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Body mass index and serum folate in childbearing age women

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Abstract. *Background*: Higher pre-pregnancy body mass index (BMI) is associated with increased risk of neural tube defects (NTDs) and possibly other negative birth outcomes in the offspring. The mechanism for this association remains unknown. Lower maternal folate level has been implicated in the etiology of NTDs in general. The association of BMI with folate level, however, has not been investigated. *Methods*: The present study examines the association of BMI with folate level in childbearing age women before and after the 1998 U.S. folate fortification program of cereal products, using data from two cross-sectional surveys of the U.S. population, the third wave of the National Health and Nutrition Examination Survey (NHANES III; 1988–1994) and the more recent wave of this survey (NHANES 1999–2000). *Results*: After controlling for intake of folate in food and nutritional supplements, increased BMI in childbearing age women was associated with a lower serum folate level in both surveys ($p < 0.001$). Using data from NHANES 1999–2000, it was estimated that women in the 30.0+ kg/m^2 BMI category would need to take an additional 350 μg/day of folate to achieve the same serum folate level as women in the < 20.0 kg/m^2 category. *Conclusion*: Lower folate level may be one mechanism linking higher maternal BMI and increased risk of NTDs in the offspring. If corroborated in future studies, findings from this study suggest a need for a higher dose of folate supplement in heavier childbearing age women.

Key words: Body mass index, Folate, Neural tube defects, Obesity

Abbreviations: BMI = body mass index; NHANES = national health and nutrition examination survey; NTDs = neural tube defects; RBC = red blood cell

Introduction

There is growing evidence for the adverse impact of maternal obesity on the outcome of pregnancy. High pre-pregnancy body mass index (BMI) is consistently associated with increased risk of neural tube defects (NTDs) [1–6] and less consistently with other birth defects [1, 3, 7–9]. An intriguing association between maternal BMI and adulthood schizophrenia in the offspring was also noted in two large birth cohorts [10, 11]. The mechanism underlying these associations, however, remains unknown.

Since a lower maternal folate intake appears to be a strong risk factor for NTDs in a number of studies [12, 13], much attention has focused on folate intake among high BMI women with NTD-affected pregnancies [1, 2, 4, 5]. These studies did not find less frequent pre-conceptional use of multi-vitamins [4] or folate [1, 2, 5] among these women. As folate stored in tissues is directly related to folate intake, it is unlikely that the level of stored folate in these women would be lower than normal either. There are suggestions, however, that abnormalities in folate distribution or metabolism which make the stored folate in maternal tissues less available to the growing embryo may be the responsible mechanism in some cases of NTD-affected pregnancies [4, 6, 14]. Studies of women with a history of NTD-affected pregnancies have noted a decreased serum/red blood cell folate ratio [15, 16] and a slowed rise of serum folate level in response to folate intake [17]. As the folate in serum is the direct source of this micronutrient to the growing embryo, variations in serum folate levels may have a profound effect on neural tube development in the first few weeks of gestation [17].

In view of the growing problem of obesity in the U.S. [18], better understanding of the association of BMI and folate distribution and metabolism has important public health implications. The Food and Nutrition Board of the Institute of Medicine has recommended that women who might become pregnant should take a daily dose of 400 μg of folate [19]. If high BMI does change folate availability, 400 μg/day might not provide the same level of protection against NTDs in heavier women as compared to less heavy women. Similar to women with previous NTD-affected pregnancies [20], women with higher BMI may require a higher dose of folate.

This study examines the association of BMI with serum folate in samples of childbearing age women drawn from two general population surveys: the third National Health and Nutrition Examination Survey (NHANES III) [21] and the National Health and Nutrition Examination Survey of 1999–2000 (NHANES 1999–2000) [22]. The study tests the hypothesis that higher BMI is associated with lower serum levels of folate after controlling for folate intake from food and supplements and for body's stores of folate (measured by red blood cell [RBC] folate levels). In addition, the study examines the association of BMI with the metabolic activity of folate by assessing the serum level of homocysteine – a substrate for methionine synthesis which requires 5-methyltetrahydrofolate for its metabolism. As the impact of folate deficiency on the development of the neural tube is limited to the first few weeks of gestation [12], examining childbearing age women may provide important information not readily available from samples of pregnant women.

