Folate status in the US population 20 y after the introduction of folic acid fortification

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ABSTRACT

Background: Enriched cereal-grain products have been fortified in the United States for >20 y to improve folate status in women of reproductive age and reduce the risk of folic acid–responsive neural tube birth defects (NTDs).

Objectives: Our objectives were to assess postfortification changes in folate status in the overall US population and in women aged 12–49 y and to characterize recent folate status by demographic group and use of folic acid–containing supplements.

Methods: We examined cross-sectional serum and RBC folate data from the NHANES 1999–2016.

Results: Serum folate geometric means increased from 2007–2010 to 2011–2016 in persons aged ≥1 y (38.7 compared with 40.6 nmol/L) and in women (35.3 compared with 37.0 nmol/L), whereas RBC folate showed no significant change. Younger age groups, men, and Hispanic persons showed increased serum and RBC folate concentrations, whereas non-Hispanic black persons and supplement nonusers showed increased serum folate concentrations. The folate insufficiency prevalence (RBC folate <748 nmol/L; NTD risk) in women decreased from 2007–2010 (23.2%) to 2011–2016 (18.6%) overall and in some subgroups (e.g., women aged 20–39 y, Hispanic and non-Hispanic black women, and supplement nonusers). After covariate adjustment, RBC folate was significantly lower in all age groups (by ∼10–20%) compared with persons aged ≥60 y and in Hispanic (by 8.2%), non-Hispanic Asian (by 12.1%), and non-Hispanic black (by 20.5%) compared with non-Hispanic white women (2011–2016). The 90th percentile for serum (∼70 nmol/L) and RBC (∼1800 nmol/L) folate in supplement nonusers aged ≥60 y was similar to the geometric mean in users (2011–2014).

Conclusions: Blood folate concentrations in the US population overall and in women have not decreased recently, and folate insufficiency rates are ∼20%. Continued monitoring of all age groups is advisable given the high folate status particularly in older supplement users. *Am J Clin Nutr* 2019;110:1088–1097.

Keywords: serum folate, RBC folate, women of reproductive age, folate deficiency, folate insufficiency, supplement use, race-ethnicity

Introduction

Since 1998, folic acid fortification of enriched cereal-grain products has been mandatory in the United States (1) and is estimated to provide a median usual intake of 115 μg of folic acid per day in women of reproductive age, contributing to a total folic acid intake of ∼240 μg/d from enriched cereal-grain products and ready-to-eat cereals (2). Overall, a 28% reduction in birth prevalence of anencephaly and spina bifida, the 2 most common neural tube defects (NTDs), was observed from pre- to postfortification using data from various participating programs; a greater reduction was found for programs with rather than without prenatal ascertainment (35% compared with 21%) (3). The postfortification prevalence of NTDs remained fairly stable during a 13-y period up to 2011; however, Hispanic compared with non-Hispanic black or white women showed a higher NTD prevalence (3). In 2016, the FDA approved voluntary corn masa flour fortification to help increase folic acid intake in the Hispanic population (4).

Folate status, monitored through NHANES, improved greatly from pre- (1988–1994) to early postfortification (1999–2004) (5, 6). A longer-term analysis capturing more than a decade of postfortification showed a small but significant linear trend of decreasing blood folate concentrations during 1999–2010

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Supplemental Methods, Supplemental Tables 1–5, and Supplemental Figure 1 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: MBA, microbiologic assay; MTHFR, 5,10-methylenetetrahydrofolate reductase; NTD, neural tube defect.

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(7). The prevalence of folate deficiency (risk of megaloblastic anemia) in the overall population aged ≥4 y was <1% for both serum and RBC folate during postfortification (1999–2010) (8). The prevalence of folate insufficiency (risk of NTDs) in women of reproductive age decreased from 59% prefortification (1988–1994) to 15% (1999–2006) and 23% (2007–2010) postfortification (8).

The goal of this study was to address some contemporary concerns regarding folate. Using the most recent NHANES data from 2011–2016, we assessed whether the decreasing trend in serum and RBC folate concentrations previously reported up to 2010 (7) continued in the overall US population and in women of reproductive age and also whether prevalence estimates of low folate concentrations changed during the postfortification period. We also provided serum and RBC folate reference data for major race-Hispanic origin groups, including new data for non-Hispanic Asians. Given that there are continued discussions regarding the safety of high folic acid intakes in older persons (9), we characterized folate status in persons aged ≥60 y by race-Hispanic origin and supplement use. The focus of this study is on newer data from 2007 to 2016 [generated with the microbiologic assay (MBA) and/or LC-MS/MS]. However, to provide context for nearly 2 decades of postfortification, we also included older radioassay data (1988–2006) after adjusting them to the MBA.

Methods

Participants and survey design

NHANES collects cross-sectional data on the health and nutritional status of the civilian noninstitutionalized US population. The National Center for Health Statistics conducts this survey, which uses a complex, stratified, multistage probability sample. Participants undergo a detailed in-home interview during which demographic characteristics, dietary supplement use, and health-related information are collected, followed by a visit to a Mobile Examination Center where a physical examination is performed and blood is collected. Prior to 1999, the survey was conducted periodically; 1988–1994 represented the last prefolic acid–fortification survey period, and folate status was assessed in persons aged ≥4 y. Since 1999, NHANES has been a continuous survey with data released in 2-y survey periods; 1999–2000 represented the first postfortification survey period. The NHANES sample design includes oversampling for particular population subgroups to increase the precision of estimates for these groups (**Supplemental Methods**). Folate status was assessed in persons aged ≥3 y during 1999–2002 and in persons aged ≥1 y during 2003–2016. All adult participants gave their informed consent. Parental consent was obtained from minors. The National Center for Health Statistics Research Ethics Review Board reviewed and approved the NHANES protocol. Interview and examination response rates for each survey period are publicly available on the NHANES website (10).

