

Trends in blood folate and vitamin B-12 concentrations in the United States, 1988–2004^{1–3}

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ABSTRACT

Background: Monitoring the folate status of US population groups over time has been a public health priority for the past 2 decades, and the focus has been enhanced since the implementation of a folic acid fortification program in the mid-1990s.

Objective: We aimed to determine how population concentrations of serum and red blood cell (RBC) folate and serum vitamin B-12 have changed over the past 2 decades.

Design: Measurement of blood indicators of folate and vitamin B-12 status was conducted in ≈23 000 participants in the prefortification third National Health and Nutrition Examination Survey (NHANES III; 1988–1994) and in ≈8000 participants in 3 postfortification NHANES periods (together covering 1999–2004).

Results: Serum and RBC folate concentrations increased substantially (by 119–161% and 44–64%, respectively) in each age group in the first postfortification survey period and then declined slightly (by 5–13% and 6–9%, respectively) in most age groups between the first and third postfortification survey periods. Serum vitamin B-12 concentrations did not change appreciably. Prevalence estimates of low serum and RBC folate concentrations declined in women of childbearing age from before to after fortification (from 21% to <1% and from 38% to 5%, respectively) but remained unchanged thereafter. Prevalence estimates of high serum folate concentrations increased in children and older persons from before to after fortification (from 5% to 42% and from 7% to 38%, respectively) but decreased later after fortification.

Conclusions: The decrease in folate concentrations observed longer after fortification is small compared with the increase soon after the introduction of fortification. The decrease is not at the low end of concentrations and therefore does not raise concerns about inadequate status. *Am J Clin Nutr* 2007;86:718–27.

KEY WORDS Nutrition survey, age, sex, race, ethnic groups, National Health and Nutrition Examination Survey, NHANES, fortification, neural tube defects

INTRODUCTION

Folate is essential for optimal growth, development, and health maintenance throughout all stages of life. Low serum concentrations of folate have been associated with atherosclerotic diseases (1), various cancers (2), psychiatric disorders (3), and cognitive impairment in the elderly (4, 5); a chronic deficiency of folate in the diet can cause anemia (2). Vitamin B-12 is an important cofactor in folate metabolism. Severe vitamin B-12

deficiency causes anemia; however, hematologic signs of deficiency are not always present, and hematologic and neurologic abnormalities are inversely correlated in vitamin B-12 deficiency (6). There is some concern that high folate intakes could mask vitamin B-12 deficiency, particularly in the elderly, who often have vitamin B-12 malabsorption (7). Monitoring the folate status of the US population over time has been a priority since the results for serum and red blood cell (RBC) folate concentrations from the second National Health and Nutrition Examination Survey (NHANES II; 1976–1980) suggested that the folate status of some population groups may be a public health concern (8, 9) and since data from the third NHANES (NHANES III; 1988–1994) confirmed these findings (10).

Subsequently, several initiatives to improve the folate status of the US population were undertaken because it was shown that, for women of childbearing age, dietary supplements containing folic acid reduced the risk that a fetus would be affected by a neural tube defect (NTD) (11). These initiatives included significantly increasing the folic acid content of the US food supply by the mandatory fortification of enriched cereal-grain products concurrent with the application of folate-related health and nutrient content claims on food and dietary supplement products (12–15). In 1999, NHANES became a continuous survey providing the opportunity to monitor blood folate and vitamin B-12 concentrations over consecutive 2-y survey periods to assess the net effect on folate status of changes in both folate composition of the food supply and changes in consumer food and supplement selection patterns. Since the introduction of fortification, several reports from regional (16, 17) and nationally representative (18–

¹ From the National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA (CMP, RBJ, and JDO); the National Center for Health Statistics, Hyattsville, MD (CLJ); the National Center for Birth Defects and Developmental Disabilities, Atlanta, GA (JM); the Food and Drug Administration, College Park, MD (JIR); and the Office of Dietary Supplements, National Institutes of Health, Bethesda, MD (EAY, MFP, and KDF).

² The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention or the Agency for Toxic Substances and Disease Registry.

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20) populations have shown that serum and RBC folate concentrations have increased in the general US population and in women of childbearing age (21) and that NTD rates have declined (22).

The focus of this report is to document trends in folate and vitamin B-12 status in the US population over a span of almost 2 decades that included a change in fortification practice. We extend the recent findings of Ganji and Kafai (20) by including a third postfortification survey period, which allows the examination of time trends. This report provides a singular source of comprehensive folate and vitamin B-12 data for multiple population strata over the extended period noted above. In addition, given that the same analytic technique was used throughout this period and that it will be changed in the near future because of the discontinuation of the Quantaphase II assay, the current time offers a unique opportunity for evaluating the data. Changes over time in blood concentrations of homocysteine and methylmalonic acid (MMA), which may be superior to serum vitamin B-12 as a marker of vitamin B-12 status, are not the subject of this report, because methodologic changes across survey periods preclude a direct comparison, and the 2003–2004 MMA data are not yet available.

SUBJECTS AND METHODS

Survey design and subjects

NHANES constitutes a series of nationally representative cross-sectional probability survey periods of the noninstitutionalized civilian population of the United States. Conducted by the National Center for Health Statistics (NCHS) at the Centers for Disease Control and Prevention, NHANES obtains a stratified, multistage probability sample designed to represent the US population on the basis of age, sex, and race-ethnicity. During each survey period, certain subpopulations are oversampled to allow for more precise estimates. The procedures for selecting participants and conducting interviews and examinations for NHANES 1999–2000, 2001–2002, and 2003–2004 were similar to those used for NHANES III (1988–1994) (23). Race-ethnicity categories [non-Hispanic white (NHW), non-Hispanic black (NHB), and Mexican American (MA)] are based on self-reported data (24). The biochemical indicators of B-vitamin status were measured for participants aged ≥ 4 y in 1988–1994 (for vitamin B-12 only in 1991–1994), ≥ 3 y in 1999–2000 and 2001–2002, and ≥ 1 y in 2003–2004.

All respondents gave written informed consent. The NHANES protocol for each survey period was reviewed and approved by the NCHS Institutional Review Board.