Use of two population samples allows for replication of the findings and for examining the possible impact of the 1998 Food and Drug Administration's (FDA) initiative that mandated folate fortification of cereal grain products sold in the U.S. While the effect of this population intervention on serum folate levels of childbearing age women has been documented [23–25], the impact of BMI on serum folate levels before and after implementation of this policy has not been examined.

Methods

Surveys

Both NHANES III and NHANES 1999–2000 used a stratified, multistage probability sampling design to survey U.S. household civilian populations. NHANES III conducted interviews with a sample of 33,994 persons ages 2 months and older between October 1988 and October 1994 (for children, interviews were conducted with parents or guardians). Young children, older persons, African-Americans, and Mexican-Americans were oversampled. NHANES 1999–2000 conducted interviews with a sample of 9965 persons of all ages between March 1999 and December 2000. Low-income persons, adolescents (ages 12–19 years), persons 60+ years of age, African-Americans, Mexican-Americans and pregnant women were oversampled. Use of sampling weights make these survey samples representative of the U.S. population. Both surveys were approved by Institutional Review Boards and both obtained written consent from adult participants and from parents or guardians for participants younger than 18 years.

Samples for this study

Of the 33,994 participants in NHANES III, 5728 were non-pregnant women aged 17–49 (pregnancy status was ascertained by questioning the participant). The sample for this study was comprised of 5018 (87.6%) of these women for whom data on BMI and serum folate level were available. Of the 9965 persons interviewed in NHANES 1999–2000, 1572 were non-pregnant women aged 17–49. The sample for this study was comprised of 1351 (85.9%) of these women for whom data on both BMI and serum folate level were available.

Assessments

BMI was computed as weight in kilograms divided by the square of height in meters (kg/m^2). The weight and height measurements were part of a standardized physical examination. The procedures, standardization of measurements, the equipment and quality control procedures have been described elsewhere [26–29]. Following recommendations of the Institute of Medicine's Committee on Nutritional Status During Pregnancy and Lactation [30], BMI was categorized into four categories: < 20.0, 20.0–26.9, 27.0–29.9, and $30.0+$. Age and race/ethnicity were self-reported.

Information on nutrient intake in both NHANES III and NHANES 1999–2000 was based on a 24-hour dietary recall interview. The interview for NHANES 1999–2000 was administered using a computer assisted dietary interview system. The primary source of food composition data was the U.S. Department of Agriculture Survey Nutrient Database [21, 31].

Use of dietary supplement was ascertained by one question that inquired if the participant had used or taken any vitamins, minerals or other dietary supplements in the past month, including those prescribed by a health professional and supplements that do not require a prescription. In both NHANES III and NHANES 1999–2000 an attempt was made to identify the supplements by name and ascertain the amount used. NHANES III created a database containing the names of the individual supplements and their contents. For this study, the daily intake of folate through supplements was computed by merging this database with individualized data from participants who reported taking supplements. At the time of this report, data on the specific supplements and their contents from the NHANES 1999–2000 survey were not yet released.

The laboratory procedures for measurement of serum and RBC folate and homocysteine have been described in detail elsewhere [32, 33]. Specimens collected in the field were frozen and then shipped to the laboratory on dry ice by overnight mail. Once

received, they were stored at −20 °C until analyzed. Serum folate is fairly stable if stored at this temperature. Folate was measured by using the Bio-Rad Laboratories Quantaphase Folate/vitamin B12 radioassay kit. In NHANES III, total homocysteine was measured in the fasting state by using reverse-phase high-performance liquid chromatography and fluorescence detection. Whereas, in NHANES 1999–2000, fluorescence polarization immunoassay from Abbott Diagnostics was used. Method comparison studies have shown equivalence of the two methods.