Laboratory methods and data adjustment

Different laboratory methods were utilized during various NHANES data collection periods to measure serum and whole-blood folate concentrations (**Supplemental Table 1**). Because the radioassay used during 1988–2006 produced much lower blood folate results than the MBA used during 2007–2010 (11, 12), assessment of long-term trends in folate status was only possible if radioassay data from 1988 to 2006 were converted to MBA-equivalent data (13, 14). The use of 5,10-methylenetetrahydrofolate reductase (*MTHFR*) genotype-specific regression equations would be preferable because the radioassay measured 31% lower than the MBA for whole-blood samples with T/T genotype but 48% lower for samples with C/C and C/T genotypes (14). However, *MTHFR* genotype information is currently not available for NHANES participants, so we used an "all genotype" equation (13, 15). During 2011–2016, LC-MS/MS was used to measure individual serum folate forms and to calculate serum total folate composed of biologically active folate forms. Excellent agreement between serum total folate measured by LC-MS/MS compared with MBA was demonstrated previously (16).

Study variables

For the postfortification analysis (1999–2016) by 2-y survey periods, we assessed folate status in persons aged ≥3 y. We categorized the overall participant data into 4 age groups (3–11 y, 12–19 y, 20–59 y, and ≥60 y) and by sex (male and female). We used the 3 main race-Hispanic origin categories (Mexican American, non-Hispanic black, and non-Hispanic white) that can be compared over the time period covered in this analysis, but we included other Hispanic persons and persons of other non-Hispanic races in overall estimates. Folic acid supplement use was dichotomized (yes/no) based on self-reported consumption of folic acid–containing supplements in the past 30 d (only available for NHANES 1999–2014). For analysis of recent years (between 2007 and 2016), we assessed folate status in persons aged ≥1 y by age group (1–5 y, 6–11 y, 12–19 y, 20–39 y, 40–59 y, and ≥60 y), sex (male and female), race-Hispanic origin [Hispanic (Mexican American + other Hispanic), non-Hispanic Asian (2011–2016 only), non-Hispanic black, and non-Hispanic white], and supplement use. We also assessed folate status in women of reproductive age (aged 12–49 y), for brevity referred to as women from here on, by age group (12–19 y, 20–39 y, and 40–49 y), race-Hispanic origin, and supplement use.

Statistical analysis

We followed a data analysis plan approved by all authors prior to conducting the statistical analysis using SAS for Windows software version 9.4 (SAS Institute) and SAS callable SUDAAN software version 11 (RTI) to account for the complex survey design. Taylor series linearization was used to calculate variance estimates. We used Mobile Examination Center survey weights to account for unequal probabilities of selection, adjustment for nonresponse, and poststratification to estimate various descriptive statistics. To provide context for the pre- and postfortification patterns, we included prefortification data from NHANES III (1988–1994) and the entire postfortification period (1999–2016) for persons aged ≥3 y (**Supplemental Figure 1**; for postfortification sample sizes by survey period and variable categories, see **Supplemental Table 2**). However, our main analysis focused on changes in the more recent postfortification period (2007–2016), both for persons aged ≥1 y and for women.

We used available-case analysis ("pairwise deletion") to handle missing or incomplete data (e.g., folic acid supplement use) and excluded pregnant and/or lactating women.

Both serum and RBC folate concentrations were right-skewed; thus, we used a log transformation to make the data more symmetric and facilitate interpretation and statistical inference through the use of geometric mean, and we evaluated selected percentiles. We used commonly accepted cutoff values of <7 nmol/L (serum folate) and <305 nmol/L (RBC folate) (17) to assess the prevalence of folate deficiency (risk of megaloblastic anemia) using MBA-equivalent data. A cutoff value of <748 nmol/L for RBC folate in women was used to assess the prevalence of folate insufficiency (risk of NTDs) (8, 18). There are no accepted cutoff values for high folate status. To evaluate the upper end of serum and RBC folate concentrations, we assessed the 90th percentile overall, by age group, and by supplement use during the postfortification period. For more details on cutoff values, see Supplemental Methods.

To evaluate whether the decreasing trend in blood folate concentrations previously observed up to 2010 (7) continued, we compared the geometric mean concentrations of serum and RBC folate as well as the prevalence of folate insufficiency (RBC folate <748 nmol/L) for NHANES 2007–2010 and 2011–2016 to obtain estimates with greater statistical reliability. The 2007–2016 data were generated by MBA and/or LC-MS/MS and hence required no data adjustment.

The statistical analysis in this study is primarily descriptive, with less emphasis on hypothesis testing; for this reason, we used no adjustment for multiple comparisons. In places where a statistical hypothesis test was conducted, such as a test for change in log-transformed concentrations or prevalence (Wald *F*-test), we defined significance as a 2-sided *P* value of ≤0.05. We used multiple linear regression (log-transformed concentrations) for 2 different purposes: *1*) to assess whether trends persisted either across the 1999–2016 survey cycles or between 2007–2010 and 2011–2016, and *2*) to examine demographic differences in the most recent 2011–2016 combined period. All regression models included age, sex (if appropriate), race-Hispanic origin, and folic acid–containing supplement use as covariates, and we reported adjusted *P* values from the Wald *F*-test as well as percentage changes in adjusted geometric mean concentrations.

Results

Long-term patterns in serum and RBC folate concentrations by survey period in persons aged ≥3 y

Median and 90th percentile concentrations of serum folate (**Figure 1**A) and RBC folate (Figure 1B) sharply increased from pre- to postfortification, followed by comparatively minor fluctuations during the postfortification survey periods (1999–2016).

