese women planning to become pregnant.

REFERENCES

1. CDC. *Defining Adult Overweight and Obesity*. [cited 2019 3319]; Available from: https://www.cdc.gov/obesity/adult/defining.html.
2. Kopelman, P.G., *Obesity as a medical problem.* Nature, 2000. **404**(6778): p. 635-43.
3. Racusin, D., et al., *Obesity and the risk and detection of fetal malformations.* Semin Perinatol, 2012. **36**(3): p. 213-21.
4. Marion Devaux, S.G., Yevgeniy Goryakin, and H.H.a.F.C. Michele Cecchini, *Obesity update 2017.* OECD, 2017.
5. Cynthia L. Ogden, P.D.M.D.C., M.S.P.H.; Cheryl D. Fryar, M.S.P.H.; and P.D. and Katherine M. Flegal, *Prevalence of Obesity Among Adults and Youth: United States, 2011–2014.* NCHS Data Brief, 2015. **219**.
6. Craig M. Hales, M.D., Margaret D. Carroll, M.S.P.H., Cheryl D. Fryar, M.S.P.H., and Cynthia L. Ogden, Ph.D., *Prevalence of Obesity Among Adults and Youth: United States, 2015–2016.* 2017.
7. Cogswell, M.E., et al., *Obesity in women of childbearing age: risks, prevention, and treatment.* Primary Care Update for OB/GYNS, 2001. **8**(3): p. 89-105.
8. Vahratian, A., *Prevalence of overweight and obesity among women of childbearing age: results from the 2002 National Survey of Family Growth.* Matern Child Health J, 2009. **13**(2): p. 268-73.
9. Flegal, K.M., et al., *Trends in obesity among adults in the united states, 2005 to 2014.* JAMA, 2016. **315**(21): p. 2284-2291.
10. O'Sullivan, A.J., *Does oestrogen allow women to store fat more efficiently? A biological advantage for fertility and gestation.* Obes Rev, 2009. **10**(2): p. 168-77.
11. Hillemeier, M.M., et al., *Transition to overweight or obesity among women of reproductive age.* J Womens Health (Larchmt), 2011. **20**(5): p. 703-10.
12. Leck, I., *Causation of neural tube defects: clues from epidemiology.* Br Med Bull, 1974. **30**(2): p. 158-63.
13. Frey, L. and W.A. Hauser, *Epidemiology of Neural Tube Defects.* Epilepsia, 2003. **44**(s3): p. 4-13.
14. Denny, K.J., et al., *Neural tube defects, folate, and immune modulation.* Birth Defects Res A Clin Mol Teratol, 2013. **97**(9): p. 602-609.
15. Zaganjor, I., et al., *Describing the Prevalence of Neural Tube Defects Worldwide: A Systematic Literature Review.* PLoS One, 2016. **11**(4): p. e0151586.
16. Blencowe, H., et al., *Estimates of global and regional prevalence of neural tube defects for 2015: a systematic analysis.* Ann N Y Acad Sci, 2018. **1414**(1): p. 31-46.
17. Grosse, S.D., et al., *Retrospective Assessment of Cost Savings From Prevention: Folic Acid Fortification and Spina Bifida in the U.S.* Am J Prev Med, 2016. **50**(5 Suppl 1): p. S74-s80.
18. Rofail, D., et al., *A review of the social, psychological, and economic burdens experienced by people with spina bifida and their caregivers.* Neurol Ther, 2013. **2**(1-2): p. 1-12.
19. Prentice, A. and G. Goldberg, *Maternal obesity increases congenital malformations.* Nutr Rev, 1996. **54**(5): p. 146-50.