Statistical analysis

Statistical analysis was conducted in three stages. First, the bivariate relationships between BMI on the one hand and age, race/ethnicity, folate from food, use of nutritional supplement, folate from supplements (for NHANES III), RBC folate, serum folate, and serum homocysteine, on the other hand, were explored. Trends along the BMI categories were assessed using linear regression for continuous variables and logistic regression for categorical variables in which BMI was entered as an ordinal independent variable. The significance test for trend, thus, was the statistical test for the regression coefficient associated with the BMI variable. Analyses were conducted separately in the NHANES III and NHANES 1999–2000 data. Direct comparisons across the two surveys is not feasible because they use different weighting methods (see below) and the data cannot be combined. However, it is possible to compare estimates from the two surveys by examining the 95, 99 and 99.9% confidence intervals (CI) for these estimates. Non-overlapping CI indicate statistically significant differences across the two surveys at $p < 0.05$, 0.01 or 0.001, respectively.

Second, the association of BMI with serum folate was assessed in multiple linear regression models in which log-transformed serum folate was the dependent variable of interest and BMI, the continuous independent variable of interest. The analyses controlled for the effect of age, race/ethnicity, folate intake from food, taking any nutritional supplement, folate from supplements (for NHANES III) and RBC folate. Log-transformation of the serum folate variable allows to examine the association of BMI with percentage change in folate level rather than with absolute change. These regression analyses were also conducted separately for NHANES III and NHANES 1999–2000.

Third, the additional amount of folate intake needed to compensate for the reduced serum levels among women with higher BMI was estimated. For this exercise, data linking folate intake and serum folate levels from 6 studies of childbearing age women compiled by Wald and colleagues [34] were used. These authors found that every 100 μg/day increase in folate intake is associated with about 1 ng/ml increase in serum folate across a broad range of doses and serum levels. The more recent NHANES 1999–2000 data were used to estimate how much more folate should be taken to compensate for the lower serum folate level in women with higher BMI.

The complex sampling design of both NHANES samples requires the use of weights and specific design elements to make the samples representative of the U.S. population and to derive correct standard errors for estimates. The two samples, however, require different procedure for such adjustment. NHANES III provides strata and primary sampling units that can be used to obtain appropriate standard errors by the Taylor linearization method. To reduce the risk to subjects of being identified, NHANES 1999–2000 only provides replicates that can be used in a balanced repeated replication or jackknife analysis. Thus, STATA 7 [35] was used for analyses of NHANES III and Wesvar 2 (Westat Inc., Rockville, MD) for analyses of NHANES 1999–2000. As described above, comparisons across the two surveys were conducted using confidence intervals obtained in separate analyses of the two datasets.

Results

The BMI of childbearing age women increased from NHANES III (Mean = 27.0, SD = 6.8) to NHANES 1999–2000 (Mean = 28.0, SD = 7.3) ($p < 0.001$, assessed by comparing CI). The bivariate associations of BMI with demographic characteristics, folate intake and folate levels are presented in Table 1 for NHANES III and Table 2 for NHANES 1999–2000. BMI was associated with age in both samples. Participants in the higher BMI categories were older than those in lower BMI categories. BMI was also associated with race/ethnicity in both samples. African-Americans and Mexican-Americans were over-represented in the higher BMI categories.

The intake of folate from food increased dramatically between NHANES III (Mean = 228.5 μg/day, SD = 179.4) and NHANES 1999–2000 (Mean-324.3 μg/day, SD = 193.6) ($p < 0.001$, assessed by comparing CI). Although folate intake from food increased in all BMI categories across the two waves of NHANES, participants in the higher BMI categories had lower intake of folate compared to participants in the lower BMI categories in both surveys.