Laboratory methods

All analyses were conducted on samples of venous serum (folate for 1988–1994 and 1999–2004; vitamin B-12 for 1991–1994 and 1999–2004) or whole blood hemolysate (RBC folate for 1988–1994 and 1999–2004) that were frozen and shipped on dry ice to the laboratory conducting the B-vitamin analyses (25). A radioassay (Quantaphase I; BioRad, Hercules, CA) was used for the serum and RBC folate measurements in NHANES 1988–1991, and Quantaphase II was used in 1991–2004 for serum and RBC folate and for serum vitamin B-12 measurements (25). Appropriate adjustments have been made to NHANES 1988–1991 folate data before their public release to account for method

differences between the Quantaphase I and II and to make the data comparable to those from NHANES 1991–1994 (26). To ensure unbiased results over time, our laboratory used well-characterized quality-control pools to bridge the transition from old to new quality-control material, analyzed World Health Organization reference material when available (RBC folate and serum vitamin B-12), and continuously participated in proficiency testing programs of the College of American Pathologists. Long-term CVs for each 2-y postfortification period were 4–7% for serum folate at 2.30–13.2 ng/mL, 3–6% for serum vitamin B-12 at 381–1570 pg/mL, and 4–6% for RBC folate at 63.0–494 ng/mL.

Statistical analysis

Statistical analyses were performed by using SAS (version 9; SAS Institute Inc, Cary, NC) and SUDAAN (version 9; RTI, Research Triangle Park, NC) software. In each survey period, sample weights were used to account for differences in nonresponse or noncoverage and to adjust for planned oversampling of some groups. The 95% CIs for all survey periods were estimated with SUDAAN software by using Taylor series linearization, a method that incorporates the sample weights and accounts for the sample design. We used the following age breakdown: ages 4–11 y (children), 12–19 y (adolescents), 20–59 y (adults), and ≥ 60 y (older persons). We did not exclude any participants from our analyses. Sample sizes for the biochemical indicators of folate and vitamin B-12 status in each of the 4 survey periods are shown in **Table 1**. We evaluated the influence of fasting on serum folate concentrations during the prefortification survey period and found that length of fasting (<1 h to ≥ 12 h) had no appreciable influence on serum folate concentrations, regardless of the session (morning, afternoon, or evening) in which participants were examined. During the postfortification period, slightly higher serum folate concentrations were observed in the morning session participants who fasted <4 h; however, this was no longer true when data from all participants (regardless of the examination session) were compared for different durations of fasting. Approximately 60% of all participants fasted for ≥ 9 h before they were examined.

To illustrate changes in the distribution of folate concentrations over time, we generated frequency distributions for serum and RBC folate concentrations in the entire population for each survey period (**Figure 1**). Because the distributions of these biomarkers were skewed, medians or geometric means (log₁₀-transformed data) should be evaluated. We opted to present medians and 95% CIs by sex, race-ethnicity, and age group for the 4 survey periods because those measures describe the population better than do geometric means (**Table 2**). Prevalence estimates (%) and 95% CIs of subjects at risk of low or high concentrations of folate and vitamin B-12 were determined for specific groups of public health interest who are at risk of either low or high intakes, such as women of childbearing age (15–45 y old), children, and older persons (**Table 3**). Prevalence estimates of persons at risk of low or high concentrations of folate and vitamin B-12 by standard subgroups of sex, race-ethnicity, and age are shown elsewhere (*See Table S1 under “Supplemental data” in the current online issue at 222.ajcn.org.*). We used the following standard cutoffs: <3 ng/mL for low serum folate (27), <140 ng/mL for low RBC folate (27), and <200 pg/mL for low serum vitamin B-12 (28). We used a cutoff of >20 ng/mL for high serum folate. Although arbitrary, this concentration is the highest



TABLE 1

Sample sizes for biochemical folate and vitamin B-12 concentrations in children, adolescents, and adults examined during the National Health and Nutrition Examination Survey (NHANES) 1988–1994, 1999–2000, 2001–2002, and 2003–2004 by sex, race-ethnicity, and age group¹

Race-ethnicity	Sex	Age group y	Survey period			
			1988–1994 ²	1999–2000	2001–2002	2003–2004
Serum folate						
All	Both	All	23 361	7411	8242	7692
All	M	All	11 130	3613	3981	3793
All	F	All	12 231	3798	4261	3899
NHW	Both	All	8 534	2575	3575	3277
NHB	Both	All	6 813	1714	1994	2056
MA	Both	All	7 017	2525	2088	1887
All	Both	4–11	4 627	1131	1317	1147
All	Both	12–19	2 957	2124	2208	2058
All	Both	20–59	10 726	2669	3189	2831
All	Both	≥60	5 051	1487	1528	1656
Red blood cell folate						
All	Both	All	23 402	7491	8336	7700
All	M	All	11 144	3644	4019	3792
All	F	All	12 258	3847	4317	3908
NHW	Both	All	8 574	2600	3611	3261
NHB	Both	All	6 869	1738	2032	2086
MA	Both	All	6 956	2545	2096	1885
All	Both	4–11	4 660	1155	1348	1153
All	Both	12–19	2 955	2136	2226	2063
All	Both	20–59	10 720	2687	3217	2828
All	Both	≥60	5 067	1513	1545	1656
Serum vitamin B-12						
All	Both	All	11 860	7409	8245	7692
All	M	All	5 380	3611	3977	3793
All	F	All	6 480	3798	4268	3899
NHW	Both	All	3 989	2574	3579	3277
NHB	Both	All	3 903	1714	1991	2057
MA	Both	All	3 365	2525	2088	1886
All	Both	4–11	2 204	1131	1316	1147
All	Both	12–19	1 593	2123	2208	2059
All	Both	20–59	5 556	2668	3193	2831
All	Both	≥60	2 507	1487	1528	1655

¹ NHW, non-Hispanic white; NHB, non-Hispanic black; MA, Mexican American; All, racial-ethnic groups were not shown separately.

² In NHANES III, serum vitamin B-12 concentrations were measured only in persons aged ≥4 y for 1991–1994.

calibration point in the BioRad assays before samples need to be diluted with protein diluent. Coincidentally, this concentration is also close to the 95th percentile for the US population before fortification in 1988–1994—ie, 17.1 ng/mL (10). In addition, Lawrence et al (29) used this concentration when they evaluated the effect of fortification by using data from Kaiser Permanente.

