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Obesity Is Associated with Increased Red Blood Cell Folate Despite Lower Dietary Intakes and Serum Concentrations[1–4]

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Abstract

Background: Folates are essential cofactors in metabolic pathways that facilitate biological methylation and nucleotide synthesis, and therefore have widespread effects on health and diseases. Although obesity is prevalent worldwide, few studies have investigated how obesity interacts with folate status.

Objective: Based on data from the NHANES, this study aims to examine the association between body mass index (BMI) and obesity-related metabolic factors with blood folate status.

Methods: A nationally representative sample of 3767 adults from the NHANES (2003–2006) was used as the study population. Regression analyses, with and without adjustment for demographic factors and dietary intakes, were performed to examine associations between BMI and metabolic factors with serum and RBC folate.

Results: The results indicate serum folate concentrations were lower in obese groups compared to the desirable BMI and overweight categories, paralleling lower intakes in this group. In contrast, RBC folate increased incrementally with BMI. Regression analyses demonstrated an inverse relation between BMI and serum folate but a positive relation for RBC folate ($P < 0.01$). Waist circumference, serum triglycerides, and fasting plasma glucose each displayed significant positive relations with RBC folate ($P < 0.01$), although relations with serum folate were not significant and consistent.

Conclusions: In summary, obesity is associated with decreased serum folate, which parallels decreased folate intakes. In contrast, obesity is positively associated with RBC folate. Therefore, RBC folate, in addition to serum folate, should also be considered as a critical biomarker for folate status, especially in the obese population. Future research is needed to understand how obesity differentially alters serum and RBC folate status because they are associated with a variety of medical complications. *J Nutr* 2015;145:79–86.

Keywords: folate metabolism, NHANES, obesity, adults, B-vitamins, biomarkers, blood

Introduction

Folate metabolism plays a critical role in diverse metabolic pathways, including biological methylation and nucleotide synthesis, and thereby inadequacies of the vitamin are linked to a variety of disease states (1–5). It is important, therefore, to identify and define factors in the general population that effect the body's ability to sustain adequate folate status.

The classical manifestations of folate deficiency remain public health issues primarily in countries without folic acid fortification (6). Although the prevalence of folate deficiency (serum folate <10 nmol/L) was greatly reduced in the United States by the introduction of mandatory folic acid fortification in enriched grain products in 1998 (7), certain environmental and endogenous factors may interfere with the ability to sustain suitable folate status. For instance, both chronic alcoholism and tobacco smoking have been shown to interfere with folate metabolism in ways that lead to higher rates of folate deficiency (8–10). Similarly, individuals homozygous for the common C677T polymorphism in the gene encoding for the methylenetetrahydrofolate reductase

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[3] Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the USDA.
[4] Supplemental Tables 1–4 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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enzyme demonstrate lower RBC folate concentrations and are more vulnerable to elevated homocysteine concentrations (11).

Overweight and obesity have reached epidemic proportions in the United States (12, 13) and are important contributors to morbidity and mortality globally, both in developed and developing countries (14). Excess adiposity may have profound consequences for how one-carbon nutrients are metabolized. In mice, a high-fat diet that produced abdominal obesity resulted in lower concentrations of liver S-adenosylhomocysteine, an intermediate in one-carbon metabolism (15). Dahlhoff et al. (16) found a down-regulation of the trans-sulfuration pathway by diet-induced obesity and an increased variability in homocysteine concentrations in obese mice. Others found that obesity was associated with marked alterations of key metabolites and enzymes in hepatic one-carbon metabolism and related pathways (17). However, to date there is little epidemiologic research that has examined whether these mechanistic observations in animals have any relevance to the effects of obesity on folate status in humans. Recently, Bradbury et al. (18) reported a 1% decrease in serum folate concentration associated with every increase of 1 unit in BMI. Serum folate concentrations were also lower in subjects with higher waist circumferences and higher BMI in a study conducted in China; the authors speculated that this might be related to liver dysfunction (19).

In this study, we sought to define the relation between human obesity and folate status in a more incisive fashion than the prior studies in humans, and we chose to do so in a nationally representative database: the NHANES data from 2003–2006.