Participants in the higher BMI categories were less likely to take nutritional supplements in both survey waves (Tables 1 and 2). As a result, their intake of folate from supplement was also lower than participants with lower BMI (data available only for NHANES III). Despite their lower intake of folate from food and supplements, in neither survey did participants with higher BMI have a lower RBC folate level, which is an indicator of body's folate stores. This finding is surprising since RBC folate levels were quite responsive to increase in folate intake from food between the two surveys. RBC folate increased dramatically between NHANES III

Table 1. Demographic characteristics, folate intake and folate and homocysteine levels in 5018 non-pregnant women aged 17–49 in NHANES III, categorized according to BMI

	BMI (kg/m^2)				Test for trend p
	< 20.0	20.0–26.9	27.0–29.9	30.0 +	
N (% of the total)	553 (11.0)	2362 (47.1)	695 (13.9)	1408 (28.1)	–
Age, % (95% CI)[a]					
17–24	37.0 (30.7, 43.8)	24.6 (21.8, 27.5)	14.1 (10.2, 19.1)	12.0 (9.8, 14.5)	< 0.001
25–32	28.0 (21.9, 34.9)	25.8 (22.7, 29.2)	19.5 (15.2, 24.5)	23.5 (19.5, 28.1)	0.14
33–40	24.2 (20.0, 29.0)	26.6 (23.4, 30.0)	33.2 (27.5, 39.5)	32.8 (28.4, 37.5)	0.001
41–49	10.9 (2.3, 16.0)	23.0 (20.1, 26.3)	33.3 (26.6, 40.7)	31.7 (28.0, 35.7)	< 0.001
Age, continuous, mean years (95% CI)	29.3 (28.4, 30.2)	32.4 (31.7, 33.1)	35.7 (34.5, 36.9)	35.5 (35.0, 36.1)	< 0.001
Race/Ethnicity, % (95% CI)[a]					
Non–Hispanic white	79.9 (73.8, 84.9)	75.6 (71.9, 79.0)	62.1 (55.5, 68.4)	63.4 (58.4, 68.1)	< 0.001
Non–Hispanic black	7.9 (6.4, 9.6)	10.2 (8.6, 12.0)	19.9 (17.3, 22.7)	18.9 (15.9, 22.3)	< 0.001
Mexican-American	3.1 (2.4, 3.9)	4.8 (3.9, 6.0)	9.0 (6.9, 11.7)	7.9 (6.3, 10.0)	< 0.001
Other	9.2 (5.1, 15.9)	9.4 (7.1, 12.4)	9.0 (4.8, 16.4)	9.8 (7.2, 13.2)	0.84
Folate from food, μg/day (95% CI)	240.1 (216.9, 263.2)	243.7 (228.1, 259.2)	218.0 (192.7, 243.3)	215.5 (203.2, 227.7)	0.004
Taking any supplement, % (95% CI)	49.5 (44.1, 54.8)	45.2 (41.8, 48.7)	38.0 (31.5, 44.9)	35.2 (31.2, 39.4)	< 0.001
Folate from supplement, μg/day (95% CI)	145.3 (103.9, 186.6)	146.0 (124.3, 167.8)	133.4 (50.6, 216.2)	103.6 (85.1, 122.0)	0.013
Red blood cell (RBC) folate, ng/ml (95% CI)	182.9 (173.3, 192.6)	184.8 (176.2, 193.3)	177.6 (165.4, 189.9)	176.5 (168.1, 184.9)	0.11
Serum folate, ng/ml (95% CI)	6.9 (6.1, 7.7)	6.6 (6.1, 7.0)	5.5 (4.9, 6.1)	5.0 (4.6, 5.3)	< 0.001
Serum/RBC folate ratio, % (95% CI)	3.7 (3.5, 4.0)	3.6 (3.4, 3.7)	3.1 (2.9, 3.3)	2.8 (2.7, 3.0)	< 0.001
Serum homocystein, μmol/l (95% CI)[b]	7.9 (7.1, 8.6)	8.1 (7.7, 8.5)	8.6 (9.4, 2.9)	8.5 (8.0, 9.0)	0.08

Note: CI stands for confidence intervals.
[a]Some percentages add up to more than 100% due to rounding error.
[b]Homocysteine testing was performed only in the second phase of NHANES III (1991–1994). The sample for this analysis was comprised of 2792 participants.