To assess whether blood folate and vitamin B-12 concentrations (Table 2) and prevalence estimates (Table 3) changed between consecutive survey periods (ie, 1988–1994 compared with 1999–2000, 1999–2000 compared with 2001–2002, and 2001–2002 compared with 2003–2004), we first tested for sex × survey period, race × survey period, and age × survey period interactions by using an analysis of variance model that included age (4 age groups), sex (male or female), racial-ethnic group (NHW, NHB, MA, or other), and the abovementioned interaction terms. We used geometric means to test for significant differences in our subgroup analysis (Table 2) because no satisfactory parametric approach exists for a statistical analysis of

complex survey data that compares medians. We found a significant ($P < 0.05$) age × survey period interaction for serum and RBC folate and significant age × survey period and race × survey period interactions for serum vitamin B-12. Because of the age × survey period interaction, neither crude nor age-adjusted indexes can be used to summarize trends. We therefore restricted our time trend analysis (using a 2-tailed, 2-group *t* test) to age-specific subgroups (4–11, 12–19, 20–59, and ≥60 y old). To adjust for multiple comparisons, the *P* value of each comparison was considered significant if it was ≤0.017 (0.05 divided by 3, the total number of comparisons). This Bonferroni multiple comparison adjustment is known to be slightly conservative, but alternative procedures are not applicable to complex survey data. For subgroups that comprised the entire age range (all, males, females, NHW, NHB, and MA), we reported only medians (Table 2) or prevalence estimates (Table 3; also see Table S1 under “Supplemental data” in the current online issue at www.ajcn.org)

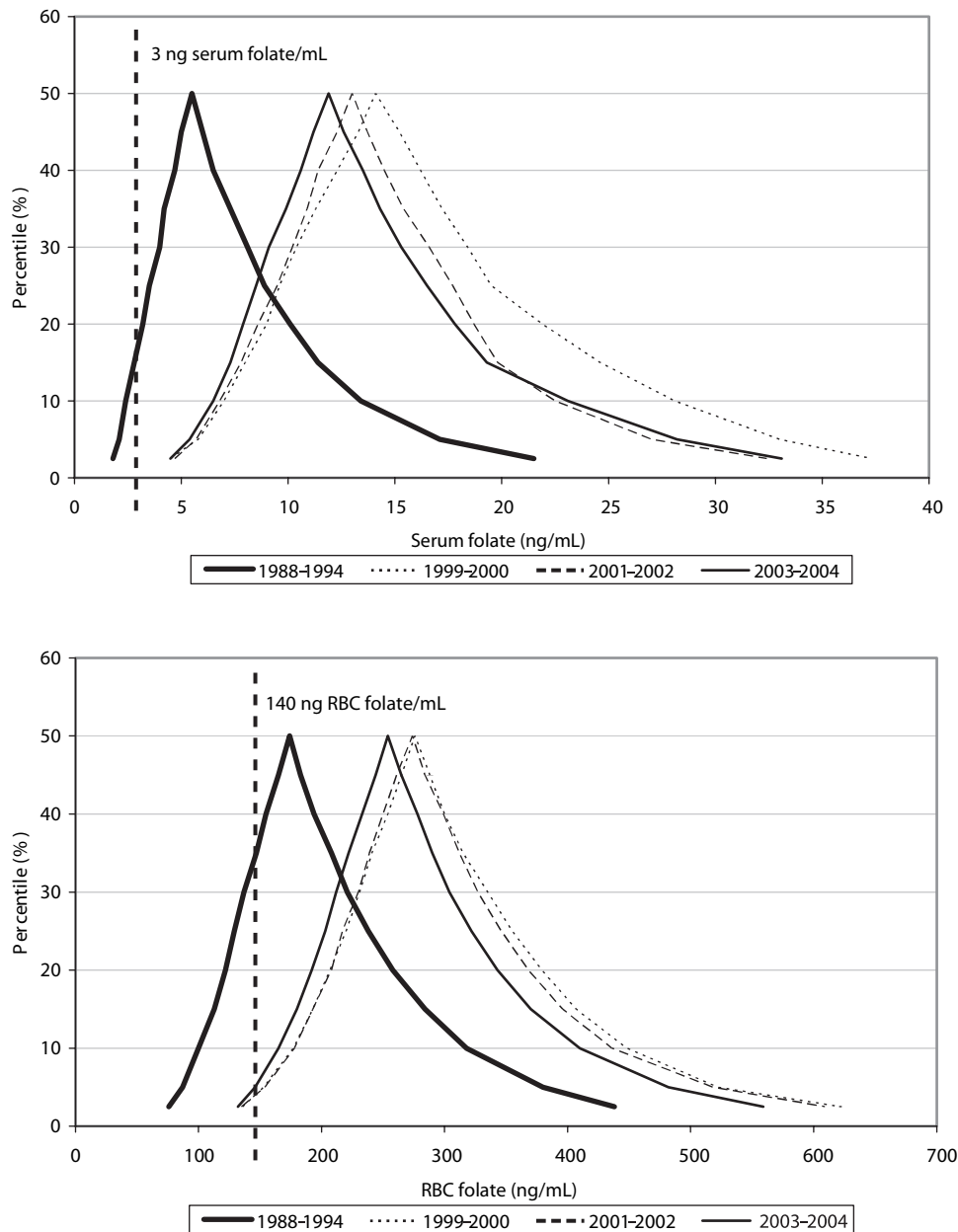


FIGURE 1. Frequency distribution of serum and red blood cell (RBC) folate among the entire population of the United States according to the National Health and Nutrition Examination Surveys spanning 1988–1994, 1999–2000, 2001–2002, and 2003–2004. The vertical dotted lines in the top and bottom panels indicate the cutoff for low values of serum folate and RBC folate, respectively.

without performing subgroup analysis to test for significant differences between survey periods.

We determined selected population percentile values (2.5th, 50th, and 97.5th) and their 95% CIs for serum and RBC folate and for serum vitamin B-12 from 6 y of data covering the postfortification period 1999–2004 and from either 3 y (for serum vitamin B-12) or 6 y (for serum and RBC folate) of data covering the prefortification period 1988–1994 (Table 4). We determined these values for the entire population and for population subgroups by sex, race-ethnicity, and age group. The outer tail percentiles are not necessarily comparable to clinical reference ranges, but they enable us to estimate circulating concentrations of folate and vitamin B-12 for 95% of the US population and to compare the US population with other populations. For selected

population percentile values for various age-sex and age-race-ethnicity combinations, see Table S2 under “Supplemental data” in the current online issue at www.ajcn.org.