The range of geometric mean serum folate concentrations across the postfortification cycles (1999–2016) was 38.0–45.7 nmol/L overall and 34.5–44.0, 30.5–38.6, and 40.5–47.9 nmol/L for Mexican-American, non-Hispanic black, and non-Hispanic white persons aged ≥3 y, respectively (**Supplemental Table 3**). We observed the highest serum folate concentrations in 1999–2000 for all 3 subgroups. Non-Hispanic black persons were the only race-Hispanic origin group that showed higher RBC folate concentrations in 2015–2016 compared with every previous cycle even after adjusting for age, sex, and supplement use (data not shown), although this difference was only statistically significant for 2001–2002, 2003–2004, 2009–2010, and 2011–2012.

When we stratified NHANES participants by use of folic acid–containing supplements, geometric mean serum folate concentrations in persons aged ≥3 y were 32.8–40.2 nmol/L (nonusers) and 51.3–61.2 nmol/L (users), whereas RBC folate concentrations were 943–1060 nmol/L (nonusers) and 1280–1490 nmol/L (users) (Supplemental Table 3). After adjusting for age, sex, race-Hispanic origin, and supplement use, serum folate concentrations in 1999–2000 were on average 8.6% (95% CI: 3.7%, 13.7%; $P_{adjusted} = 0.0005$) higher compared with concentrations in 2001–2002 (model data not shown). After that, concentrations remained relatively stable with smaller fluctuations, showing an average concentration of 34.8 nmol/L (95% CI: 34.3, 35.2 nmol/L; nonusers) and 53.3 nmol/L (95% CI: 52.5, 54.2 nmol/L; users) for 2001–2014, respectively (model data not shown).

During the course of the 9 postfortification survey periods, the 90th percentile of serum folate concentrations ranged from 70.2 to 82.7 nmol/L for persons aged ≥3 y, whereas RBC folate concentrations ranged from 1710 to 1890 nmol/L (**Supplemental Table 4**). Persons aged ≥60 y had the highest serum and RBC folate concentrations compared to other age groups (Supplemental Table 4). Users of folic acid–containing supplements showed noticeably higher serum and RBC folate concentrations at the 90th percentile compared with nonusers (Supplemental Table 4).

Changes in postfortification blood folate concentrations from 2007–2010 to 2011–2016 in persons aged ≥1 y and in women

Serum folate geometric mean concentrations increased significantly from 2007–2010 to 2011–2016 in persons aged ≥1 y (38.7 compared with 40.6 nmol/L; **Table 1**) and in women (35.3 compared with 37.0 nmol/L; **Table 2**), whereas RBC folate concentrations showed no significant change. Some subgroups (e.g., younger age groups, males, and Hispanic persons) showed increases in both biomarkers, whereas non-Hispanic black persons and nonusers of folic acid–containing supplements showed increases in serum folate only. In no instance did either biomarker show significantly lower concentrations in the most recent period. After adjusting for age, sex, race-Hispanic origin, and supplement use, serum folate concentrations in 2011–2014 were on average 7.0% (95% CI: 4.0%, 10.0%; $P_{adjusted} < 0.0001$) and 5.2% (95% CI: 1.6%, 9.0%; $P_{adjusted} = 0.0052$) higher compared with concentrations in 2007–2010 in persons aged ≥1 y and in women, respectively (model data not shown). Adjusted RBC folate concentrations were not significantly different between the 2 time periods ($P_{adjusted} = 0.09$ in persons aged ≥1 y and 0.33 in women; model data not shown).

Postfortification prevalence of low folate concentrations

The prevalence of folate deficiency (serum folate <7 nmol/L or RBC folate <305 nmol/L) representing risk of megaloblastic