20. Waller, D.K., et al., *Are obese women at higher risk for producing malformed offspring?* Am J Obstet Gynecol, 1994. **170**(2): p. 541-8.
21. Watkins, M.L., et al., *Is maternal obesity a risk factor for anencephaly and spina bifida?* Epidemiology, 1996. **7**(5): p. 507-12.
22. Shaw, G.M., E.M. Velie, and D. Schaffer, *Risk of neural tube defect-affected pregnancies among obese women.* Jama, 1996. **275**(14): p. 1093-6.
23. Rasmussen, S.A., et al., *Maternal obesity and risk of neural tube defects: a metaanalysis.* Am J Obstet Gynecol, 2008. **198**(6): p. 611-9.
24. Huang, H.Y., H.L. Chen, and L.P. Feng, *Maternal obesity and the risk of neural tube defects in offspring: A meta-analysis.* Obes Res Clin Pract, 2017. **11**(2): p. 188-197.
25. Gao, L.J., et al., *Maternal overweight and obesity and the risk of neural tube defects: a case-control study in China.* Birth Defects Res A Clin Mol Teratol, 2013. **97**(3): p. 161-5.
26. McMahon, D.M., et al., *Maternal obesity, folate intake, and neural tube defects in offspring.* Birth Defects Res A Clin Mol Teratol, 2013. **97**(2): p. 115-22.
27. Viswanathan, M., et al., *Folic acid supplementation for the prevention of neural tube defects: An updated evidence report and systematic review for the us preventive services task force.* JAMA, 2017. **317**(2): p. 190-203.
28. Greenberg, J.A., et al., *Folic Acid supplementation and pregnancy: more than just neural tube defect prevention.* Rev Obstet Gynecol, 2011. **4**(2): p. 52-9.
29. Czeizel, A.E. and I. Dudas, *Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation.* N Engl J Med, 1992. **327**(26): p. 1832-5.
30. *Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. MRC Vitamin Study Research Group.* Lancet, 1991. **338**(8760): p. 131-7.
31. Williams, J., et al., *Updated estimates of neural tube defects prevented by mandatory folic Acid fortification - United States, 1995-2011.* MMWR Morb Mortal Wkly Rep, 2015. **64**(1): p. 1-5.
32. Crider, K.S., et al., *Population red blood cell folate concentrations for prevention of neural tube defects: Bayesian model.* Bmj, 2014. **349**: p. g4554.
33. Tinker, S.C., et al., *U.S. women of childbearing age who are at possible increased risk of a neural tube defect-affected pregnancy due to suboptimal red blood cell folate concentrations, National Health and Nutrition Examination Survey 2007 to 2012.* Birth Defects Res A Clin Mol Teratol, 2015. **103**(6): p. 517-26.
34. Piyathilake, C., et al., *Determinants of neural tube defect (NTD)-protective circulating concentrations of folate in women of child-bearing age in the US post-folic acid fortification era.* Nutr Res Pract, 2013. **7**(4): p. 315-25.
35. Stern, S.J., et al., *Dosage Requirements for Periconceptional Folic Acid Supplementation: Accounting for BMI and Lean Body Weight.* Journal of Obstetrics and Gynaecology Canada, 2012. **34**(4): p. 374-378.
36. Ray, J.G., et al., *Greater maternal weight and the ongoing risk of neural tube defects after folic acid flour fortification.* Obstet Gynecol, 2005. **105**(2): p. 261-5.
37. Stahl, S.M., *L-methylfolate: a vitamin for your monoamines.* J Clin Psychiatry, 2008. **69**(9): p. 1352-3.