RESULTS

Trends in circulating folate and vitamin B-12 concentrations, 1988–2004

Frequency distributions for serum and RBC folate for the entire population in each of the 4 survey periods illustrate how the distribution in blood concentrations changed (Figure 1). Whereas a remarkable upward shift occurred in the entire distribution of serum and RBC folate concentrations from before



TABLE 2

Trends in biochemical folate and vitamin B-12 concentrations in the US population by sex, race-ethnicity, and age group during the National Health and Nutrition Examination Survey (NHANES), 1988–2004¹

Race-ethnicity	Sex	Age group	Median (95% CIs)				p ²		
			1988–1994 ³	1999–2000	2001–2002	2003–2004	1988–1994 vs 1999–2000 ⁴	1999–2000 vs 2001–2002 ⁵	2001–2002 vs 2003–2004 ⁶
y									
Serum folate (ng/mL) ⁷									
All	Both	All	5.5 (5.2, 5.7)	14.1 (13.3, 14.9)	13.0 (12.5, 13.4)	11.9 (11.4, 12.3)	—	—	—
All	M	All	5.3 (5.0, 5.5)	13.3 (12.5, 14.2)	12.5 (12.0, 12.9)	11.5 (11.0, 11.9)	—	—	—
All	F	All	5.7 (5.5, 6.0)	14.7 (14.0, 15.9)	13.5 (13.1, 13.9)	12.5 (11.9, 12.9)	—	—	—
NHW	Both	All	5.8 (5.5, 6.1)	14.8 (13.8, 16.2)	13.5 (13.0, 14.0)	12.6 (12.0, 13.3)	—	—	—
NHB	Both	All	4.6 (4.4, 4.7)	11.6 (10.9, 12.3)	10.8 (10.1, 11.3)	10.1 (9.30, 10.8)	—	—	—
MA	Both	All	5.1 (4.7, 5.3)	13.4 (12.8, 13.9)	11.7 (10.9, 12.5)	11.1 (10.7, 11.6)	—	—	—
All	Both	4–11	8.8 (8.3, 9.3)	19.3 (18.5, 19.9)	17.0 (16.4, 17.7)	15.6 (15.0, 16.3)	<0.001	<0.001	0.01
All	Both	12–19	5.1 (4.9, 5.5)	13.3 (12.7, 14.0)	12.6 (11.9, 13.3)	11.0 (10.4, 11.4)	<0.001	0.014	0.002
All	Both	20–59	4.8 (4.5, 5.0)	12.5 (11.6, 13.6)	11.8 (11.2, 12.2)	11.0 (10.5, 11.3)	<0.001	NS	0.011
All	Both	≥60	6.9 (6.6, 7.3)	17.4 (16.4, 18.2)	16.6 (15.4, 17.5)	15.6 (14.8, 16.4)	<0.001	0.009	NS
Red blood cell folate (ng/mL)									
All	Both	All	174 (169, 180)	276 (266, 289)	274 (265, 282)	254 (246, 262)	—	—	—
All	M	All	172 (166, 176)	270 (258, 280)	265 (254, 276)	248 (239, 258)	—	—	—
All	F	All	177 (169, 184)	286 (272, 299)	281 (273, 291)	262 (251, 271)	—	—	—
NHW	Both	All	184 (177, 189)	293 (275, 308)	287 (282, 295)	268 (257, 279)	—	—	—
NHB	Both	All	135 (132, 139)	226 (219, 232)	214 (209, 220)	210 (205, 214)	—	—	—
MA	Both	All	164 (156, 170)	255 (250, 261)	252 (240, 264)	236 (230, 244)	—	—	—
All	Both	4–11	200 (190, 210)	287 (279, 292)	274 (263, 284)	256 (247, 266)	<0.001	NS	0.002
All	Both	12–19	150 (143, 156)	244 (237, 256)	237 (226, 248)	224 (215, 230)	<0.001	NS	0.003
All	Both	20–59	164 (160, 171)	269 (256, 285)	271 (260, 280)	248 (239, 258)	<0.001	NS	<0.001
All	Both	≥60	211 (204, 220)	344 (323, 363)	335 (325, 349)	321 (310, 337)	<0.001	NS	NS
Serum vitamin B-12 (pg/mL)									
All	Both	All	466 (453, 478)	482 (472, 492)	483 (472, 492)	483 (466, 499)	—	—	—
All	M	All	464 (449, 479)	484 (474, 497)	483 (467, 495)	483 (468, 497)	—	—	—
All	F	All	466 (449, 484)	476 (465, 493)	484 (473, 493)	484 (462, 504)	—	—	—
NHW	Both	All	444 (427, 460)	465 (452, 476)	471 (458, 485)	466 (447, 486)	—	—	—
NHB	Both	All	567 (553, 579)	578 (566, 597)	551 (535, 566)	545 (520, 581)	—	—	—
MA	Both	All	495 (471, 522)	525 (502, 539)	492 (465, 529)	517 (496, 535)	—	—	—
All	Both	4–11	667 (636, 703)	710 (677, 747)	714 (687, 745)	724 (708, 748)	NS	NS	NS
All	Both	12–19	480 (464, 507)	506 (494, 518)	515 (496, 537)	504 (481, 528)	NS	NS	NS
All	Both	20–59	436 (423, 456)	447 (438, 457)	449 (439, 459)	452 (435, 470)	NS	NS	NS
All	Both	≥60	413 (398, 430)	469 (458, 482)	480 (465, 493)	477 (453, 494)	<0.001	NS	NS

¹ NHW, non-Hispanic white; NHB, non-Hispanic black; MA, Mexican American; All, racial-ethnic groups were not shown separately. Serum and red blood cell folate concentrations were measured in ng/mL; serum vitamin B-12 concentrations were measured in pg/mL. SI conversion: to convert serum folate to nmol/L, multiply by 2.266; to convert red blood cell folate to nmol/L, multiply by 2.266; to convert serum vitamin B-12 to pmol/L, multiply by 0.7378.