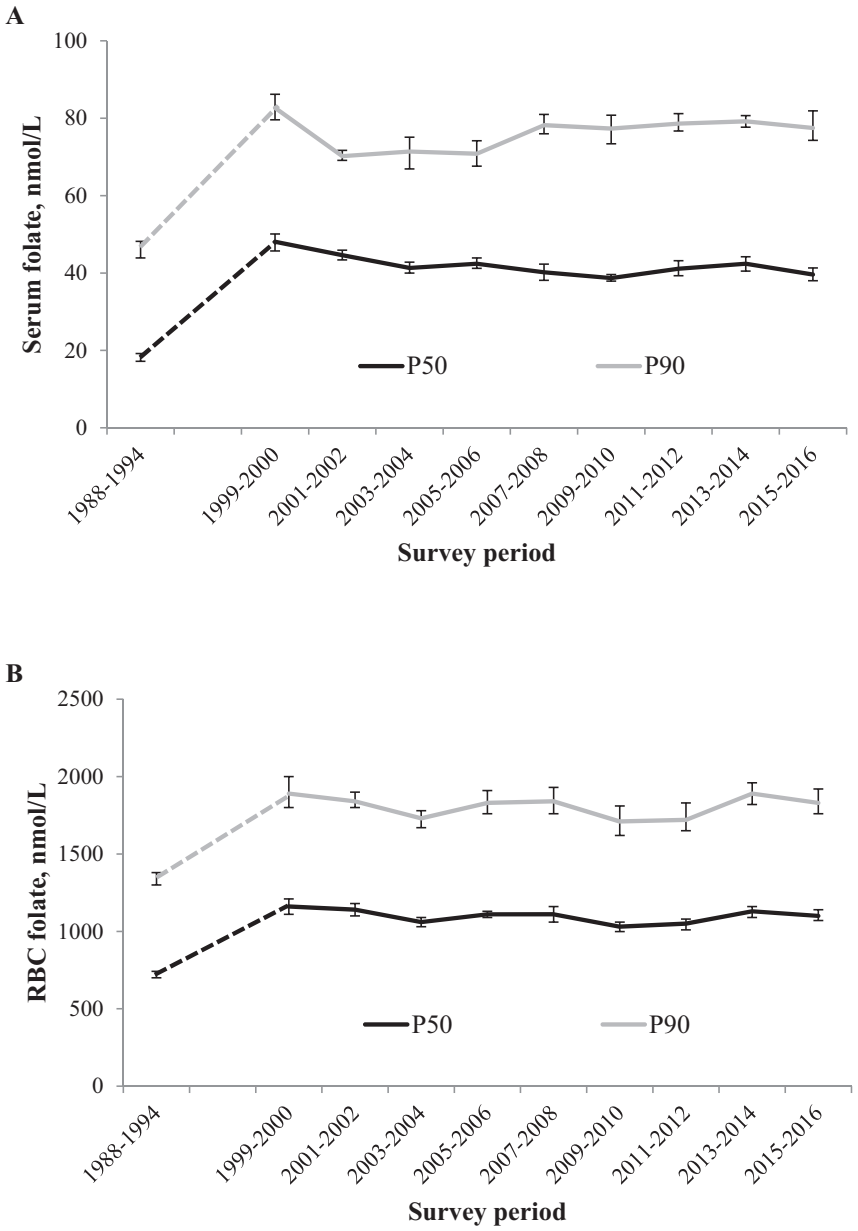

FIGURE 1 Pre- and postfortification median and 90th percentile concentrations of serum (A) and RBC (B) folate in persons aged ≥3 y by survey period, NHANES 1988–2016. Folic acid fortification became mandatory in 1998. Lines between 1988–1994 and 1999–2000 are dashed to indicate that there were several years between these 2 surveys. Error bars represent 95% CIs. For NHANES 1988–1994, biological samples were collected from persons aged ≥4 y only. The 1988–1994 and 1999–2006 radioassay data were adjusted (13) to make them comparable to the 2007–2016 RBC microbiologic assay, 2007–2010 serum microbiologic assay, and 2011–2016 serum LC-MS/MS data. For sample sizes (*n*), see Supplemental Table 2 for 1999–2016; sample sizes for 1988–1994 were 22,939 (serum folate) and 22,438 (RBC folate).

anemia was <1% in persons aged ≥3 y in each survey cycle during the period 1999–2016 (data not shown). The prevalence of folate insufficiency in women (RBC folate <748 nmol/L; NTD risk) ranged from 13.5% to 26.6% during the period 1999–2016, with similar prevalence estimates for Mexican-American (12.0–25.5%) and non-Hispanic white (9.46–21.3%) women but higher estimates for non-Hispanic black women (26.5–43.4%) (data not shown). Where data were available for Hispanic and non-Hispanic Asian women, the prevalence of folate insufficiency ranged from 14.6% to 25.2% (2007–2016) and from 18.7% to 23.4% (2011–2016), respectively (data not shown). The prevalence of folate insufficiency in women decreased from 2007–2010 (23.2%) to 2011–2016 (18.6%) overall and in some subgroups (e.g., 20- to 39-y-old women, Hispanic and

TABLE 1 Postfortification serum and RBC folate concentrations by demographic group in persons aged ≥1 y, NHANES 2007–2010 compared with 2011–2016[1]

Matrix	2007–2010		2011–2016		
Population	*n*	nmol/L	*n*	nmol/L	*P* value
Serum					
All	16,741	38.7 (37.7, 39.7)	23,684	40.6 (39.8, 41.4)	0.0039
Age group, y					
1–5	1606	52.0 (50.6, 53.5)	2118	58.3 (56.6, 60.1)	<0.0001
6–11	1995	55.4 (54.0, 56.9)	3022	57.5 (56.1, 58.9)	0.0468
12–19	2254	37.9 (36.5, 39.2)	3436	40.3 (39.1, 41.6)	0.0088
20–39	3448	31.2 (30.3, 32.2)	5017	34.2 (33.4, 34.9)	<0.0001
40–59	3636	36.2 (34.7, 37.8)	5145	37.2 (36.2, 38.3)	0.26
≥60	3802	48.1 (46.7, 49.6)	4946	47.3 (45.9, 48.7)	0.41
Sex					
Male	8451	36.3 (35.2, 37.4)	11,840	38.8 (37.9, 39.7)	0.0007
Female	8290	41.3 (40.3, 42.3)	11,844	42.5 (41.6, 43.5)	0.08
Race-Hispanic origin[2]					
Hispanic	5498	36.0 (35.0, 37.1)	6648	39.2 (38.3, 40.0)	<0.0001
Non-Hispanic Asian		NA	2599	40.2 (38.8, 41.7)	NA
Non-Hispanic black	3268	31.5 (30.7, 32.5)	5475	33.8 (33.0, 34.7)	0.0005
Non-Hispanic white	7100	40.9 (39.4, 42.5)	7948	42.5 (41.6, 43.4)	0.08
Supplement use[3]					
Yes	4403	53.4 (52.0, 54.7)	4079	54.6 (53.1, 56.2)	0.22
No	12,229	33.3 (32.5, 34.0)	11,543	36.4 (35.5, 37.2)	<0.0001
RBC[4]					
All	16,829	1070 (1050, 1100)	24,150	1110 (1090, 1130)	0.0536
Age group, y					
1–5	1648	1040 (1020, 1060)	2301	1120 (1090, 1140)	<0.0001
6–11	2018	1080 (1060, 1100)	3134	1120 (1090, 1140)	0.0283
12–19	2252	935 (904, 968)	3491	982 (961, 1000)	0.0183
20–39	3455	962 (940, 985)	5047	999 (974, 1020)	0.0294
40–59	3642	1090 (1060, 1140)	5187	1120 (1100, 1140)	0.29
≥60	3814	1330 (1300, 1370)	4990	1310 (1280, 1340)	0.32
Sex					
Male	8491	1050 (1020, 1080)	12,091	1090 (1070, 1110)	0.0435
Female	8338	1100 (1070, 1120)	12,059	1120 (1100, 1150)	0.10
Race–Hispanic origin[2]					
Hispanic	5526	969 (939, 1000)	6778	1010 (1000, 1030)	0.0098
Non-Hispanic Asian		NA	2673	1010 (981, 1030)	NA
Non-Hispanic black	3289	884 (864, 905)	5656	906 (887, 926)	0.12
Non-Hispanic white	7138	1150 (1110, 1190)	8006	1180 (1160, 1210)	0.10
Supplement use[3]					
Yes	4420	1330 (1290, 1370)	4189	1340 (1300, 1380)	0.66
No	12,299	970 (950, 991)	11,850	999 (977, 1020)	0.05