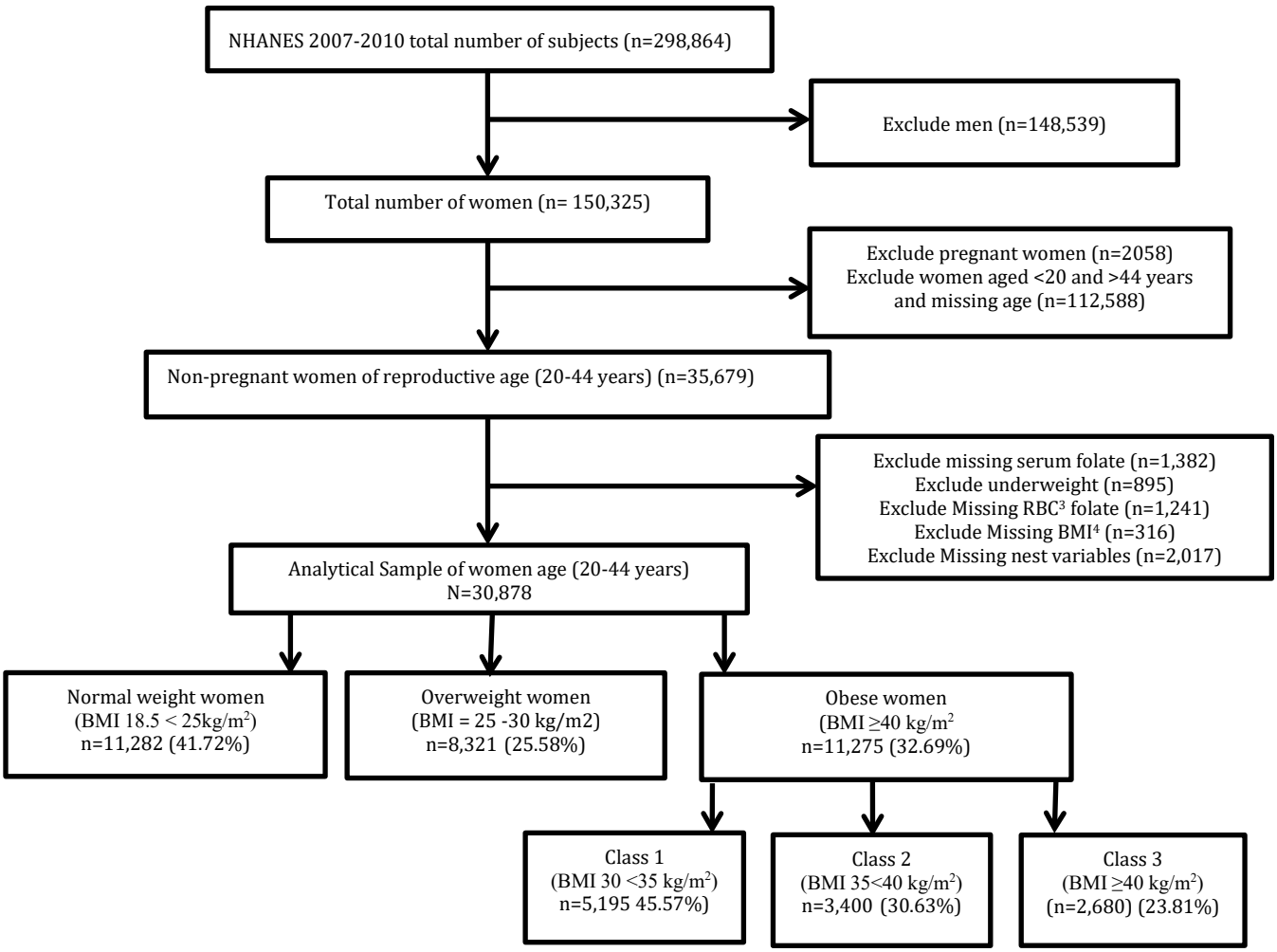
38. Pitkin, R.M., *Folate and neural tube defects.* Am J Clin Nutr, 2007. **85**(1): p. 285s-288s.
39. *Folate status in women of childbearing age, by race/ethnicity--United States, 1999-2000.* MMWR Morb Mortal Wkly Rep, 2002. **51**(36): p. 808-10.
40. Wilson, R.D., *Pre-conceptional vitamin/folic acid supplementation 2007: the use of folic acid in combination with a multivitamin supplement for the prevention of neural tube defects and other congenital anomalies.* J Obstet Gynaecol Can, 2007. **29**(12): p. 1003-1013.
41. Mojtabai, R., *Body mass index and serum folate in childbearing age women.* Eur J Epidemiol, 2004. **19**(11): p. 1029-36.
42. Farah, N., et al., *Maternal obesity and pre-pregnancy folic acid supplementation.* Obes Facts, 2013. **6**(2): p. 211-5.
43. Crider, K.S., L.B. Bailey, and R.J. Berry, *Folic acid food fortification-its history, effect, concerns, and future directions.* Nutrients, 2011. **3**(3): p. 370-84.
44. Yang, Q.-H., et al., *Race-ethnicity differences in folic acid intake in women of childbearing age in the United States after folic acid fortification: findings from the National Health and Nutrition Examination Survey, 2001–2002.* The American Journal of Clinical Nutrition, 2007. **85**(5): p. 1409-1416.
45. *Spina bifida and anencephaly before and after folic acid mandate--United States, 1995-1996 and 1999-2000.* MMWR Morb Mortal Wkly Rep, 2004. **53**(17): p. 362-5.
46. Miller, A.L., *The methylation, neurotransmitter, and antioxidant connections between folate and depression.* Altern Med Rev, 2008. **13**(3): p. 216-26.
47. Scott, J.M., et al., *Folic acid metabolism and mechanisms of neural tube defects.* Ciba Found Symp, 1994. **181**: p. 180-7; discussion 187-91.
48. Bailey, L.B. and I.I.I.J.F. Gregory, *Folate Metabolism and Requirements.* The Journal of Nutrition, 1999. **129**(4): p. 779-782.
49. WHO, *Guideline: Optimal serum and red blood cell folate concentrations in women of reproductive age*
for prevention of neural tube defects. Geneva: World Health Organization; 2015, (2015).
50. Kancherla, V. and R.E. Black, *Historical perspective on folic acid and challenges in estimating global prevalence of neural tube defects.* Ann N Y Acad Sci, 2018. **1414**(1): p. 20-30.
51. Bailey, S.W. and J.E. Ayling, *The pharmacokinetic advantage of 5-methyltetrahydrofolate for minimization of the risk for birth defects.* Sci Rep, 2018. **8**(1): p. 4096.
52. Ryan-Harshman, M. and W. Aldoori, *Folic acid and prevention of neural tube defects.* Canadian Family Physician, 2008. **54**(1): p. 36-38.
53. Shen, M., et al., *Serum and red-blood-cell folate demonstrate differential associations with BMI in pregnant women.* Public Health Nutr, 2016. **19**(14): p. 2572-9.
54. McDowell, M.A., et al., *Blood folate levels: the latest NHANES results.* NCHS Data Brief, 2008(6): p. 1-8.
55. da Silva, V.R., et al., *Obesity affects short-term folate pharmacokinetics in women of childbearing age.* Int J Obes (Lond), 2013. **37**(12): p. 1608-10.