(Mean = 165.0 ng/ml, SD = 81.2) and NHANES 1999–2000 (Mean = 271.7 ng/ml, SD = 110.8) ($p < 0.001$, assessed by comparing CI).

Serum folate levels also increased dramatically from NHANES III (Mean = 5.5 ng/ml, SD = 4.5) to NHANES 1999–2000 (Mean = 13.5 ng/ml, SD = 7.6) ($p < 0.001$, assessed by comparing CI). Furthermore, serum folate levels showed a consistent association with BMI in both surveys. Participants in the higher BMI categories had lower serum folate levels. Similarly, serum/RBC folate ratios showed an increase from NHANES III to NHANES 1999–2000 and a decrease with increasing BMI in both surveys. The association of RBC and serum folate levels with BMI categories in the two surveys is also presented in Figure 1.

Serum homocysteine levels declined between NHANES III (Mean = 8.0 μmol/l, SD = 3.7) and NHANES 1999–2000 (Mean = 6.4 μmol/l, SD = 2.5) ($p < 0.001$, assessed by comparing CI). However, in neither survey was there an association between BMI and serum homocysteine level.

Results of the multiple regression analyses for the association of BMI and log-transformed serum folate level are presented in Table 3. BMI was entered as a continuous variable into these models. As the data presented in Table 3 indicate, the association of BMI and serum folate persisted even after controlling for age, race/ethnicity, folate intake and RBC folate levels. Each 10 kg/m^2 increase in BMI was associated with 15.6% reduction in serum folate in NHANES III and 11.3% reduction in NHANES 1999–2000. When the analyses were repeated with raw data on serum folate, each 10 kg/m^2 increase in BMI was associated with 0.92 ng/ml reduction in serum folate in NHANES III and 1.91 ng/ml reduction in serum folate in NHANES 1999–2000. Using these data and the

Table 2. Demographic characteristics, folate intake and folate and homocysteine levels in 1351 non-pregnant women aged 17–49 in NHANES 1999–2000, categorized according to BMI