² For serum and red blood cell folate, time trend analysis of groups comprising the entire age range could not be performed because of a significant age × survey period interaction. For serum vitamin B-12, time trend analysis of groups comprising the entire age range and all races could not be performed because of significant age × survey period and race × survey period interactions.

³ In NHANES III, serum vitamin B-12 concentrations were measured only for all persons aged ≥4 y in 1991–1994.

⁴ Pairwise comparison (*t* test) of geometric means from 1988–1994 and 1999–2000.

⁵ Pairwise comparison (*t* test) of geometric means from 1999–2000 and 2001–2002.

⁶ Pairwise comparison (*t* test) of geometric means from 2001–2002 and 2003–2004.

⁷ Folate measurements were performed by using the Quantaphase II radioassay (BioRad Diagnostics, Hercules, CA), which measures, on average, ≈35% lower than does the microbiologic assay.

fortification to the first postfortification survey period, an apparent decline was seen mainly at the upper end of the distribution in the 2 most recent postfortification survey periods with no apparent decline at the lower end of the distribution. The lowest postfortification distributions of serum and RBC folate concentrations were seen for 2003–2004, and yet the distribution curve

for that survey period was still much higher than that for the prefortification survey. Frequency distributions for serum vitamin B-12 for the entire population for each of the 4 survey periods did not appear to change significantly (data not shown).

Median concentrations of serum and RBC folate and serum vitamin B-12 by sex, race-ethnicity, and age group in 1988–



TABLE 3

Trends in the prevalence of the risk of low or high blood folate and vitamin B-12 concentrations in the entire US population and in groups of special public health interest during the National Health and Nutrition Examination Survey (NHANES), 1988–2004¹

Race-ethnicity	Sex	Age group	Prevalence (95% CI)				P			
			1988–1994 ²	1999–2000	2001–2002	2003–2004	1988–1994 vs 1999–2000 ³	1999–2000 vs 2001–2002 ⁴	2001–2002 vs 2003–2004 ⁵	
			y	%						
Serum folate < 3 ng/mL										
All	F	15–45	20.6 (18.6, 22.8)	0.8 (0.3, 1.7)	0.3 (0.1, 1.0)	0.6 (0.2, 1.5)	<0.001	NS	NS	
NHW	F	15–45	20.0 (17.4, 22.8)	1.1 (0.5, 2.4)	0.4 (0.1, 1.6)	0.7 (0.2, 2.1)	<0.001	NS	NS	
NHB	F	15–45	29.7 (27.6, 31.9)	0.4 (0.1, 2.7)	0.4 (0.1, 1.8)	0.8 (0.2, 3.2)	<0.001	NS	NS	
MA	F	15–45	21.7 (18.6, 25.2)	0.0	0.4 (0.1, 2.3)	0.3 (0.0, 2.4)	<0.001	NS	NS	
Red blood cell folate < 140 ng/mL										
All	F	15–45	37.6 (34.4, 40.9)	5.1 (3.4, 7.5)	4.0 (2.6, 5.9)	5.5 (4.5, 6.6)	<0.001	NS	NS	
NHW	F	15–45	34.5 (30.6, 38.6)	4.4 (2.3, 8.4)	2.7 (1.3, 5.7)	3.5 (2.7, 4.6)	<0.001	NS	NS	
NHB	F	15–45	59.6 (56.5, 62.6)	11.9 (7.6, 18.3)	14.1 (9.7, 20.1)	14.3 (10.8, 18.8)	<0.001	NS	NS	
MA	F	15–45	38.7 (33.4, 44.2)	1.6 (0.6, 3.8)	2.8 (1.4, 5.7)	4.7 (3.0, 7.5)	<0.001	NS	NS	
Serum folate > 20 ng/mL										
All	Both	4–11	5.4 (3.7, 7.6)	42.4 (35.7, 49.3)	26.8 (23.4, 30.6)	18.6 (15.2, 22.5)	<0.001	<0.001	0.002	
All	Both	≥60	7.1 (5.8, 8.8)	38.0 (34.5, 41.7)	31.5 (28.4, 34.9)	31.8 (28.2, 35.6)	<0.001	0.009	NS	
All	M	≥60	5.0 (3.6, 6.9)	29.2 (25.2, 33.6)	25.3 (22.0, 28.9)	27.5 (22.8, 32.8)	<0.001	NS	NS	
All	F	≥60	8.8 (7.0, 11.1)	45.2 (41.2, 49.3)	36.2 (31.6, 41.0)	35.1 (31.5, 38.9)	<0.001	0.005	NS	
Serum vitamin B-12 < 200 pg/mL										
All	Both	≥60	4.8 (3.6, 6.2)	2.7 (2.0, 3.7)	3.6 (2.8, 4.6)	3.9 (2.9, 5.2)	0.012	NS	NS	
All	M	≥60	5.2 (3.7, 7.4)	2.6 (1.7, 4.0)	3.4 (2.6, 4.5)	3.8 (2.4, 5.9)	NS	NS	NS	
All	F	≥60	4.4 (2.9, 6.5)	2.8 (1.9, 4.1)	3.7 (2.3, 5.7)	4.0 (2.4, 6.4)	NS	NS	NS	
NHW	Both	≥60	5.1 (3.8, 6.8)	2.7 (1.8, 4.1)	3.6 (2.8, 4.6)	4.1 (3.1, 5.3)	NS	NS	NS	
NHB	Both	≥60	1.8 (0.9, 3.7)	1.4 (0.6, 3.2)	1.0 (0.3, 3.3)	2.0 (1.0, 3.9)	NS	NS	NS	
MA	Both	≥60	6.3 (4.0, 9.8)	4.0 (2.0, 7.8)	1.9 (0.6, 5.9)	1.7 (1.1, 2.8)	NS	NS	NS	

¹ NHW, non-Hispanic white; NHB, non-Hispanic black; MA, Mexican American; All, racial-ethnic groups were not shown separately. Folate measurements were performed by using the Quantaphase II radioassay (BioRad Diagnostics, Hercules, CA), which measures, on average, ≈35% lower than does the microbiologic assay.

² In NHANES III, serum vitamin B-12 concentrations were measured only for all persons aged ≥4 y in 1991–1994.

³ Pairwise comparison (*t* test) of prevalence estimates from 1988–1994 and 1999–2000.

⁴ Pairwise comparison (*t* test) of prevalence estimates from 1999–2000 and 2001–2002.