56. Kose, S., et al., *Obesity is associated with folate metabolism.* Int J Vitam Nutr Res, 2019: p. 1-12.
57. Bird, J.K., et al., *Obesity is associated with increased red blood cell folate despite lower dietary intakes and serum concentrations.* J Nutr, 2015. **145**(1): p. 79-86.
58. Subramanian, V.S., N. Chatterjee, and H.M. Said, *Folate uptake in the human intestine: promoter activity and effect of folate deficiency.* J Cell Physiol, 2003. **196**(2): p. 403-8.
59. Zhou, S.S., et al., *Vitamin paradox in obesity: Deficiency or excess?* World J Diabetes, 2015. **6**(10): p. 1158-67.
60. Daly, L.E., et al., *Folate levels and neural tube defects: Implications for prevention.* JAMA, 1995. **274**(21): p. 1698-1702.
61. Maffoni, S., et al., *Folate status in women of childbearing age with obesity: a review.* Nutr Res Rev, 2017. **30**(2): p. 265-271.
62. Scholing, J.M., et al., *Association between pre-pregnancy weight status and maternal micronutrient status in early pregnancy.* Public Health Nutrition, 2018: p. 1-10.
63. Watkins, M.L., et al., *Maternal obesity and risk for birth defects.* Pediatrics, 2003. **111**(5 Pt 2): p. 1152-8.
64. Kim, H., et al., *Relationship between body-mass index and serum folate concentrations in pregnant women.* Eur J Clin Nutr, 2012. **66**(1): p. 136-8.
65. Tinker, S.C., et al., *Does obesity modify the association of supplemental folic acid with folate status among nonpregnant women of childbearing age in the United States?* Birth Defects Res A Clin Mol Teratol, 2012. **94**(10): p. 749-55.
66. Case, A.P., et al., *Folic acid supplementation among diabetic, overweight, or obese women of childbearing age.* J Obstet Gynecol Neonatal Nurs, 2007. **36**(4): p. 335-41.
67. Perry, C.A., et al., *Ethnicity and Race Influence the Folate Status Response to Controlled Folate Intakes in Young Women.* The Journal of Nutrition, 2004. **134**(7): p. 1786-1792.
68. Marchetta, C.M. and H.C. Hamner, *Blood folate concentrations among women of childbearing age by race/ethnicity and acculturation, NHANES 2001-2010.* Matern Child Nutr, 2016. **12**(1): p. 39-50.
69. Mukhtar, A., et al., *Race and ethnicity and preconception folic acid supplement use among pregnant women in Georgia, PRAMS 2009 to 2011.* Birth Defects Res, 2017. **109**(1): p. 38-48.
70. Miller, E.C., et al., *Why do Canadian women fail to achieve optimal pre-conceptional folic acid supplementation? An observational study.* J Obstet Gynaecol Can, 2011. **33**(11): p. 1116-1123.
71. Canfield, M.A., et al., *Folic acid awareness and supplementation among Texas women of childbearing age.* Prev Med, 2006. **43**(1): p. 27-30.
72. Shojania, A.M., *Oral contraceptives: effect of folate and vitamin B12 metabolism.* Can Med Assoc J, 1982. **126**(3): p. 244-7.
73. Crider, K.S., et al., *Modeling the impact of folic acid fortification and supplementation on red blood cell folate concentrations and predicted neural tube defect risk in the United States: have we reached optimal prevention?* Am J Clin Nutr, 2018. **107**(6): p. 1027-1034.

74. Pfeiffer, C.M., et al., *Applying inappropriate cutoffs leads to misinterpretation of folate status in the US population.* Am J Clin Nutr, 2016. **104**(6): p. 1607-1615.
75. Sternberg, M.R., et al., *Applying inappropriate cutoffs leads to misinterpretation of folate status in the US population.* The American Journal of Clinical Nutrition, 2016. **104**(6): p. 1607-1615.
76. WHO. *BMI classification*. [cited 2019 3/3/19]; Available from: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html.
77. Mirel LB, M.L., Dohrmann SM, et al., *National Health and Nutrition Examination Survey: Estimation Procedures, 2007–2010*. 2013, National Center for Health Statistics. Vital Health Stat.
78. Yetley, E.A., et al., *Biomarkers of folate status in NHANES: a roundtable summary.* Am J Clin Nutr, 2011. **94**(1): p. 303S-312S.
79. Pfeiffer, C.M., et al., *Estimation of trends in serum and RBC folate in the U.S. population from pre- to postfortification using assay-adjusted data from the NHANES 1988-2010.* J Nutr, 2012. **142**(5): p. 886-93.
80. CDC. 2009-2010; Available from: https://wwwn.cdc.gov/Nchs/Nhanes/2009-2010/FOLATE_F.htm.
81. Deputy NP, D.B., Sharma AJ. . , *Prevalence and Trends in Prepregnancy Normal Weight — 48 States, New York City, and District of Columbia, 2011–2015.* MMWR Morb Mortal Wkly Rep 2018;: p. 66:1402–1407.

TABLES AND FIGURES

Figure 1. Consort diagram showing Study Population Eligibility Criteria (based on the unweighted sample) [1], NHANES[2], United States, 2007-2010.



[1] The analytical sample might be affected by potential overlap of data and multiple criteria for exclusion, which might have resulted in differences in weighted and unweighted samples.
[2] National Health and Nutrition Examination Survey
[3] Red Blood Cells
[4] Body Mass Index
[5] Weighted frequencies and percentages

Table 1: Descriptive analysis of normal weight, overweight, and obese non-pregnant women of reproductive age (20-44 years), NHANES (2007-2010)