[1] Values are weighted geometric means (95% CI). The 2007–2010 serum and RBC folate data were generated by microbiologic assay. The 2011–2016 serum folate data were generated by LC-MS/MS, and the RBC folate data were generated by microbiologic assay. NA, not applicable because no data are available for non-Hispanic Asians prior to 2011.

[2] Persons of other non-Hispanic races are not shown but were included in overall estimates.

[3] Use of folic acid–containing dietary supplements. Data are limited to 2011–2014 because information on dietary supplement use is not available for 2015–2016.

[4] RBC folate estimates rounded to 3 significant figures.

non-Hispanic black women, and nonusers of folic acid–containing supplements), whereas it did not change significantly in other subgroups (**Table 3**).

Postfortification reference data for serum and RBC folate for 2011–2016 in persons aged ≥1 y, in women, and in older persons

After combining the serum folate data from the 3 most recent survey periods, we observed a U-shaped age pattern, higher concentrations in females compared with males, and lowest concentrations in non-Hispanic black persons compared with other race-Hispanic origin groups at the geometric mean and throughout the entire distribution (Table 1, **Supplemental Table 5**). For RBC folate, we also observed the same age and race-Hispanic origin patterns, but the sex differences were less pronounced. We quantified the magnitude of demographic differences relative to a reference category after covariate adjustment (**Table 4**). Females had 1.8% higher RBC folate concentrations and 6.9% higher serum folate concentrations

TABLE 2 Postfortification serum and RBC folate concentrations by demographic group in women aged 12–49 y, NHANES 2007–2010 compared with 2011–2016[1]

Matrix	2007–2010		2011–2016		
Population	*n*	nmol/L	*n*	nmol/L	*P* value
Serum					
All	3792	35.3 (34.2, 36.5)	5530	37.0 (36.2, 38.0)	0.0204
Age group, y					
12–19	1049	38.1 (36.5, 39.7)	1681	41.2 (39.8, 42.7)	0.0040
20–39	1728	34.0 (32.6, 35.4)	2442	36.0 (35.1, 37.0)	0.0174
40–49	1015	35.8 (33.8, 38.0)	1407	36.0 (34.7, 37.4)	0.85
Race-Hispanic origin[2]					
Hispanic	1297	34.0 (32.9, 35.1)	1602	37.1 (35.9, 38.3)	0.0002
Non-Hispanic Asian		NA	728	40.4 (38.7, 42.3)	NA
Non-Hispanic black	759	28.6 (27.4, 29.9)	1274	30.6 (29.4, 31.9)	0.0231
Non-Hispanic white	1524	37.4 (35.5, 39.4)	1692	38.2 (37.2, 39.3)	0.46
Supplement use[3]					
Yes	867	46.6 (44.3, 49.1)	897	46.1 (44.3, 47.9)	0.71
No	2904	31.6 (30.8, 32.4)	2781	34.3 (33.5, 35.3)	<0.0001
RBC[4]					
All	3800	995 (972, 1020)	5583	1020 (998, 1040)	0.13
Age group, y					
12–19	1047	937 (907, 968)	1710	978 (952, 1010)	0.0428
20–39	1735	983 (958, 1010)	2460	1010 (986, 1040)	0.11
40–49	1018	1060 (1010, 1110)	1413	1070 (1030, 1100)	0.80
Race–Hispanic origin[2]					
Hispanic	1301	956 (925, 987)	1613	1000 (979, 1020)	0.0176
Non-Hispanic Asian		NA	740	981 (953, 1010)	NA
Non-Hispanic black	758	843 (814, 873)	1298	872 (845, 900)	0.15
Non-Hispanic white	1529	1050 (1020, 1090)	1694	1070 (1040, 1100)	0.52
Supplement use[3]					
Yes	869	1210 (1170, 1250)	908	1170 (1130, 1210)	0.20
No	2909	920 (900, 941)	2820	947 (923, 971)	0.09

[1] Values are weighted geometric means (95% CI). The 2007–2010 serum and RBC folate data were generated by microbiologic assay. The 2011–2016 serum folate data were generated by LC-MS/MS, and the RBC folate data were generated by microbiologic assay. NA, not applicable because no data are available for non-Hispanic Asians prior to 2011.

[2] Persons of other non-Hispanic races are not shown but were included in overall estimates.

[3] Use of folic acid–containing dietary supplements. Data are limited to 2011–2014 because information on dietary supplement use is not yet available for 2015–2016.

[4] RBC folate estimates rounded to 3 significant figures.

compared with males after adjusting for age, race-Hispanic origin, and supplement use. Non-Hispanic Asian and Hispanic persons had similar serum folate concentrations compared with non-Hispanic white persons ($P_{adjusted}$ = 0.80 and 0.36, respectively), whereas non-Hispanic black persons had 17.3% lower concentrations after adjusting for age, sex, and supplement use. In contrast, adjusted RBC folate concentrations were significantly lower in all 3 race-Hispanic origin groups compared with concentrations in non-Hispanic white persons by 8.2% (Hispanics), 12.1% (non-Hispanic Asians), and 20.5% (non-Hispanic blacks).