Methods

Study population. The NHANES is a nationally representative sample of the general noninstitutionalized population living in the United States. Health and nutritional status have been monitored via an intensive series of interviews, examinations, and laboratory measurements on a continuous basis since 1999. A unique feature of this survey is that the sampling approaches, interviews, and examination methods are standardized across the studied population. The description of basic sample design and exact methods have been described elsewhere (20). The data for this analysis come from 2 cycles of the surveys, 2003–2004 and 2005–2006 (21, 22). To exclude potentially confounding effects, children and adolescents were excluded, and only adults aged ≥19 y were examined. Pregnant women were also excluded from the dataset because BMI is not an accurate metric of obesity during pregnancy, and the metabolism of pregnant women is altered compared to the nonpregnant state. Dixon's Q test using a Q level of 0.1 was used to identify outliers in the vitamin status variables; values identified were marked as missing (23). From the total 2003–2006 dataset of 20,470 subjects, 6708 subjects were included in the fasting subsample. A total of 2341 pregnant women, children, and adolescents aged <19 y were excluded, as were 538 adults who were included in the fasting dataset because of their diabetic status but were nonfasting. Furthermore, because we were interested primarily in the effect of obesity compared with healthy weight individuals, 62 underweight adult subjects were excluded. These subjects are most likely underweight because of various medical complications, serious illness, abnormal metabolism, extreme dieting, etc. A total of 3767 adults were therefore included in the final dataset for analysis.

Serum and RBC folate status. Serum and RBC folate concentrations in the NHANES were measured with the Bio-Rad radioassay before 2006 and with the microbiologic assay using *Lactobacillus rhamnosus* (formerly known as *Lactobacillus casei*) for the period after 2006 (7). The data from the present study were all derived from 2003–2006 during which the data were entirely generated by Bio-Rad radioassay. Therefore, by choosing data from 2003–2006, we eliminated any concerns about mixing data from 2 different assay methods.

Dietary intake data for riboflavin (mg), folate [dietary folate equivalent (DFE)], and vitamins B-6 (mg) and B-12 (μg) that were used for adjustment in the dietary and full models to determine the impact of obesity on serum and RBC folate status were based on the mean of 2 dietary recall days to determine the usual intake. The data reflects vitamin intakes from the diet (including fortified foods) but not dietary supplements. Because subjects with a higher BMI are likely to have greater absolute intakes of nutrients, vitamin intakes were adjusted to a standard 2000-kcal diet. In instances where only 1 day's worth of data were available, a single determination was used.

Demographic variables. Demographic data, including age, gender, race/ethnicity, and poverty-income ratio (as a measure of socioeconomic status) were derived from the demographics dataset. Dietary supplement usage was provided by self-report in the 30-d dietary supplement use dataset. Alcohol use was calculated from reported alcoholic intakes by averaging alcohol intakes from the two 24-h dietary recalls in the dietary data, then categorized in the following groups: none, <1 drink/d, 1 to <2 drinks/d, and ≥2 drinks/d (24). One drink was equivalent to 14-g ethanol, as is standard in the United States. Serum cotinine concentrations in the laboratory dataset were used to classify smoking status: subjects were defined as nonsmokers if concentrations were <1 ng/dL, light smokers or heavy-passive smokers if concentrations were 1–10 ng/dL, and heavy smokers if serum cotinine concentrations were >10 ng/dL (25).

Metabolic characteristics. Measured (not self-reported) BMI was obtained from data in the examination dataset. Many overweight and obese individuals exhibit metabolic abnormalities, although some do not (26). Therefore, in addition to BMI, we also selected several additional metabolic factors (waist circumference, fasting plasma glucose, and TG concentrations) from the International Diabetes Federation (27) that might enable us to understand the relations between obesity, metabolic factors, and biomarkers of folate status. Waist circumference was obtained from data in the examination dataset. Fasting plasma glucose and TG concentrations were obtained from the laboratory dataset. The methods are described in more detail in the NHANES analytic notes (21, 22).

The method for TG analysis is based on a series of coupled reactions that hydrolyze TGs to glycerol with use of Hitachi 717 or Hitachi 912 (Roche Diagnostics). Fasting plasma glucose concentration used the hexokinase method, and the method for glucose determination changed between 2003 and 2004 (Roche Cobas Mira; Roche Diagnostics) and 2005 and 2006 (Roche/Hitachi 911; Roche Diagnostics); however, the difference between the samples was <2%, and therefore, no correction factor was applied. Because the continuous variables of vitamin status, BMI, TGs, and fasting plasma glucose were skewed, they were log-transformed to approximate a normal distribution for the linear regression analyses.