	Obese vs. Normal weight				Overweight vs. Normal weight		
Characteristics	Normal weight N=11282(%)	Obese N=11275(%)	*P* value[4]	Prevalence Crude Odds Ratio (95% CI)	Overweight N=8321(%)	*P* value[4]	Prevalence Crude Odds Ratio (95% CI)
Serum folate concentration [1]			**0.0024**			0.355	
Low (<3.19nmol/l)	2183(18.38)	3446(28.27)		**0.60(0.39,0.93)**	2047(20.36)		1.03(0.67,1.59)
Medium (3.19-3.88 nmol/l)	5639 (47.70)	5658(50.31)		Ref	4252(51.27)		Ref
High (>3.88 nmol/l)	3460(33.92)	2171(21.42)		1.46(0.96,2.20)	2022(28.37)		0.78(0.54,1.11)
RBC folate concentration[1]			**0.0245**			0.8697	
Low (<6.63nmol/l)	2976(23.53)	2335(18.35)		1.22(0.90,1.66)	2330(22.23)		0.95(0.63,1.43)
Medium (6.63-7.17 nmol/l)	5552(48.03)	5530(47.37)		Ref	4196(49.72)		Ref
High (> 7.17 nmol/l)	2754(28.44)	3410(34.28)		0.79(0.63,1.00)	1795(28.04)		0.91(0.72,1.16)
Serum folate (nmol/l) (mean+/-SE)	3.63(0.03)	3.47(0.03)	**0.0007**	0.53(0.37,0.76)	3.59(0.03)	0.2981	0.84(0.6,1.18)
RBC folate (nmol/l) (mean+/-SE)	6.93(0.02)	6.99(0.02)	**0.027**	**1.45(1.03,2.03)**	6.94(0.02)	0.8369	1.04(0.74,1.46)
RBC folate concentrations by NTD risk category[2]			0.179			0.5545	
High Risk (≤585 nmol/l)[3]	1001(10.34)	905(11.31)		0.75(0.53,1.06)	773(11.22)		0.85(0.63,1.16)
Elevated risk (586-747 nmol/l)[3]	1892(23.67)	1343(18.75)		1.03(0.69,1.54)	1452(20.81)		1.05(0.68, 1.65)
Optimal Risk (748-1215 nmol/l)[3]	5124(66.00)	4939(69.94)		Ref	3972(67.97)		Ref
Age (years)	31.67(0.56)	33.63(0.37)	0.0078	1.04(1.01,1.07)	33.17(0.37)	0.0362	1.03(1.00,1.06)
Race			**<.0001**			**<0.0001**	
Non-Hispanic White	5995(72.16)	4237(57.96)		Ref	3101(61.04)		Ref
Non-Hispanic Black	1192(6.24)	2628(17.81)		**3.56(2.49,5.08)**	1467(12.62)		**2.39(1.58, 3.61)**
Mexican -American	1772(6.89)	2687(12.79)		**2.31(1.60,3.33)**	2225(13.22)		**2.27(1.52,3.38)**
Other	2323(14.71)	1723(11.43)		0.97(0.61,1.53)	1528(13.12)		1.06(0.68,1.63)
Education			**<.0001**				
< 12th grade/ GED	1655(9.69)	2928(18.87)		**1.53(1.04,2.24)**	1645(17.75)	**0.0015**	**0.65(0.47,0.89)**
12th grade/GED	2158(16.09)	2324(20.50)		Ref	2173(16.41)		Ref
> 12th grade/GED	7467(74.22)	6023(60.63)		**0.64(0.46,0.90)**	4503(65.84)		**0.52(0.35,0.78)**
Marital Status			0.2849			0.5279	
Married	5489(52.55)	5108(49.51)			4298(54.79)		0.91(0.68,1.23)
Single (divorced/separated/ Widowed/Single/ Living with partner)	5793(47.45)	6167(50.49)		1.13(0.89,1.42)	4023(45.21)		
Employment			0.1284			0.3365	
Employed	7570(69.88)	6824(65.42)		1.23(0.93,1.61)	5243(67.33)		1.13(0.88,1.45)
Unemployed	3690(30.12)	4451(34.58)			3078(32.67)		
Household income			**<.0001**			**0.0236**	
<$24,999	2856(18.13)	4121(28.87)		1.26(0.89,1.79)	2650(24.59)		1.32(0.94,1.86)
$25,000-$74,999	4395(38.54)	4941(48.60)		Ref	3478(39.62)		Ref
$≥ 75,000	3674(43.32)	1892(22.53)		0.41(0.25,0.69)	1820(35.79)		0.80(0.56,1.14)

Average number of alcohol drinks/day in past 12 months. (mean+/-SE)	2.16(0.11)	2.41(0.13)	0.22	1.07(0.96,1.18)	2.46(0.13)	0.1236	1.09(0.97,1.22)
Smoking status			0.243			0.3249	
Everyday smoker	1687(42.79)	2118(50.16)		1.43(0.93,2.2)	1486(44.51)		1.22(0.69,2.11)
Someday smoker	415(10.82)	555(11.74)		Ref	445(15.76)		Ref
Not a smoker	1294(46.39)	1379(38.10))		1.32(0.65,2.68)	1002(39.73)		1.7(0.84,3.46)
Cigarettes per day during past 30 days (mean+/-SE)	11.85(0.92)	12.53(0.87)	0.59	1.01(0.98,1.03)	11.24(1.21)	0.6657	0.99(0.95,1.03)
Diabetes							
Diabetic	109(0.89)	868(7.54)	**<.0001**	**9.06(3.56,23.08)**	74(0.61)	0.6249	**0.68(0.14,3.37)**
Non-Diabetic	11167(99.11)	10387(92.46)			8229(99.39)		
Currently taking Birth Control Pills			**0.0669**			**0.0145**	
Yes	1038(35.84)	503(20.27)		0.46(0.18,1.15)	514(20.76)		**0.47(0.25,0.89)**
No	2706(64.15)	3021(79.73)			2555(79.24)		
Perform moderate physical activity							
Yes	4648(43.93)	3985(36.96)	0.0271	1.34(1.02,1.75)	2769(34.32)	0.0392	1.49(0.99,2.25)
No	6634(56.07)	7290(63.04)			5552(65.68)		
Use of folic acid diet supplement							
≥400 mcg	2066(20.67)	1328(14.78)	**0.036**		1251(18.21)	0.5081	1.17(0.75,1.82)
<400 mcg or non users	9205(79.33)	9947(85.22)		1.50(1.00,2.25)	7070(81.79)		
Diet supplement taken in past 30 days (mean+/-SE)	20.22(0.61)	18.91(0.86)	0.24	0.99(0.97,1.01)	19.79(0.92)	0.7082	0.99(0.98,1.02)
Length of diet supplement taken in days (mean+/-SE)	1246.67(154.71)	1287.58(158.98)	0.84	1(1,1)	1114.63(165.49)	0.5733	1(1,1)
Eat fortified cereal						0.3439	
Yes	381(3.68)	332(3.40)	0.574		240(3.16)		1.17(0.83,1.64)
No	10027(96.32)	10056(96.60)		1.09(0.81,1.46)	7454(96.84)		