In the combined 3 most recent survey periods, the central 95% reference intervals for serum and RBC folate were 14.0–107 and 505–2510 nmol/L in persons aged ≥1 y, respectively, and 14.2–87.7 and 466–2270 nmol/L in women, respectively (Supplemental Table 5). We observed large differences in the reference intervals by age group (e.g., serum folate for persons aged 1–5 y compared with persons aged 20–39 y was 24.6–129 nmol/L compared with 12.7–81.5 nmol/L) and by supplement use (e.g., serum folate for users compared with nonusers was 19.5–136 nmol/L compared with 13.6–89.4 nmol/L).

During 2011–2014, geometric mean serum folate concentrations in persons aged ≥60 y were nearly twice as high in supplement users compared with nonusers, whereas RBC folate concentrations were ∼50% higher (**Table 5**). In fact, the 90th percentile in nonusers was just slightly higher than the geometric mean in supplement users, both for serum and for RBC folate. The 90th percentile serum and RBC folate concentrations in supplement users were ∼100 nmol/L and between 2000 and ∼3000 nmol/L, respectively, depending on the race-Hispanic origin group.

Discussion

This study presents new information on postfortification concentrations of serum and RBC folate over nearly 2 decades (1999–2016) in a representative sample of the US population. The previously shown decline in blood folate concentrations

TABLE 3 Postfortification prevalence of folate insufficiency by demographic group in women 12–49 y, NHANES 2007–2010 compared with 2011–2016[1]

Matrix	2007–2010		2011–2016		
Population	*n*	%	*n*	%	*P* value
All	3800	23.2 (21.0, 25.4)	5583	18.6 (16.8, 20.6)	0.0028
Age group, y					
12–19	1047	23.9 (20.0, 28.3)	1710	19.2 (16.4, 22.2)	0.07
20–39	1735	24.2 (21.6, 27.0)	2460	19.0 (17.0, 21.1)	0.0029
40–49	1018	21.0 (16.8, 25.8)	1413	17.7 (14.8, 21.2)	0.24
Race–Hispanic origin[2]					
Hispanic	1301	22.7 (19.6, 26.2)	1613	17.0 (15.1, 18.8)	0.0017
Non-Hispanic Asian		NA	740	21.3 (18.5, 24.3)	NA
Non-Hispanic black	758	39.0 (36.0, 42.1)	1298	33.8 (30.7, 37.1)	0.0223
Non-Hispanic white	1529	18.8 (16.2, 21.8)	1694	15.6 (13.2, 18.2)	0.09
Supplement use[3]					
Yes	869	9.55 (7.42, 12.2)	908	9.69 (7.40, 12.6)	0.94
No	2909	28.8 (26.1, 31.6)	2820	23.8 (21.2, 26.5)	0.0089

[1] Values are weighted percentage RBC folate concentrations <748 nmol/L (95% CI). The RBC folate data were generated by microbiologic assay. NA, not applicable because no data are available for non-Hispanic Asians prior to 2011.

[2] Persons of other non-Hispanic races are not shown but were included in overall estimates.

[3] Use of folic acid–containing dietary supplements. Data are limited to 2011–2014 because information on dietary supplement use is not yet available for 2015–2016.

from 1999–2010 (6) did not continue into more recent years. Serum folate concentrations in the overall population and in women showed minor statistically significant increases from 2007–2010 to 2011–2016, whereas RBC folate concentrations trended in the same direction but were borderline nonsignificant (for both crude and covariate-adjusted estimates). Similarly, the prevalence of folate insufficiency decreased from 2007–2010 to 2011–2016 in all women and in subgroups of women who showed an increase in blood folate concentrations. Yet despite the decreases in insufficiency prevalence, disparities among race-Hispanic origin groups remain, with non-Hispanic black women having the highest prevalence (34% in 2011–2016). Still, this group generally had the lowest prevalence of NTDs during 1995–2011 compared with other race-Hispanic origin groups (3), which may be explained by the multifactorial etiology of NTDs, with factors such as vitamin B-12 status and genetic background playing additional roles (19). Compared with non-Hispanic whites, non-Hispanic blacks have better vitamin B-12 status (20, 21) and a lower population frequency of the *MTHFR C677T* polymorphism (22).

The prevalence of folate insufficiency in US women has been, on average, 18.6% (2011–2016). Unfortunately, there is a paucity of global data on folate insufficiency due to issues with noncomparability of assays and cutoff values used in national surveys (23). Nonetheless, we compared our NHANES estimate to data from 5 countries that used the MBA and corresponding cutoff values (<748 or <906 nmol/L for calibration with 5-methyltetrahydrofolate or folic acid, respectively). Two Central American countries with voluntary wheat flour fortification (1.8 mg folic acid/kg), Guatemala [47% (24)] and Belize [49% (25)], had lower insufficiency prevalence compared with Ireland [64% (26)] and New Zealand [73% (27)], where voluntary folic acid fortification is permitted. Of note, the 2009–2010 Guatemala National Micronutrient Survey reported a wide range of folate insufficiency—19% in the Metropolitan region (similar to the US data) and 81% in the Northern region—suggesting problems with limited access to fortified products (24). The United Kingdom has no folic acid fortification, and the UK National Diet and Nutrition Survey Rolling Programme Years 7 and 8 reported an insufficiency prevalence of 91% (28). This indicates that a mandatory and well-controlled folic acid fortification program can be effective in reducing the prevalence of folate insufficiency and keeping it at a fairly constant level of ∼20%.