Statistical methods. Statistical analyses were performed with use of SAS version 9.3 (SAS Institute). Procedures SURVEYMEANS, SURVEYFREQ, CORR, and SURVEYREG were employed to calculate means, Rao-Scott chi-square tests, Pearson correlation coefficients, proportions, and to perform multiple linear regressions with use of the strata and cluster variables provided by the CDC within the dataset. The fasting sample weight, recalculated to reflect 4 years' worth of data, were used to reweight the results to be representative of the U.S. population.

For the regression analyses, potential confounding variables were divided into 2 blocks, similar to the method described by Sternberg et al. (24), containing respectively demographic factors and dietary vitamin intakes. Analysis of dietary vitamin intakes that appear in the tables and figures are all adjusted for energy intake because this is the convention (28), but we also performed sensitivity analyses with total dietary folate intake without adjustment for energy intakes. Because the SURVEYREG procedure does not have an appropriate model selection method, all factors were kept in the full model. Higher-order interactions were not included in the model. P values < 0.05 were considered significant. In addition to reporting β-coefficients and significance levels, both the outcome and predictor are log-transformed back, and the β-coefficients are explained as a percentage change in the predictor associated with a percentage change in the outcome.

Results

Demographic characteristics according to BMI category. A total of 3767 adults were included in the final dataset. Age, gender, and race/ethnicity categories are nationally representative. Alcohol use is likely to be an underestimate of normal drinking habits because a proportion of occasional drinkers would be classified as nondrinkers if, by chance, they did not consume alcohol on the two 24-h dietary recall days. **Table 1** describes the demographic data based on BMI categories. Although the prevalence of overweight and obesity was similar between women and men (66% vs. 69%), the proportion of obesity was 7% higher in women than men (37% vs. 30%). The older population (≥51 y of age) was more likely to be overweight and obese (73% compared with 63% for subjects 19–50 y of age). The proportion of obesity was higher for the non-Hispanic black population (43%) compared with the non-Hispanic white (30%) and Mexican American (33%) populations. Incidence of overweight and obesity was lower in the heavy smoking group (61%) compared to the light and nonsmoking groups (70%). Obese individuals (BMI ≥30) were less likely to use dietary vitamin supplements and less likely to drink alcohol compared with the combination of underweight, normal weight, and overweight groups. In the population using dietary supplements, obese individuals only accounted for 29.6% compared with 36.2% in the population not using supplements. In the population that never drank alcohol, obese individuals accounted for as much as 41.9% compared with only 21.6% to ~27.9% in populations that drank alcohol.

TABLE 1 Demographic variables by BMI category, NHANES 2003–2006[1]

	BMI (kg/m^2)			
	18.5 to <25	25 to <30	30–50	P^2
Total	1141 (32.3)	1268 (33.3)	1236 (32.7)	<0.001
Gender				<0.001
Female	566 (36.7)	515 (28.6)	662 (34.8)	
Male	575 (28.9)	753 (39.3)	574 (31.7)	
Age, y				<0.001
19–50	727 (36.1)	677 (32.7)	644 (31.1)	
≥51	414 (27.3)	591 (35.9)	592 (36.8)	
Race/ethnicity				<0.001
Non-Hispanic white	616 (33.7)	645 (34.3)	566 (32.0)	
Non-Hispanic black	215 (24.2)	230 (29.0)	347 (46.8)	
Mexican American	194 (25.5)	306 (40.6)	256 (33.9)	
Other Hispanic	35 (30.7)	43 (37.2)	33 (32.1)	
Other race	81 (50.5)	44 (27.1)	34 (22.4)	
Family Poverty Index ratio				0.022
Low <1.85	473 (36.0)	445 (29.2)	484 (34.8)	
Medium 1.85–3.49	265 (30.5)	341 (33.8)	339 (35.7)	
High ≥3.5	340 (31.9)	417 (36.9)	354 (31.3)	
Smoking status				<0.001
Nonsmoker	690 (30.0)	891 (35.7)	842 (34.3)	
Light smoker	60 (27.9)	68 (37.3)	67 (34.8)	
Heavy smoker	375 (39.5)	302 (29.8)	317 (30.7)	
Dietary supplement user				<0.001
Yes (last 30 d)	568 (34.1)	672 (35.8)	560 (30.1)	
No	571 (31.3)	596 (31.7)	674 (37.0)	
Alcohol use[3]				<0.001
None	304 (23.9)	445 (33.5)	555 (42.6)	
<1 drink/d	369 (37.3)	396 (34.3)	316 (28.4)	
1 to <2 drinks/d	78 (39.3)	82 (34.9)	66 (25.8)	
≥2 drinks/d	70 (41.8)	68 (36.1)	44 (22.1)	