[1] log transformed Serum and RBC folate concentration is calculated as quartiles, low level is first quartile, medium level is a combination of second quartile and third quartile, and high level is third quartile.

[2] Risk categories based on neural tube defect risk at particular RBC folate concentrations. (Ref. Tinker et.al.)

[3] NHANES method–derived RBC folate concentration [= (Molloy method RBC folate concentration x 0.7876) + 34.2802] (Ref. Crider et. al)

[4] Rao-Scott Chi-square *p* value for categorical variables and ttest score for continuous variables

Table 2: Descriptive analysis of obesity sub classes vs. normal weight women of non-pregnant reproductive age (20-44 years), NHANES cycles (2007-2010)

Characteristics	Normal weight	Class 1 Obesity[4] vs. Normal weight (BMI 30- 34 kg/m^2)			Class 2 Obesity[4] vs. Normal weight (BMI 35-39 kg/m^2)			Class 3 Obesity[4] vs. Normal weight (BMI ≥40 kg/m^2)		
	N (%)	N (%)	*P* value[5]	Prevalence Crude Odds Ratio (95% CI)	N (%)	*P* value[5]	Prevalence Crude Odds Ratio (95% CI)	N (%)	*P* value[5]	Prevalence Crude Odds Ratio (95% CI)
Serum folate concentration [1]			0.2002			**0.0251**			**<.0001**	
Low (<3.19nmol/l)	2183(18.38)	1273(22.80)		1.15(0.72,1.81)	1140(31.58)		**1.77(1.01,3.09)**	1033(34.47)		**1.69(1.11,2.57)**
Medium (3.19-3.88 nmol/l)	5639 (47.70)	2683(51.61)		Ref	1648(46.31)		Ref	1327(52.97)		Ref
High (>3.88 nmol/l)	3460(33.92)	1239(25.59)		0.69(0.41,1.18)	612(22.11)		0.67(0.35,1.29)	320(12.55)		0.33(0.17,0.66)
RBC folate concentration[1]			0.1766			0.1719			0.3691	
Low (<6.63nmol/l)	2976(23.53)	1137(18.81)		0.81(0.59,1.13)	584(16.84)		0.72(0.42,1.23)	614(19.40)		0.84(0.57,1.25)
Medium (6.63-7.17 nmol/l)	5552(48.03)	2530(47.22)		Ref	1738(47.88)		Ref	1262(46.99)		Ref
High (> 7.17 nmol/l)	2754(28.44)	1528(33.96)		1.21(0.84,1.75)	1078(35.28)		1.24(0.76,2.04)	804(33.61)		1.21(0.73,1.99)
Serum folate (nmol/l) (mean+/-SE)	3.63(0.03)	3.51(0.04)	**0.027**	**0.64(0.44,0.94)**	3.48(0.06)	**0.0461**	**0.59(0.35,0.99)**	3.36(0.05)	**<.0001**	**0.36(0.25,0.51)**
RBC folate (nmol/l) (mean+/-SE)	6.93(0.02)	6.97(0.03)	0.3039	1.27(0.79,2.06)	7.02(0.04)	**0.0369**	**1.81(1.01,3.25)**	6.98(0.03)	0.1728	1.37(0.86,2.18)
RBC folate concentrations by NTD risk category[2]			0.4623			0.1034			0.6031	
High Risk (≤585 nmol/l)[3]	1001(10.34)	430(11.21)		1.03(0.56,1.90)	290(14.43)		1.31(0.77,2.21)	185(7.62)		0.69(0.34,1.44)
Elevated risk (586-747 nmol/l)[3]	1892(23.67)	676(19.21)		0.77(0.52,1.14)	293(14.99)		0.59(0.29,1.20)	374(22.53)		0.90(0.54,1.51)
Optimal Risk (748-1215 nmol/l)[3]	5124(66.00)	2277(69.58)		Ref	1507(70.57)		Ref	1155(69.85)		Ref
Age (years) (mean+/-SE)	31.67(0.56)	33.97(0.44)	**0.0039**	**1.05(1.02,1.08)**	33.65(0.65)	0.8276	**1.04(1.00,1.08)**	32.97(0.59)	0.123	1.03(0.99,1.06)
Race			**<.0001**			**<.0001**			**<.0001**	
Non-Hispanic White	5995(72.16)	1885(56.29)		Ref	1293(60.31)		Ref	1059(58.13)		Ref
Non-Hispanic Black	1192(6.24)	1068(16.23)		**3.34(2.24,4.98)**	646(14.41)		**2.76(1.64,4.67)**	914(25.21)		**5.02(3.09,8.16)**
Mexican -American	1772(6.89)	1339(13.67)		**2.54(1.59,4.07)**	951(15.16)		**2.63(1.64,4.23)**	397(8.20)		1.46(0.86,2.48)
Other	2323(14.7)	903(13.81)		1.20(0.69,2.11)	510(10.12)		0.82(0.43,1.59)	310(8.56)		0.72(0.32,1.63)
Education			**0.0009**			**<0.0001**			**0.00033**	
< 12th grade/ GED	1655(9.69)	1340(18.17)		1.49(0.97,2.31)	951(21.19)		**1.84(1.09,3.09)**	637(17.19)		1.25(0.64,2.16)
12th grade/GED	2158(16.09)	1017(20.20)		Ref	697(19.19)		Ref	610(22.79)		Ref
> 12th grade/GED	7467(74.23)	2838(61.63)		0.66(0.41,1.07)	1752(59.62)		0.67(0.38,1.19)	1433(60.01)		0.57(0.32,1.02)
Marital Status			0.5207			0.92			0.0640	
Married	5489(52.55)	2339(50.24)		1.09(0.89,1.47)	1734(52.96)		0.98(0.71,1.37)	1035(43.69)		1.43(0.97,2.11)
Single (divorced/separated/ Widowed/Single/ Living with partner)	5793(47.45)	2856(49.76)			1666(47.04)			1645(56.30)		