	BMI (kg/m^2)				Test for trend p
	< 20.0	20.0–26.9	27.0–29.9	30.0 +	
N (% of the total)	114 (8.4)	621 (46.0)	180 (13.3)	436 (32.3)	–
Age, % (95%)[a]					
17–24	34.8 (21.7, 47.9)	27.8 (21.9, 33.6)	19.6 (12.4, 26.7)	13.4 (9.5, 17.2)	< 0.001
25–32	15.3 (7.3, 23.4)	22.0 (17.5, 26.6)	26.2 (15.6, 36.8)	22.2 (16.3, 28.1)	0.39
33–40	21.6 (9.3, 33.8)	26.6 (21.5, 31.7)	24.5 (15.7, 33.3)	30.7 (25.5, 35.9)	0.17
41–49	28.3 (16.1, 40.6)	23.6 (17.8, 29.5)	29.8 (20.2, 39.3)	33.7 (27.0, 40.5)	0.09
Age, continuous, mean years (95% CI)	31.8 (29.3, 34.4)	32.36 (31.0, 33.6)	34.5 (32.6, 36.3)	35.7 (34.6, 36.8)	< 0.001
Race/ethnicity, % (95% CI)[a]					
Non-Hispanic white	75.1 (64.9, 85.2)	69.4 (63.0, 75.8)	67.5 (57.6, 77.1)	58.4 (50.4, 66.4)	0.009
Non-Hispanic black	5.3 (2.0, 8.6)	8.9 (7.1, 10.7)	11.8 (7.8, 15.8)	20.0 (15.8, 24.2)	< 0.001
Mexican-American	3.5 (1.8, 5.2)	6.7 (5.4, 8.0)	9.3 (6.0, 12.7)	8.1 (6.5, 9.8)	0.03
Other	16.1 (6.8, 25.4)	15.0 (8.6, 21.4)	11.6 (3.5, 19.7)	13.4 (5.7, 21.2)	0.59
Folate from food, μg/day (95%CI)	360.8 (299.7, 421.9)	332.1 (301.5, 362.6)	314.4 (279.1, 349.8)	301.0 (277.3, 324.7)	0.02
Taking any supplement, % (95% CI)	49.9 (35.5, 64.2)	54.2 (48.4, 60.0)	48.4 (36.8, 60.0)	39.5 (34.5, 44.5)	0.006
Red blood cell (RBC) folate, ng/ml (95% CI)	262.1 (232.4, 291.8)	287.4 (270.0, 304.9)	291.5 (265.9, 317.1)	275.9 (261.1, 290.6)	0.95
Serum folate, ng/ml (95% CI)	14.6 (11.9, 17.3)	15.9 (14.7, 17.2)	14.8 (12.8, 16.8)	11.6 (11.0, 12.3)	< 0.001
Serum/RBC folate ratio, % (95% CI)	5.7 (5.0, 6.5)	5.7 (5.4, 6.0)	5.1 (4.5, 5.7)	4.4 (4.1, 4.6)	< 0.001
Serum homocystein, μmol/l (95% CI)	7.3 (6.2, 8.3)	6.6 (6.3, 6.8)	6.7 (6.1, 7.4)	6.8 (6.5, 7.1)	0.98

Note: CI stands for confidence intervals.
[a]Some percentages add up to more than 100% due to rounding error.

formula proposed by Wald and colleagues [34], to compensate for the reduction in serum folate level associated with a 10 kg/m^2 increase in BMI, an average woman in NHANES 1999–2000 sample would need to take an additional 190 μg/day of folate. Everything else being equal, to achieve the same serum folate level as women in the lowest BMI category (< 20.0 kg/m^2), women in the highest BMI category (30.0 + kg/m^2) would need to take an additional 350 μg/day of folate (mean BMI in the lowest category = 18.4 kg/m^2 and in the highest category = 36.4 kg/m^2).

To assess whether the observed relationship between BMI and serum folate is consistent across age groups, the regression analyses were repeated after stratifying the sample of reproductive age women in each survey into 3 age groups: < 20, 20–37 and > 37 years. These analyses controlled for the same variables included in the main analyses in Table 3. The regression coefficients for BMI in these further analyses were significantly different from 0 in the 20–37 year age group (NHANES III: $B = -0.152$, $SE = 0.025$, $p < 0.001$; NHANES 1999–2000: $B = -0.111$, $SE = 0.032$, $p = 0.001$) and the > 37 year age group (NHANES III: $B = -0.166$, $SE = 0.020$, $p < 0.001$; NHANES 1999–2000: $B = -0.126$, $SE = 0.040$, $p < 0.002$). While the coefficients in the < 20 years age groups were also negative, they were smaller than in the other age groups and did not reach a statistically significant level (NHANES III: $B = -0.077$, $SE = 0.050$, $p = 0.133$; NHANES 1999–2000: $B = -0.081$, $SE = 0.050$, $p = 0.113$).