⁵ Pairwise comparison (*t* test) of prevalence estimates from 2001–2002 and 2003–2004.

1994, 1999–2000, 2001–2002, and 2003–2004 are shown in Table 2. Because of the age × survey period interaction, we present only the findings from time trend analysis for the age-specific subgroups. Concentrations of serum and RBC folate increased substantially in every age group after the introduction of folic acid fortification in 1998: serum folate concentrations more than doubled and RBC folate concentrations increased by ≈50%. Serum vitamin B-12 concentrations showed a small increase (14%) from before fortification to the first postfortification survey period in older persons. From the first to the second postfortification survey period, we saw a small decrease in serum folate concentrations in children (12%), adolescents (5%), and older persons (5%); from the second to the third postfortification period, we saw a small decrease in children (8%), adolescents (13%), and adults (7%). We saw no change in RBC folate from the first to the second postfortification survey period, but we saw a small decrease from the second to the third postfortification period in children (7%), adolescents (6%), and adults (9%). Overall, serum folate concentrations decreased more than did

RBC folate concentrations (by 10–20% and 7–11%, respectively); however, decreases in both analytes were highest in children and adolescents. Serum vitamin B-12 concentrations remained unchanged between the first and third postfortification survey periods.

Trends in prevalence estimates for persons at risk of low or high blood folate and vitamin B-12 concentrations, 1988–2004

Prevalence estimates for the 4 survey periods covering 1988–2004 in age-specific groups of special public health interest are shown in Table 3. For prevalence estimates in subgroups by standard sex, race-ethnicity, and age group breakdown, see Table S1 under “Supplemental data” in the current online issue at www.ajcn.org. The prevalence of low serum folate concentrations (<3 ng/mL) decreased substantially for women of child-bearing age (from 21% to <1%) after fortification was introduced. No change occurred between the first and second or between the second and third postfortification periods in women

TABLE 4

Selected population percentile values for biochemical folate and vitamin B-12 concentrations by sex, race-ethnicity, and age group during the National Health and Nutrition Examination Survey (NHANES), 1988–1994 and 1999–2004¹

Race-ethnicity	Sex	Age group	Survey	Subjects	Percentile (95% CI)		
					2.5th	50th	97.5th
					<i>y</i>		
					<i>n</i>		
Serum folate²							
All	Both	All	1988–1994	23 361	1.7 (1.6, 1.8)	5.5 (5.2, 5.7)	21.3 (19.9, 22.6)
			1999–2004	23 345	4.6 (4.5, 4.9)	13 (12.7, 13.2)	34.8 (34.1, 36.0)
All	M	All	1988–1994	11 130	1.8 (1.6, 1.9)	5.3 (5.0, 5.5)	18.6 (17.6, 20.2)
			1999–2004	11 387	4.5 (4.3, 4.7)	12.3 (12.0, 12.7)	32.2 (31.2, 33.6)
All	F	All	1988–1994	12 231	1.7 (1.6, 1.8)	5.7 (5.5, 6.0)	23.2 (21.6, 25.7)
			1999–2004	11 958	4.8 (4.6, 5.1)	13.6 (13.2, 13.9)	37.3 (35.7, 39.5)
NHW	Both	All	1988–1994	8534	1.7 (1.6, 1.8)	5.8 (5.5, 6.1)	22.4 (20.6, 24.2)
			1999–2004	9427	4.9 (4.6, 5.1)	13.6 (13.1, 13.9)	36.5 (35.0, 37.8)
NHB	Both	All	1988–1994	6813	1.6 (1.5, 1.6)	4.6 (4.4, 4.7)	16.3 (15.3, 16.8)
			1999–2004	5764	4.1 (3.8, 4.4)	10.7 (10.4, 11.1)	30.1 (27.7, 32.8)
MA	Both	All	1988–1994	7017	1.7 (1.7, 1.8)	5.1 (4.7, 5.3)	17.0 (15.1, 19.3)
			1999–2004	6500	4.7 (4.2, 4.9)	11.9 (11.4, 12.2)	28.7 (27.2, 30.6)
All	Both	4–11	1988–1994	4627	3.3 (3.1, 3.6)	8.8 (8.3, 9.3)	26.9 (22.6, 29.5)
			1999–2004	3595	8.6 (8.2, 9.0)	17.2 (16.9, 17.6)	37.7 (34.7, 41.0)
All	Both	12–19	1988–1994	2957	1.7 (1.6, 1.9)	5.1 (4.9, 5.5)	16.1 (14.3, 18.1)
			1999–2004	6390	5 (4.8, 5.2)	12.1 (11.7, 12.5)	27.2 (25.6, 28.2)
All	Both	20–59	1988–1994	10 726	1.6 (1.4, 1.6)	4.8 (4.5, 5.0)	18.5 (17.1, 20.4)
			1999–2004	8689	4.4 (4.1, 4.5)	11.6 (11.3, 11.9)	31.0 (30.1, 32.6)
All	Both	≥60	1988–1994	5051	2.1 (1.9, 2.3)	6.9 (6.6, 7.3)	28.1 (25.2, 30.3)
			1999–2004	4671	5.6 (5.2, 5.9)	16.6 (16.0, 17.0)	45.8 (43.7, 48.4)
Red blood cell folate²							
All	Both	All	1988–1994	23 402	77 (74, 79)	174 (169, 180)	438 (423, 456)
			1999–2004	23 527	135 (131, 138)	269 (263, 274)	600 (581, 618)
All	M	All	1988–1994	11 144	79 (74, 82)	172 (166, 176)	412 (393, 439)
			1999–2004	11 455	136 (131, 140)	260 (255, 266)	566 (545, 580)
All	F	All	1988–1994	12 258	75 (71, 77)	177 (169, 184)	455 (439, 473)
			1999–2004	12 072	132 (128, 136)	276 (272, 282)	630 (598, 657)
NHW	Both	All	1988–1994	8574	79 (76, 84)	184 (177, 189)	455 (437, 473)
			1999–2004	9472	145 (138, 150)	284 (277, 290)	636 (608, 657)
NHB	Both	All	1988–1994	6869	58 (53, 60)	135 (132, 139)	317 (305, 330)
			1999–2004	5856	111 (108, 114)	216 (213, 219)	433 (411, 462)
MA	Both	All	1988–1994	6956	79 (74, 82)	164 (156, 170)	368 (342, 401)
			1999–2004	6526	135 (129, 141)	247 (242, 252)	519 (494, 539)
All	Both	4–11	1988–1994	4660	101 (95, 107)	200 (190, 210)	386 (358, 409)
			1999–2004	3656	172 (164, 176)	271 (266, 275)	507 (473, 523)
All	Both	12–19	1988–1994	2955	72 (67, 76)	150 (143, 156)	360 (332, 384)
			1999–2004	6425	131 (127, 135)	235 (230, 240)	454 (425, 490)
All	Both	20–59	1988–1994	10 720	74 (70, 77)	164 (160, 171)	414 (395, 433)
			1999–2004	8732	131 (127, 134)	263 (256, 269)	567 (539, 595)
All	Both	≥60	1988–1994	5067	83 (80, 88)	211 (204, 220)	573 (525, 611)
			1999–2004	4714	145 (140, 152)	333 (325, 344)	744 (718, 789)
Serum vitamin B-12³							
All	Both	All	1991–1994	11 860	196 (181, 212)	466 (453, 478)	1070 (1020, 1120)
			1999–2004	23 346	209 (206, 212)	483 (475, 490)	1180 (1150, 1210)
All	M	All	1991–1994	5380	200 (179, 220)	464 (449, 479)	1050 (993, 1100)
			1999–2004	11 381	219 (212, 226)	484 (476, 491)	1100 (1080, 1140)
All	F	All	1991–1994	6480	196 (179, 214)	466 (449, 484)	1090 (1030, 1160)
			1999–2004	11 965	202 (199, 207)	482 (473, 491)	1260 (1200, 1300)
NHW	Both	All	1991–1994	3989	200 (183, 213)	444 (427, 460)	986 (918, 1070)
			1999–2004	9430	203 (201, 208)	469 (460, 476)	1100 (1080, 1140)
NHB	Both	All	1991–1994	3903	237 (218, 260)	567 (553, 579)	1280 (1200, 1360)
			1999–2004	5762	238 (226, 252)	561 (547, 574)	1340 (1310, 1390)
MA	Both	All	1991–1994	3365	205 (193, 228)	495 (471, 522)	1290 (1160, 1470)
			1999–2004	6499	234 (219, 243)	513 (498, 524)	1450 (1240, 1760)
All	Both	4–11	1991–1994	2204	346 (321, 377)	667 (636, 703)	1350 (1250, 1460)
			1999–2004	3594	337 (326, 350)	715 (700, 732)	1470 (1380, 1540)
All	Both	12–19	1991–1994	1593	248 (224, 274)	480 (464, 507)	1010 (909, 1150)
			1999–2004	6390	236 (228, 243)	508 (497, 520)	1050 (1000, 1080)
All	Both	20–59	1991–1994	5556	188 (172, 207)	436 (423, 456)	935 (870, 1030)
			1999–2004	8692	207 (203, 211)	451 (443, 458)	1060 (1010, 1100)
All	Both	≥60	1991–1994	2507	158 (145, 179)	413 (398, 430)	1060 (977, 1200)
			1999–2004	4670	184 (168, 194)	474 (467, 483)	1250 (1170, 1330)