[1] Values are *n* (%) and crude counts; percentiles are weighted to the U.S. population using fasting mobile examination center subsample weights. Totals do not necessarily equal the total study population size of 3767 because of missing values, which may differ for each measurement.
[2] *P* values are from the Rao-Scott chi-square test.
[3] 1 drink = 14 g ethanol.

Serum and RBC folate status among the 4 BMI categories. **Figure 1** describes how serum and RBC folate concentrations and folate intakes differ between BMI categories. Although the mean serum folate concentrations between normal weight and overweight groups are similar, serum folate concentrations decrease as BMI category increases from normal weight to obese. This relation is similar to the relation between BMI and the energy-adjusted dietary folate intake (μg DFE/2000 kcal, Figure 1A), or unadjusted total folate intake (μg DFE/d, data shown in **Table 2**). Correlation analysis indicated a statistically significant association between serum folate and folate intakes ($P < 0.05$) for both energy-adjusted dietary folate intake and unadjusted total folate intake, indicating serum folate is parallel with folate intakes. In contrast, regardless of a decrease in serum folate and folate intake, RBC folate increases continuously throughout the BMI categories (Figure 1B). The actual values of folate intakes, serum, and RBC folate are shown in Table 2.

Association between BMI and folate status. The results of the linear regression analyses between BMI and serum and RBC folate are displayed in **Table 3**. The regression analysis indicates an inverse relation between serum folate and BMI ($P < 0.05$) regardless of the adjustment for demographic variables, vitamin intakes, or both (crude model, model I, II, and III). Therefore, we demonstrate that serum folate is inversely associated with BMI among normal, overweight, and obese populations. In contrast, RBC folate has a significant ($P < 0.01$) and positive relation with BMI across all models (Table 3). Stratified results regarding the relations between BMI and blood folate status are shown in the online supplemental materials (**Supplemental Table 1**). Total dietary folate intake without adjustment for calorie intake was also calculated, and the values across the 3 BMI categories are presented in Table 2. Analysis using total dietary folate intakes without adjustment for energy intake did not change the associations between BMI and measures of folate status (data not shown).

Associations between metabolic factors and folate status. The results of the regression analyses between metabolic factors and serum and RBC folate are shown in **Table 4** (without adjustment for demographic factors and dietary vitamin intakes), and the data for adjusted models are shown in **Supplemental Tables 2–4**. The associations between waist circumference and serum and RBC folate agree closely with the relations that were observed with BMI. Thus, both BMI and waist circumference are predictors of systemic metrics of folate status. The relations between serum TGs and fasting plasma glucose and folate status were not significant for serum folate ($P > 0.05$), but a positive association was clear ($P < 0.01$) for RBC folate and higher concentrations of TGs and fasting plasma glucose (Table 4). Adjustment for demographic factors (model I), dietary vitamin intakes (model II), or their combination (model III) did not significantly alter the association patterns (Supplemental Tables 2–4). Thus, RBC folate is positively correlated with BMI, waist circumference, TGs, and fasting plasma glucose, but there is a significant inverse relation between serum folate only with the 2 direct measures of obesity, BMI, and waist circumference.