Discussion

The results of this study support the hypothesis that higher BMI is associated with decreased serum folate levels. Women with higher BMI were less likely to use nutritional supplements and to receive folate through diet. However, even after controlling for these differences in folate intake, the association of higher BMI with lower serum folate persisted. This association was consistent across the two surveys despite the drastic increase in the serum levels in NHANES 1999–2000 following the FDA mandated folate

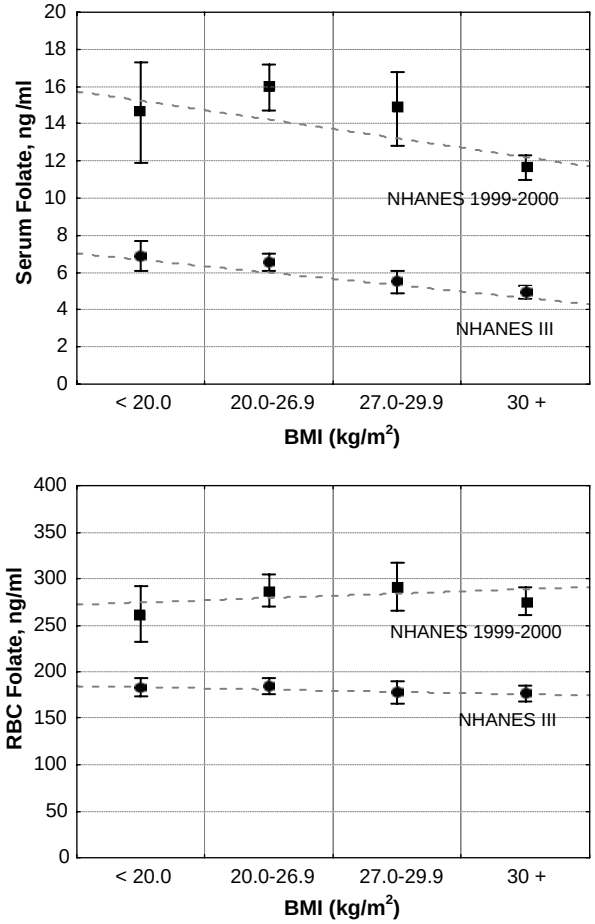

Figure 1. Association between BMI and serum and red blood cell (RBC) folate levels in non-pregnant women aged 17–49 in NHANES III and NHANES 1999–2000.

fortification. The results also suggest that the association of BMI and serum folate level may be stronger in women who are 20 years old or older. These findings tentatively suggest that the decrease in serum folate level may mediate the association of higher BMI and increased risk for NTD-affected pregnancies. If corroborated in future research, these findings extend past research that has mostly focused on the deficiency in stored (RBC) folate as a risk factor for NTDs; the findings suggest that factors involved in the release of folate into serum may also play a role.

In addition, the finding of responsiveness of serum folate levels to folate intake across the range of BMI suggests that by increasing the intake of folate in food or as dietary supplements, the lower serum levels in heavier women may be compensated. Heavier women may simply need to take a larger dose of folate to achieve the same serum folate level as their lighter counterparts. If so, the current recommendations for pre-conceptional folate supplement use may need to take the higher needs of heavier women into account. Based on the NHANES 1999–2000 data, it was estimated that women in the highest BMI category need to use an additional dose of approximately 350 μg/day to achieve the same serum level as their counterparts in the lowest BMI category.

However, drawing such policy implications from the data presented here is based on two assumptions. First, one has to assume that lower serum folate level is, in fact, the mechanism responsible for the observed association of BMI and the risk of NTD-affected pregnancies. While this study did show an association between BMI and serum folate levels, it did not examine the risk of NTD-affected pregnancies. Establishing the role of folate in the association of maternal BMI and NTD-affected pregnancies requires further studies.

A second assumption for deriving policy implications from these data is that the association of serum folate level and risk of NTD-affected pregnancies is present across the range of serum folate levels. Studies of the association of folate and the risk of NTD-affected pregnancies mostly predate the 1998 FDA folate fortification mandate. As the data presented here indicate, serum folate levels dramatically rose following the FDA mandate. Although the association of BMI with serum folate levels did not change following the increase in serum folate levels, the association of folate levels with the risk of NTD-affected pregnancies might have changed and predictions based on data from before 1998 may no longer apply. Furthermore, the optimal level of folate in the prevention of NTs is not known. Without data on the association of NTD-affected pregnancies and folate in more recent times and on the optimal level of folate for prevention of NTDs, caution is required in drawing policy implications from the data presented here.