Employment			0.3225			0.3571			0.1596	
Employed	7570(69.88)	3319(66.26)		1.18(0.84,1.66)	1958(66.50)		1.17(0.83,1.65)	1547(62.42)		1.39(0.86,2.28)
Unemployed	3690(30.12)	1876(33.74)			1442(33.50)			1133(37.58)		
Household income			**<.0001**			**0.006**			**0.0008**	
<$24,999	2856(18.13)	1655(25.46)		1.17(0.79,1.74)	1108(24.36)		1.01(0.61,1.68)	1170(35.37)		**1.92(1.14,3.26)**
$25,000-$74,999	4395(31.06)	2533(54.94)		Ref	Ref		Ref	920(39.08)		Ref
$≥ 75,000	3674(43.32)	862(19.60)		**0.34(0.18,0.67)**	537(24.49)		**0.43(0.20,0.90)**	493(25.54)		0.58(0.27,1.25)
Average number of alcohol drinks/day in past 12 months. (mean+/-SE)	2.16(0.11)	2.08(0.13)	0.6411	0.97(0.86,1.10)	2.87(0.37)	0.1056	1.15(0.98,1.36)	2.35(0.34)	0.5765	1.05(0.89,1.22)
Smoking status			0.1837			0.6647			0.6176	
Everyday smoker	1687(42.79)	900(51.46)		1.59(0.90,2.78)	737(48.87)		1.28(0.79,2.05)	481(49.59)		1.39(0.65,2.96)
Someday smoker	415(10.82)	253(13.36)		1.63(0.71,3.75)	154(9.74)		1.01(0.37,2.78)	148(11.76)		1.30(0.42,4.03)
Not a smoker	1294(46.39)	621(35.18)		Ref	519(41.39)		Ref	239(38.66)		Ref
Cigarettes per day during past 30 days (mean+/-SE)	11.85(0.92)	12.05(1.59)	0.9031	1.00(0.97,1.03)	11.22(1.43)	0.7425	0.99(0.94,1.05)	16.01(2.37)	0.1381	1.04(0.99,1.08)
Diabetes			**0.0004**			**0.0006**			**<0.0001**	
Diabetic	109(0.89)	309(4.33)		**5.03(1.79,14.13)**	201(6.03)		**7.14(1.83,27.87)**	358(15.84)		**20.95(8.86,49.51)**
Non-Diabetic	11167(99.11)	4886(95.67)			3199(93.97)			2302(84.15)		
Currently taking Birth Control Pills			0.1508			0.2543			**0.0280**	
Yes	1038(35.84)	244(23.13)		0.54(0.21,1.4)	151(20.11)		0.45(0.09,2.04)	108(15.05)		0.32(0.11,0.92)
No	2706(64.15)	1303(76.87)			1034(79.89)			684(84.95)		
Perform moderate physical activity			0.0536			0.1895			**0.0432**	**1.43(1.00,2.05)**
Yes	4648(43.93)	1807(37.14)		1.33(0.98,1.79)	1243(37.95)		1.28(0.86,1.98)	935(35.34)		
No	6634(56.07)	3388(62.86)			2157(62.05)			1745(64.66)		
Use of folic acid diet supplement			0.4042			0.6622			**<.0001**	**3.22(1.58,6.55)**
≥400 mcg	2066(20.67)	652(17.14)		1.26(0.72,2.22)	481(16.945)		1.28(0.69,2.36)	195(7.49)		
<400 mcg or non-users	9205(79.33)	4543(82.86)			2919(83.05)			2485(92.51)		
Diet supplement taken in past 30 days (mean+/-SE)	20.22(0.61)	18.82(1.01)	0.2273	0.99(0.97,1.01)	19.86(1.36)	0.8276	0.99(0.97,1.03)	17.55(2.46)	0.3006	0.98(0.94,1.02)
Length of diet supplement taken in days (mean+/-SE)	1246.67(154.71)	1375.08(133.81)	0.5280	1(1,1)	1401(375.99)	0.7004	1(1,1)	910.19(204.96)	**0.0435**	1(1,1)
Eat fortified cereal			0.3069			0.3909			0.1255	
Yes	381(3.68)	159(3.16)		1.17(0.85,1.62)	110(4.36)		0.84(0.55,1.28)	63(2.64)		1.41(0.88,2.26)
No	10027(96.32)	4632(96.84)			3014(95.64)			2410(97.36)		