Minor fluctuations in blood folate concentrations across survey periods are expected because the policies that regulate folic acid fortification are not the only factors associated with blood folate concentrations. In addition to folic acid intake from fortification and supplements, blood folate concentrations depend on folate intake from natural foods and genetic variation in the population. Shifts in eating habits [e.g., low-carbohydrate diets (29)] and demographic shifts with resulting changes in frequencies of the *MTHFR* variant [e.g., indigenous Mexican populations (30)] are some of the factors that may contribute to the variation and obscure underlying shifts. Consistent with our findings of small changes in blood folate concentrations since 2007, USDA statistics on wheat flour supply and disappearance showed similar market disappearance rates between 2004 and 2016 (range: 134.6–138.3 pounds/capita) (31). Similarly, USDA's "What We Eat in America" NHANES data tables on nutrient intakes from foods showed relatively stable mean folic acid intakes for persons aged ≥2 y by survey period during 2005–2012 (199, 193, 196, and 202 μg/d) followed by a slight decrease during 2013–2016 (186 and 180 μg/d) (32).

Nonusers of folic acid–containing supplements showed slight increases in serum (statistically significant) and RBC (borderline nonsignificant) folate concentrations from 2007–2010 to 2011–2014, whereas supplement users showed no change. Given the debate about high folic acid intakes in supplement users, this is a welcome finding and it confirms that these 2 groups should be reviewed separately when evaluating nutrition policies because they likely behave differently. In the most recent 2011–2014 combined period, we observed 46.3% higher serum

TABLE 4 Estimated percentage difference in serum and RBC folate concentrations relative to a reference category in persons aged ≥1 y and women aged 12–49 y, NHANES 2011–2016[1]

Population subgroup	Serum folate, %	$P_{adjusted}$	RBC folate, %	$P_{adjusted}$
Persons aged ≥1 y				
Age group, y				
1–5	29.4 (23.9, 35.1)	<0.0001	−10.5 (−13.8, −7.0)	<0.0001
6–11	28.4 (22.9, 34.2)	<0.0001	−10.1 (−13.4, −6.7)	<0.0001
12–19	−6.3 (−9.5, −2.9)	0.0004	−18.6 (−21.0, −16.1)	<0.0001
20–39	−23.1 (−25.8, −20.4)	<0.0001	−19.7 (−22.4, −17.0)	<0.0001
40–59	−19.2 (−22.1, −16.3)	<0.0001	−12.4 (−15.3, −9.4)	<0.0001
≥60	Reference		Reference	
Sex				
Female	6.9 (5.0, 8.9)	<0.0001	1.8 (0.6, 2.9)	0.0028
Male	Reference		Reference	
Race–Hispanic origin				
Hispanic	−1.5 (−4.7, 1.8)	0.36	−8.2 (−10.7, −5.7)	<0.0001
Non-Hispanic Asian	−0.4 (−3.7, 3.0)	0.80	−12.1 (−14.6, −9.5)	<0.0001
Non-Hispanic black	−17.3 (−19.9, −14.6)	<0.0001	−20.5 (−22.7, −18.3)	<0.0001
Non-Hispanic white	Reference		Reference	
Supplement use[2]				
Yes	46.3 (42.6, 50.3)	<0.0001	28.6 (25.9, 31.5)	<0.0001
No	Reference		Reference	
Women aged 12–49 y				
Age group, y				
12–19	23.6 (17.6, 30.0)	<0.0001	−4.0 (−7.8, 0.0)	0.0501
20–39	4.2 (−0.3, 8.8)	0.07	−2.4 (−6.3, 1.8)	0.26
40–49	Reference		Reference	
Race–Hispanic origin				
Hispanic	2.7 (−1.6, 7.3)	0.22	−3.3 (−7.4, 0.9)	0.12
Non-Hispanic Asian	9.2 (3.7, 15.0)	0.0011	−7.2 (−11.5, −2.7)	0.0026
Non-Hispanic black	−17.7 (−22.3, −12.9)	<0.0001	−18.4 (−22.3, −14.3)	<0.0001
Non-Hispanic white	Reference		Reference	
Supplement use[2]				
Yes	35.9 (30.8, 41.2)	<0.0001	21.6 (18.2, 25.0)	<0.0001
No	Reference		Reference	

[1] Values are percentage difference (95% CI) in weighted geometric mean concentrations derived from a multiple linear regression model (log-transformed concentrations) that adjusted for age, sex (if appropriate), race–Hispanic origin, and folic acid–containing supplement use. Wald *F*- test was used to determine the adjusted *P* value ($P_{adjusted}$). The sample size and R^2 for the 4 models were $n = 15{,}622$, $R^2 = 24.3\%$ (serum folate in persons aged ≥1 y); $n = 16{,}039$, $R^2 = 20.0\%$ (RBC folate in persons aged ≥1 y); $n = 3678$, $R^2 = 12.5\%$ (serum folate in women aged 12–49 y); $n = 3728$, $R^2 = 9.6\%$ (serum folate in women aged 12–49 y). The 2011–2016 serum folate data were generated by LC-MS/MS, and the RBC folate data were generated by microbiologic assay.

[2] Use of folic acid–containing dietary supplements. Data are limited to 2011–2014 because information on dietary supplement use is not yet available for 2015–2016.

folate but only 28.6% higher RBC folate concentrations in supplement users compared with nonusers after adjusting for covariates, exemplifying differences between these 2 biomarkers. In older persons, we observed even larger differences between supplement users and nonusers for both folate biomarkers, but the sex and race-Hispanic origin patterns were similar in supplement users and nonusers. The serum folate concentrations we observed at the 90th percentile in older persons (∼100 nmol/L) were comparable to the median (103 nmol/L) reported in older German adults after a 3-wk intervention with 5 mg folic acid/d in a country with no folic acid fortification but much lower than the 90th percentile (281 nmol/L) (33). Thus, in the presence of continuous folic acid intake from fortified foods and supplements, serum folate concentrations are much lower than those observed after short-term high-dose folic acid supplementation.