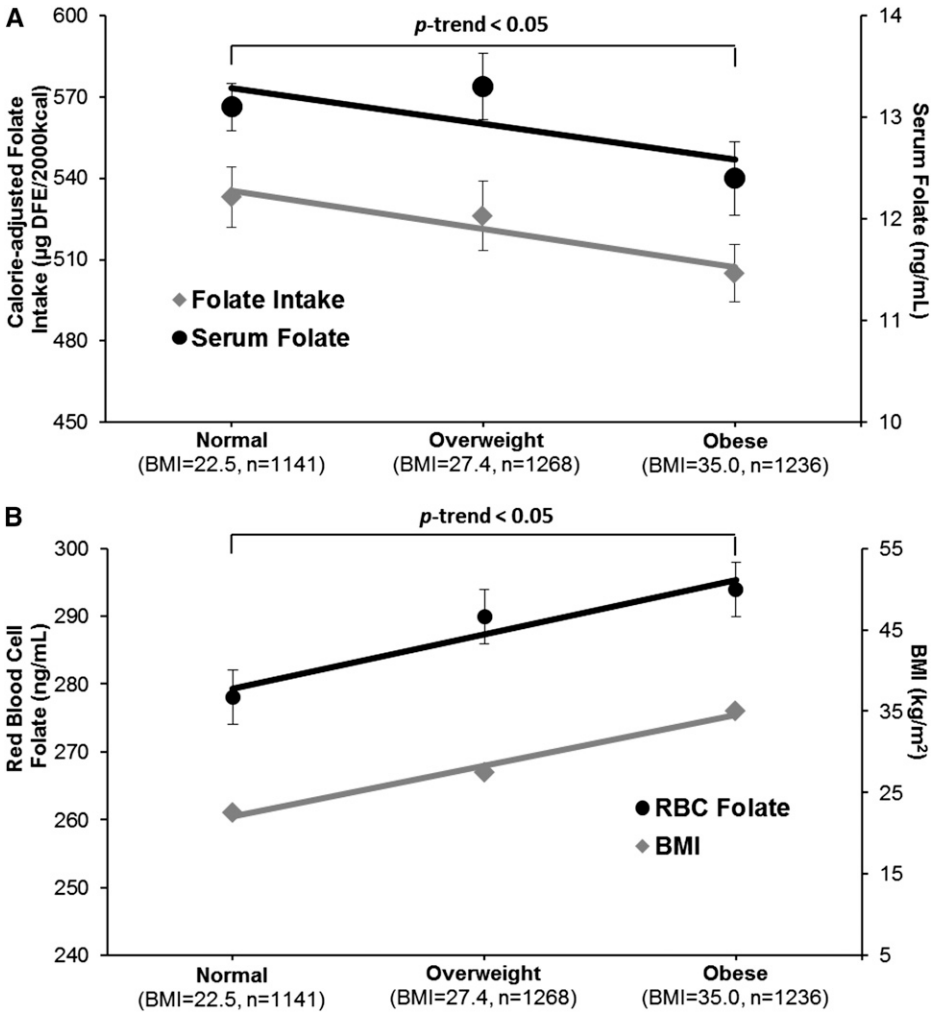

FIGURE 1 Dietary folate intake and serum and RBC folate according to BMI categories (normal weight, overweight, and obese) among U.S. adults >19 y of age in the NHANES 2003–2006. (A) Serum folate is associated with folate intake and both decrease as BMI increases from the healthy weight group to the obese group; (B) RBC folate increases as BMI increases from the healthy weight group to the obese group. Values are means ± SEs. DFE, dietary folate equivalent.

When the relations between BMI, waist circumference, serum TG, fasting plasma glucose, and blood folate status were stratified by age, the results show that both BMI and waist circumference have a statistically significant inverse association with serum folate and a positive association with RBC folate for young and middle-aged adults (19–50 y of age, $P < 0.001$), but not for older adults (≥51 y of age, $P > 0.05$) (**Table 5**). When the data were stratified by gender, it is noteworthy that the associations between BMI, waist circumference, and serum folate concentration lose significance in men ($P > 0.05$), whereas the positive relations with RBC folate remained significant ($P < 0.01$) (Table 5). For women, the significant relations remained for both serum and RBC folate. This observation recapitulates that RBC folate is a critical biomarker for folate status in obese individuals. The stratified data for TG and fasting plasma glucose, adjusted for demographic factors and/or dietary vitamin intakes, also show a significant pattern for RBC folate but not for serum folate (Supplemental Tables 3 and 4). For instance, a significant relation between TG concentration and RBC folate was found for all models with and without adjustment for demographic factors and dietary vitamin intakes, as well as for all subgroups of age and gender ($P < 0.05$), but only a few significant relations with serum folate were observed, and there

TABLE 2 Dietary folate intakes (total and calorie-adjusted), serum, and RBC folate across BMI categories among U.S. adults aged >19 y, NHANES 2003–2006[1]