[1] Serum folate concentration is calculated as quartiles, low level is first quartile, medium level is a combination of second quartile and third quartile, and high level is third quartile.
[2] Risk categories based on neural tube defect risk at particular RBC folate concentrations. (Ref. Tinker et.al.)
[3] NHANES method–derived RBC folate concentration [= (Molloy method RBC folate concentration x 0.7876) + 34.2802] (Ref. Crider et. al)
[4] Subclasses of obesity based on CDC definition.
[5] Rao-Scott Chi-square *p* value for categorical variables and ttest score for continuous variables

Table 3: Adjusted a priori multivariable analysis to study the association of folate insufficiency and obesity/ overweight vs. Normal weight non-pregnant reproductive age women (20-44 years), NHANES (2007-2010)

Characteristics	Overweight vs. Normal weight aPOR(95 % CI)[1]	Overall obese vs Normal weight aPOR(95 % CI)[1]
RBC folate concentrations by NTD risk category[2]		
High Risk (≤585 nmol/l)[3]	0.79(0.48,1.32)	0.79(0.50,1.23)
Elevated risk (586-747 nmol/l)[3]	0.76(0.55,1.05)	0.67(0.45,1.00)
Optimal Risk (748-1215 nmol/l)[3]	Ref	Ref
Race		
Non-Hispanic White	Ref	Ref
Non-Hispanic Black	**2.54(1.70,3.79)**	**3.66(2.47,5.42)**
Mexican -American	**1.77(1.18,2.68)**	**1.72(1.09,2.73)**
Other	1.09(0.66,1.80)	0.90(0.54,1.51)
Education		
< 12th grade/ GED	0.83(0.50,1.36)	1.07(0.60,1.91)
12th grade/GED	Ref	Ref
> 12th grade/GED	**0.51(0.32,0.80)**	**0.48(0.29,0.79)**
Age (years)	1.02(0.99,1.05)	1.03(0.99,1.07)

[1]Adjusted Prevalence Odds Ratio (aPOR) 95% Confidence interval (CI)

[2] Risk categories based on neural tube defect risk at particular RBC folate concentrations. (Ref. Tinker et.al.)

[3] NHANES method–derived RBC folate concentration [= (Molloy method RBC folate concentration x 0.7876) + 34.2802] (Ref. Crider et. al)

Table 4: Adjusted a priori multivariable analysis to study the association of folate insufficiency and subclasses of obesity vs. normal weight non-pregnant reproductive age women (20-44 years), NHANES (2007-2010)

	Class 1 obese vs. Normal weight aPOR(95 % CI)[1]	Class 2 obese vs. Normal weight aPOR(95 % CI)[1]	Class 3 obese vs. Normal weight aPOR(95 % CI)[1]
RBC folate concentrations by NTD risk category[2]			
High Risk (≤585 nmol/l)[3]	0.77(0.39,1.52)	1.00(0.53,1.90)	**0.46(0.24,0.89)**
Elevated risk (586-747 nmol/l)[3]	0.69(0.45,1.07)	0.58(0.26,1.26)	0.82(0.48,1.39)
Optimal Risk (748-1215 nmol/l)[3]	Ref	Ref	Ref
Race			
Non-Hispanic White	Ref	Ref	Ref
Non-Hispanic Black	**3.38(2.15,5.32)**	**2.76(1.26,6.03)**	**4.74(2.50,8.98)**
Mexican -American	**1.79(1.07,3.02)**	1.92(0.99,3.69)	1.43(0.75,2.73)
Other	1.17(0.59,2.31)	0.83(0.36,1.93)	0.54(0.23,1.27)
Education			
< 12th grade/ GED	1.04(0.58,1.84)	1.44(0.67,3.09)	0.75(0.39,1.45)
12th grade/GED	Ref	Ref	Ref
> 12th grade/GED	0.47(0.25,0.89)	0.58(0.31,1.08)	0.48(0.23,0.97)
Age (years)	**1.05(1.02,1.09)**	1.02(0.98,1.07)	1.00(0.96,1.05)

[1]Adjusted Prevalence Odds Ratio (aPOR) 95% Confidence interval (CI)
[2] Risk categories based on neural tube defect risk at particular RBC folate concentrations. (Ref. Tinker et.al.)
[3] NHANES method–derived RBC folate concentration [= (Molloy method RBC folate concentration x 0.7876) + 34.2802] (Ref. Crider et. al)