The new data for non-Hispanic Asian persons showed lower geometric mean serum folate concentrations compared with those of non-Hispanic white persons, but this difference disappeared after adjusting for covariates. Furthermore, at higher percentiles (e.g., 75th and 97.5th), non-Hispanic Asian persons had more similar serum folate concentrations compared with non-Hispanic white persons. Others have shown that supplement use in NHANES 2011–2014 was not statistically different between non-Hispanic Asians and non-Hispanic whites for older adults (aged ≥60 y: 67.7% and 72.5%, respectively) and for women (aged 20–44 y: 52.0% and 53.1%, respectively) (34, 35).

Our analysis is subject to commonly known limitations pertaining to cross-sectional study data. One caveat is that we adjusted the early postfortification radioassay data in order to assess long-term folate status. Using regression equations to adjust survey data has limitations, such as an underestimation of the SEs impacting statistical inferences and potentially biased reference intervals or prevalence estimates at a defined cutoff around the tails of the distribution (36). Such data adjustment should not be used to associate individual participant data with health outcomes because this would likely result in

TABLE 5 Postfortification serum and RBC folate concentrations by sex and race-Hispanic origin and stratified by supplement use in persons aged ≥60 y, NHANES 2011–2014[1]

Matrix	Supplement use[2]					
	Nonuser			User		
Population	*n*	Geometric mean, nmol/L	90th percentile, nmol/L	*n*	Geometric mean, nmol/L	90th percentile, nmol/L
Serum						
All	2077	37.4 (35.9, 38.9)	74.3 (70.5, 79.1)	1106	70.7 (67.5, 74.0)	117 (110, 124)
Sex						
Male	1066	35.6 (33.3, 37.9)	69.4 (62.3, 75.2)	493	64.7 (59.8, 70.1)	109 (100, 122)
Female	1011	39.1 (37.5, 40.8)	78.1 (73.3, 85.1)	613	75.4 (72.6, 78.3)	124 (116, 131)
Race–Hispanic origin[3]						
Hispanic	464	38.8 (36.3, 41.4)	68.8 (64.7, 72.9)	148	62.3 (58.7, 66.2)	97.7 (89.5, 114)
Non-Hispanic Asian	205	41.4 (37.8, 45.4)	75.9 (67.3, 96.6)	93	71.0 (67.3, 74.9)	107[4] (98.8, 121)
Non-Hispanic black	512	32.4 (31.2, 33.6)	64.3 (59.0, 67.3)	215	57.2 (53.0, 61.7)	103 (99.9, 115)
Non-Hispanic white	862	37.7 (35.8, 39.8)	76.9 (71.3, 85.1)	636	72.2 (68.3, 76.4)	120 (111, 126)
RBC[5]						
All	2110	1100 (1070, 1130)	1870 (1790, 1970)	1112	1680 (1590, 1760)	2750 (2570, 2920)
Sex						
Male	1083	1070 (1020, 1130)	1850 (1670, 1970)	494	1610 (1500, 1720)	2610 (2440, 2910)
Female	1027	1120 (1090, 1160)	1930 (1790, 2070)	618	1730 (1650, 1820)	2820 (2640, 2960)
Race–Hispanic origin [2]						
Hispanic	472	1020 (968, 1080)	1620 (1490, 1780)	151	1440 (1350, 1530)	2140 (2010, 2820)
Non-Hispanic Asian	210	1030 (959, 1110)	1670 (1530, 2030)	92	1590 (1510, 1680)	2240[4] (2080, 2710)
Non-Hispanic black	524	924 (887, 963)	1560 (1490, 1700)	220	1360 (1280, 1450)	2240 (2090, 2500)
Non-Hispanic white	869	1140 (1100, 1190)	1940 (1850, 2070)	634	1720 (1620, 1830)	2810 (2620, 2980)

[1]Values are weighted geometric means (95% CI) and 90th percentiles (95% CI). The 2011–2014 serum folate data were generated by LC-MS/MS, and the RBC folate data were generated by microbiologic assay.

[2]Use of folic acid–containing dietary supplements. Data are limited to 2011–2014 because information on dietary supplement use is not yet available for 2015–2016.

[3]Persons of other non-Hispanic races are not shown but were included in overall estimates.

[4]Estimates are subject to greater variability due to small cell size.

[5]RBC folate estimates rounded to 3 significant figures.

misclassification and biased or incorrect findings, particularly for RBC folate without adjustment for *MTHFR* genotype (37). Furthermore, other laboratories should not adjust their radioassay data using the reported regression equations because these equations describe the relation between the Bio-Rad radioassay and the CDC MBA (13).

This study has several strengths. We used originally measured blood folate data (2007–2016) to assess more recent changes in serum and RBC folate concentrations, and we based our interpretation of change on combined data from multiple cycles to improve the reliability of inferences. The availability of postfortification folate status data during 9 survey periods and the new information for non-Hispanic Asian persons during 3 survey periods provide unique and important data to US public health officials but also to countries that are considering folic acid fortification. Furthermore, the current data on folate status of Hispanic persons will serve as an important "baseline" for an impact assessment of corn masa flour fortification once new NHANES data become available.

In summary, the long-term monitoring of folate status in NHANES has provided critical data to policymakers and the research community. Although our results indicate that blood folate concentrations have not decreased in recent years and folate insufficiency rates appear to be stable at ∼20%, monitoring of folate status should be continued in all age groups, particularly given the high folate status in older supplement users.

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