Variable (unit)	Normal weight (18.5 ≤ BMI < 25)	Overweight (25 ≤ BMI < 30)	Obese (30 ≤ BMI ≤ 50)	*P*-trend[2]
n	1141	1268	1236	—
BMI	22.5 ± 0.06	27.4 ± 0.04	35.0 ± 0.13	<0.001
Total folate intake, μg DFE/d	559 ± 12.7	557 ± 14.5	517 ± 10.5	<0.001
Total folate intake, μg DFE/2000 kcal	533 ± 11.1	526 ± 12.8	505 ± 10.6	0.024
Serum folate, μg/L	13.1 ± 0.23	13.3 ± 0.33	12.4 ± 0.36	0.002
RBC folate, μg/L RBC	278 ± 4.0	290 ± 4.4	294 ± 3.8	<0.001

[1] Values are means ± SDs. DFE, dietary folate equivalent.
[2] *P*-trend values for folate intakes (total or calorie-adjusted), serum, and RBC folate are derived from the 2-sided t value between BMI and each factor.

TABLE 3 The relation between BMI and blood folate status in U.S. adults aged ≥19 y, NHANES 2003–2006

	Crude model[1]	Model I[2]	Model II[3]	Model III[4]
Serum folate β-coefficient[5]	−0.161 ± 0.047	−0.193 ± 0.061	−0.134 ± 0.048	−0.159 ± 0.060
% Change with 50% change in BMI[6]	−6.31	−7.49	−5.63	−6.24
P	0.002	0.004	0.009	0.013
RBC folate β-coefficient[5]	0.152 ± 0.031	0.157 ± 0.041	0.170 ± 0.032	0.179 ± 0.039
% Change with 50% change in BMI[6]	6.36	6.57	7.14	7.53
P	<0.001	<0.001	<0.001	<0.001

[1] Crude model: Without adjustment.
[2] Model I: Adjusted for demographic factors: age (continuous), gender (M/F), ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other Hispanic, other, and mixed race), alcohol use (based on two 24-h dietary recall days: none, <1 drink/d, 1 to <2 drinks/d, ≥2 drinks/d), family poverty-income ratio (<1.85, 1.85–3.5, >3.5), use of dietary supplements (last month: yes/no), and smoking (serum cotinine indicating: nonsmoker, light smoker, heavy smoker).
[3] Model II: Adjusted for intakes of riboflavin, folate, and vitamins B-6 and B-12; continuous data, all adjusted to a 2000-kcal diet and log-transformed.
[4] Model III: Adjusted for both demographic factors and vitamin intakes per models I and II.
[5] Values are regression β-coefficients ± SEs, based on the whole studied population (*n* = 3767).
[6] Values are percentage changes of serum or RBC folate in association with a 50% BMI change, e.g., from BMI = 20 (normal) to BMI = 30 (obese).

were no trends with some positive and some inverse relations (Supplemental Table 3).

Considering the higher prevalence of overweight and obesity in Mexican Americans and the non-Hispanic black population compared with the non-Hispanic white population (Table 1), and neural tube defect rate disparities among different race/ethnicities, we tested whether race/ethnicity contributes to the associations between BMI and serum and RBC folate concentrations. The stratified analysis shows that the paradoxic effects exist in non-Hispanic whites ($P < 0.05$), RBC folate is significantly associated with BMI in non-Hispanic blacks, and nonsignificant trends in the same direction are seen for Mexican Americans and other Hispanics for which the sample size was considerably smaller (Supplemental Table 1).

Discussion

We have demonstrated—within the context of a large, nationally representative dataset—that BMI values exceeding the desirable range are positively associated with RBC folate concentrations, but inversely associated with serum folate concentrations. These associations remained robust even after adjusting for demographic confounding factors (model I), dietary one-carbon vitamin intakes (riboflavin, B-6, B-12, and folate; model II), or in combination (model III). These associations are only significant for adults aged 19–50 y, indicating aging is an effect modifier for these relations.