The data presented here also pose new questions about the mechanisms for the association of BMI and serum folate levels. One candidate mechanism is hyperglycemia. Obesity is consistently associated with increased risk of non-insulin dependent diabetes mellitus and pre-diabetic hyperinsulinemia. Past research has found an association between congenital malformations and diabetes [36–38] as well as non-diabetic hyperglycemia [39, 40]. A recent study in Mexican-American women found that hyperinsulinemia partly explained the effect of BMI on the risk of NTD-affected pregnancies [36]. Interestingly, a recent large case–control study of diabetic women found that pre-conceptional use of multivitamin supplements reduces the risk of birth defects, although the content of supplement was not known [41].

To assess the potential mediating effect of hyperinsulinemia or hyperglycemia in the relationship of BMI and folate levels in the present study, the regression analyses in Table 3 were repeated after including both insulin and glucose levels in the models. Even after adding these variables, however, the relationship of BMI and serum folate level remained statistically significant (NHANES III: $B = -0.142$, $SE = 0.020$, $p < 0.001$; NHANES 1999–2000: $B = -0.060$, $SE = 0.007$, $p < 0.001$).

Table 3. Multiple regression analyses of the association between BMI and log-transformed serum folate level in non-pregnant women aged 17–49 in NHANES III and NHANES 1999–2000

Variables	NHANES III		NHANES 1999–2000	
	Regression Coefficient (SE)	*p*	Regression Coefficient (SE)	*P*
BMI (per 10 kg/m^2 increase)	−0.153 (0.016)	< 0.001	−0.116 (0.025)	< 0.001
Age (per 10-year increase)	0.012 (0.013)	0.35	−0.022 (0.016)	0.20
Race/Ethnicity				
Non-Hispanic white	reference		reference	
Non-Hispanic black	0.052 (0.022)	0.03	0.023 (0.047)	0.63
Mexican-American	−0.025 (0.020)	0.22	−0.017 (0.035)	0.62
Other	0.006 (0.050)	0.90	0.037 (0.043)	0.39
Folate from food (per 100 μg/day increase)	0.020 (0.006)	0.001	0.045 (0.010)	< 0.001
Taking any supplement	0.154 (0.023)	< 0.001	0.196 (0.036)	< 0.001
Folate from supplement (per 100 μg/day increase)	0.020 (0.006)	0.001	–[a]	–
Red blood cell (RBC) folate, (per 100 ng/ml increase)	0.388 (0.029)	< 0.001	0.227 (0.018)	< 0.001

Note: SE stands for standard error.
[a]At the time of this report data on folate content of supplements for NHANES 1999–2000 were not released.

Similarly, after adding the variable of diagnosis of diabetes given by a physician, the relationship of BMI and serum folate level remained significant (NHANES III: $B = -0.156$, $SE = 0.017$, $p < 0.001$; NHANES 1999–2000: $B = -0.115$, $SE = 0.003$, $p < 0.001$). Thus, hyperglycemia and diabetes do not appear to fully explain the association of BMI and serum folate levels. Future studies need to examine other potential mediating mechanisms.

In summary, these data suggest that higher BMI in childbearing age women is associated with lower serum folate levels, which, in turn, may explain the higher rates of NTD-affected pregnancies in heavier women. While at present there is no sufficient data to justify any change in recommendations for folate supplement use in childbearing age women, the highly suggestive findings call for additional studies to investigate whether the decrease in serum folate level is responsible for the increased risk of NTD-affected pregnancies in heavier women. Studies are also needed to establish whether the association between folate and the risk of NTD-affected pregnancies persisted after the general rise in the serum folate levels in the U.S. following the 1998 FDA mandated folate fortification of cereals.

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