¹ NHW, non-Hispanic white; NHB, non-Hispanic black; MA, Mexican American; All, racial-ethnic groups were not shown separately.

² Folate measurements (in ng/mL) were performed by using the Quantaphase II radioassay (BioRad Diagnostics, Hercules, CA), which measures, on average, ≈35% lower than does the microbiologic assay.

³ In NHANES III, serum vitamin B-12 concentrations were measured (in pg/mL) only for all persons aged ≥4 y in 1991–1994.

of childbearing age, whether they were considered as an entire group or by racial-ethnic groups. Similarly, the prevalence of low RBC folate concentrations (<140 ng/mL) decreased significantly from 38% before fortification to 5% after fortification. As with serum folate, we saw no change during the years spanning the 3 postfortification survey periods.

The prevalence of high serum folate concentrations (>20 ng/mL) increased substantially—from 5% to 42% in children and from 7% to 38% in older persons—after fortification was introduced. We saw a significant decrease in the prevalence of high serum folate concentrations from the first to the second postfortification survey period in children and older persons (women only) but a further decrease from the second to the third period only in children. Overall, children experienced a greater decrease in the prevalence of high serum folate concentrations between 1999 and 2004 (from 42% to 19% prevalence) than did older persons (from 38% to 32% prevalence). The prevalence of low serum vitamin B-12 concentrations (<200 pg/mL) changed only for older persons from before fortification to after fortification (from 5% to 3%).

Selected population percentile values for blood folate and vitamin B-12 concentrations in the prefortification and postfortification periods

The 2.5th, 50th, and 97.5th percentiles for 6 y of prefortification and postfortification survey periods by sex, race-ethnicity, and age group are shown in Table 4. The same percentiles by sex-age group and race-ethnicity-age group combinations are shown in Table S2 under “Supplemental data” in the current online issue at www.ajcn.org. The upward shift in the entire distribution after fortification is readily apparent.

DISCUSSION

We present here the history of blood folate and vitamin B-12 concentrations in a nationally representative sample of the US population over a long enough period of time and across enough different survey periods that both the trends associated with implementation of folate fortification and the subsequent trends unrelated to this fortification program can be assessed. The present study offers a unique opportunity to assess these trends, because the same analytic technique was used from 1988 to 2004, but a new technique will, of necessity, be introduced later in 2007.

Our analyses significantly expand on an earlier report by Ganji and Kafai (20) by including a third postfortification survey period, which allows the assessment of time trends; presenting the results in a format more useful to practitioners and the public health community (medians rather than geometric means); and providing selected population percentile values for sex-age groups to document the skewed nature of the distributions and how they changed over time. Modeling of fortification scenarios before the implementation of fortification suggested that it would have a greater effect on intakes at the upper end of the distribution curve than at the lower end (30). Our results are consistent with this prediction. The prefortification (1988–1994) to postfortification (1999–2000) increases in serum folate were 2.7, 8.7, and 15.9 ng/mL at the 2.5th, 50th, and 97.5th percentiles, respectively, and the corresponding increases in RBC folate were 59, 102, and 182 ng/mL (data not shown). Comparisons of postfortification trends show that the changes in serum folate from the

first to the third survey period at the same percentiles were 0.1, –2.3, and –4.2 ng/mL, respectively; corresponding changes in RBC folate were –4, –23, and –63 ng/mL (data not shown). We saw no changes in the prevalence of low serum folate concentrations during the overall downward trend from 1999–2004 but substantial changes in high serum folate concentrations. It is also noteworthy that we did not see appreciable changes in vitamin B-12 status over this extended period. Because there is controversy as to whether the serum vitamin B-12 concentration is a sensitive indicator of vitamin B-12 status, that question should be revisited when the 2003–2004 MMA data become available.