One of the most interesting observations of this study is the pronounced discrepancy in the relations between BMI and the 2 metrics of systemic folate status and serum and RBC folate. An inverse association between serum folate and BMI has been documented in several previous studies (29–32). For instance, in a feeding study of postmenopausal women, increased BMI, percentage body fat, and absolute amounts of central and peripheral fat were all significantly associated with decreased serum folate with 12% for overweight and 22% for obese individuals (31). Similarly, 3 studies based on NHANES data reported inverse associations between BMI and serum folate in premenopausal women or women of childbearing age (29, 30, 32). However, to our knowledge, studies looking into the relation to RBC folate are very limited. One study, which primarily investigated how obesity affects short-term folate pharmacokinetics, observed that RBC folate was higher in the obese group at baseline compared with the nonobese group, but this observation was based on only 32 women of childbearing age (33). Another study reported higher RBC folate concentrations in obese women of childbearing age, but the relation was limited to obese nonpregnant women without folic acid supplement use. Moreover, an incremental increase of RBC folate with BMI was not shown for this specific population; although high RBC folate was shown in the obese nonpregnant women, the overweight individuals, instead of having a high RBC folate concentration as we found, had a low concentration of RBC folate

TABLE 4 The relation between metabolic syndrome factors and blood folate status in U.S. adults aged ≥19 y, NHANES 2003–2006

	Metabolic syndrome factors		
	Waist circumference, cm	Serum TGs, mg/dL	Fasting plasma glucose, mg/dL
Serum folate β-coefficient[1]	−0.150 ± 0.067	0.011 ± 0.021	0.069 ± 0.044
% Change with 50% change in BMI[2]	−6.27	0.45	2.84
P	0.032	0.61	0.13
RBC folate β-coefficient[1]	0.252 ± 0.043	0.097 ± 0.015	0.169 ± 0.049
% Change with 50% change in BMI[2]	10.8	4.01	7.09
P	<0.001	<0.001	0.002

[1] Values are regression β-coefficients ± SEs from regression model without adjustment, based on the whole studied population (*n* = 3767).
[2] Values are percentage changes of serum or RBC folate in association with a 50% change in metabolic syndrome factors.

TABLE 5 The relation between BMI, waist circumference, and blood folate status in U.S. adults aged ≥19 y, stratified by age and gender, NHANES 2003–2006

	Blood status			
	Serum folate		RBC folate	
Factors	BMI, kg/m^2	Waist circumference, cm	BMI, kg/m^2	Waist circumference, cm
Age				
19–50 y (n = 2094)				
β-coefficient[1]	−0.246 ± 0.059	−0.356 ± 0.080	0.135 ± 0.039	0.167 ± 0.044
% Change with 50% change in BMI[2]	−10.5	−15.5	5.63	7.01
P	<0.001	<0.001	0.002	0.002
≥51 y (n = 1613)				
β-coefficient[1]	−0.127 ± 0.095	−0.228 ± 0.119	0.095 ± 0.070	0.108 ± 0.105
% Change with 50% change in BMI[2]	−5.28	−9.69	3.93	4.48
P	0.19	0.06	0.19	0.31
Gender				
Females (n = 1779)				
β-coefficient[1]	−0.213 ± 0.058	−0.217 ± 0.085	0.146 ± 0.042	0.245 ± 0.066
% Change with 50% change in BMI[2]	−9.02	−9.20	6.10	10.4
P	<0.001	0.016	0.003	<0.001
Males (n = 1928)				
β-coefficient[1]	−0.084 ± 0.072	0.104 ± 0.097	0.162 ± 0.053	0.381 ± 0.059
% Change with 50% change in BMI[2]	−3.46	4.31	6.79	16.7
P	0.25	0.29	0.004	<0.001

[1] Values are regression β-coefficients ± SEs from regression model without adjustment.
[2] Values are percentage changes of serum or RBC folate in association with a 50% change in metabolic syndrome factors.

compared with individuals of normal weight (32). In the present study, we examined the association between BMI and RBC folate in a large dataset for the general U.S. adult population, including both genders. In doing so, we demonstrated a clear, incremental, and positive association between BMI and RBC folate in this cohort, which is representative of the general population.

It is noteworthy that the association between BMI and RBC folate was considerably more robust than that for serum folate. Based on the crude model, in men, there are no significant associations between serum folate and BMI nor with the metabolic factors that were investigated (waist circumference, fasting serum TGs, and glucose), but in both men and women significantly positive associations are consistently present for RBC folate and BMI as well as those metabolic determinants. In particular, the serum TG concentration is associated with RBC folate, but not with serum folate (Supplemental Table 3). It is feasible that this observation may partly explain the inconsistent results of epidemiologic studies that have examined the association between folate and cancer with use of serum folate as a biomarker (34). Our analyses demonstrated that the associations between serum and RBC folate with obesity are different. Given the high prevalence of obesity, it will be important in future studies to determine how these 2 metrics accurately reflect intracellular folate status in tissues of interest. Indeed, because it is well known that folate status can differ widely between tissues (35), it is also feasible that obesity has different effects on intracellular folate metabolism in different tissues.

The understanding of the mechanism underlying these paradoxic effects of overweight and obesity on folate metabolism is limited. The low serum folate status associated with obesity may be due to a volumetric dilution of the blood in obese individuals and/or low folate intake in the obese population, as observed in this study. Another explanation may be that adiposity influences folate uptake by the intestinal epithelium: 2 studies indicate that obesity affects short-term folate pharmacokinetics with diminished uptake of orally administered folic acid (33, 36). In contrast, in obesity, low serum folate may stimulate folate uptake by red blood cells. In an animal model, dietary folate deficiency was demonstrated to lead to an increase in mRNA and protein concentrations of folate carriers, an increase in the activity of folate hydrolase, and thereby, a marked up-regulation in intestinal folate uptake, suggesting the possible involvement of a transcriptional regulatory mechanism (37). Another study on colon-derived Caco-2 cells demonstrated that folate deficiency leads to a significant up-regulation in folate uptake that is accompanied by a parallel increase in reduced folate carrier protein and mRNA concentrations (38). The up-regulation of transepithelial transport of folic acid in intestinal epithelial cells during folate deficiency provides a possible and speculative mechanism for obtaining higher cellular folate concentrations in RBCs when serum folate concentration is low in the obese condition. Considering the higher prevalence of overweight and obesity in Mexican Americans and non-Hispanic black individuals compared with non-Hispanic white individuals (Table 1), and neural tube defect rate disparities among different races/ethnicities (39), we tested whether race/ethnicity contributes to the differential associations between BMI and serum and RBC folate concentrations. The stratified analysis shows that the paradoxic effects exist in non-Hispanic white individuals and non-Hispanic black individuals. Mexican Americans did not show significant associations, but this was mainly due to a large variation within this group (Supplemental Table 1). Thus race/ethnicity is not a factor responsible for the paradoxic associations: a negative association between serum folate and BMI, but a positive association between RBC folate and BMI.

Another interesting observation is that associations between obesity and serum and RBC folate were only evident in younger adults aged 19–50 y but not in those ≥51 y of age. Because folate status has been linked to the risk of many cancers, including those of the breast (40–42), this age-related phenomenon may

help partially explain why obesity appears to protect against premenopausal breast cancer but enhances the risk of postmenopausal breast cancer (43–45), and the latter may be explained by other mechanisms (46). Nevertheless, this is just a speculative explanation, but it warrants further investigation.

The opposite associations between BMI, waist circumference, and metabolic determinants with serum and RBC folate are important observations for several reasons. First, obesity is associated with numerous epigenetic changes (47). Folates are essential enzymatic cofactors in one-carbon metabolic pathways that are required for the provision of one-carbon moieties for epigenetic processes (48). This discovery provides a potential mechanism whereby obesity mediates changes in epigenetic markers. Second, serum folate is generally used in epidemiologic studies to define the association of folate status with diseases, giving inconsistent results (49, 50). The observation in this study that RBC folate is higher in obese individuals even though they have low serum folate concentrations and folate intake raises the question of whether serum folate is a suitable biomarker of folate status in obese individuals when we examine the associations between folate status and disease.

Although the results of this study are from a large, well-defined, and nationally representative dataset of U.S. adults, they are cross-sectional and represent measurements taken at a single point in time; therefore, we are not able to assess the temporality of the association. Another limitation is that the cross-sectional nature of the NHANES does not allow an examination of causal relations, and further longitudinal and clinical studies are needed to verify the findings. Thus, the underlying physiologic mechanisms responsible for the relations that we describe here cannot be delineated by our study but certainly are deserving of further research that could reveal these mechanisms.

Overweight and obesity have reached epidemic levels; even taking into consideration the recent leveling off in the trend, it is predicted that there will be a 33% increase in obesity prevalence and a 130% increase in severe obesity over the next 2 decades (13, 51). By using the nationally representative NHANES dataset, this study demonstrated a significant association between obesity and indicators of systemic folate status, particularly the unexpected and paradoxic associations between BMI and serum and RBC folate. Folates are critical nutrients that are linked to a variety of disease states. Future research is needed to understand how obesity differentially alters serum and RBC folate status. Investigations into folate metabolism in the obese population should continue to be extremely important for public health.

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