The marked increase in blood folate concentrations after fortification has generated considerable interest. Before implementing folate fortification, FDA noted that the effect of fortification on folate intakes likely was underestimated because of survey participants' underreporting of foods consumed, food-composition table underestimates of the folate content of foods, increased consumer selection of folate-rich foods because of health claims and other publicity, and increasing availability of the numbers and types of nonstandardized folate-fortified foods—eg, breakfast cereals (13). The effects of these factors likely are cumulative and interactive in nature, which illustrates the value of monitoring the prefortification and postfortification biochemical indicators of folate status that reflect the net effect of a number of uncertainties surrounding the fortification decisions.

The recent decreases in blood folate concentrations may be due in part to changes in consumer behaviors. For example, US per capita data on the disappearance of wheat flour from the US food supply showed steady increases from ≈1985 to 2000 (from 124.6 pounds per capita in 1990 to 146.3 pounds in 2000) and a reversal of this trend after 2000, to 134.3 pounds per capita in 2004 (31). This change may have been a response to publicity about the purported benefits of low-carbohydrate diets in weight loss or due to more general concerns about calorie reduction because of publicity about the obesity epidemic. The number of low-carbohydrate products introduced increased year-by-year from 15 products in 2000 to 661 through 9 April 2004 (32). There has been no recent systematic analysis of enriched cereal-grain products, but one study suggested that the mean folate content of enriched breads may have been reduced during 2000–2003 (33).

Currently, we do not know whether the slight downward postfortification trend in serum and RBC folate concentrations is functionally important. In evaluating this question, method differences between the BioRad and the microbiologic assay have to be considered. The BioRad assay gives results that are, on average, ≈35% lower than those obtained with the microbiologic assay (26). Therefore, when cutoffs derived from the microbiologic assay are applied to results produced by the BioRad assay, the prevalence of the risk of folate inadequacy is overestimated. In the present analyses, we used cutoffs for low serum and RBC folate that were based on microbiologic assay results derived through an expert consultation process (27). When we applied adjusted cutoffs (10) to our prevalence analysis (ie, cutoffs of <2 ng/mL for low serum folate and of <95 ng/mL for low RBC folate), we found <4 participants with low serum folate and <40 participants with low RBC folate out of ≈8000 participants in each of the 3 postfortification survey periods. Thus, from the perspective of nutritional adequacy relative to generally accepted nutritional status criteria, the slight downward trend after fortification seems unlikely to be functionally important.

An evaluation of whether this slight downward trend after fortification may have any effect on the risk of NTDs is confounded by observations that the rank order of NTD incidence rates among racial-ethnic groups—ie, NHB < NHW < MA (34)—differs from that for serum and RBC folate concentrations—ie, NHW > MA > NHB—which makes it problematic to use RBC folate concentrations to predict NTD rates for the US population. Moreover, there was no apparent decline in blood folate concentrations at the low end of the distribution curves, where women are presumably at the greatest risk of suboptimal folate status. Even after the modest decrease in biochemical folate concentrations, serum folate concentrations in 2003–2004 were still approximately twice prefortification concentrations, and RBC folate concentrations were $\approx 50\%$ higher.

Fortification programs must balance the need to increase the intakes of target groups (eg, women of childbearing age) with the need to guard against excessive intakes for other consumers (eg, children and older persons). Yet, fortification decisions almost always have to be made with some uncertainties in the available data. We still lack population-based data on dose-response relations between folate intake and the risk of NTDs, systematic evaluations of potential safety problems, and data on the potential role of folate intakes and status with respect to the risk of certain chronic diseases. Observational studies have suggested potential benefits of folic acid fortification with respect to declines in the incidence of stroke (35) and neuroblastoma (36). Conversely, early results from randomized clinical trials on folic acid supplements and reduced risk of cardiovascular disease have not been promising (37–41). Potentially greater risks associated with very high folic acid intakes have also been suggested. In a preliminary cross-sectional study of postmenopausal women, an inverse U-shaped relation was found between plasma folic acid from dietary sources and supplements and natural killer cell toxicity (42). Folate appears to possess dual modulatory effects on colorectal carcinogenesis, depending on the timing and dose of the folate intervention (43, 44). There is controversy over the effects of folate and vitamin B-12 as they relate to cognition. In a prospective cohort of elderly who participated in the Chicago Health and Aging Project, those in the highest quintile of folate intake (who mostly were supplement users) had a significantly more pronounced cognitive decline over the course of 6 y than did those in the lowest quintile (45). Among seniors participating in NHANES 1999–2002, a high serum folate concentration was associated with anemia and cognitive impairment in those with low vitamin B-12 status, whereas it was associated with protection against cognitive impairment in those with normal vitamin B-12 status (46). In the Folic Acid and Carotid Intima-media Thickness trial, daily oral folic acid supplementation for 3 y beneficially affected global cognitive function in older adults (47). Finally, in the Sacramento Area Latino Study on Aging, the risk of cognitive decline increased in subjects with elevated baseline homocysteine concentrations, especially in conjunction with low serum vitamin B-12 concentrations (48). Results from ongoing research on the relation between folate intake, folate and vitamin B-12 status, and an altered risk of some chronic diseases should be carefully considered within the context of population-monitoring results.

We have presented a detailed history of folate and vitamin B-12 status in the US population from the years before and after folate fortification. After the relatively large initial increase in serum and RBC folate concentrations that followed fortification,

blood concentrations decreased by $\approx 10\%$ between 1999 and 2004, mainly at the high end of the distribution curves. It is too early to tell whether concentrations will stabilize. However, these changes underscore the need for further monitoring of the fortified-food supply as well as blood concentrations.

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