



The vitamin D status of the US population from 1988 to 2010 using standardized serum concentrations of 25-hydroxyvitamin D shows recent modest increases^{1–3}

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ABSTRACT

Background: Temporal trends in the US population's vitamin D status have been uncertain because of nonstandardized serum 25-hydroxyvitamin D [25(OH)D] measurements.

Objective: To accurately assess vitamin D status trends among those aged ≥ 12 y, we used data from the cross-sectional NHANESs.

Design: A liquid chromatography–tandem mass spectrometry (LC-MS/MS) method for measuring 25(OH)D (sum of 25-hydroxyvitamin D₂ and 25-hydroxyvitamin D₃), calibrated to standard reference materials, was used to predict LC-MS/MS–equivalent concentrations from radioimmunoassay data (1988–2006 surveys; $n = 38,700$) and to measure LC-MS/MS concentrations (2007–2010 surveys; $n = 12,446$). Weighted arithmetic means and the prevalence of 25(OH)D above or below cutoff concentrations were calculated to evaluate long-term trends.

Results: Overall, mean predicted 25(OH)D showed no time trend from 1988 to 2006, but during 2007–2010 the mean measured 25(OH)D was 5–6 nmol/L higher. Those groups who showed the largest 25(OH)D increases (7–11 nmol/L) were older, female, non-Hispanic white, and vitamin D supplement users. During 1988–2010, the proportions of persons with 25(OH)D < 40 nmol/L were 14–18% (overall), 46–60% (non-Hispanic blacks), 21–28% (Mexican Americans), and 6–10% (non-Hispanic whites).

Conclusions: An accurate method for measuring 25(OH)D showed stable mean concentrations in the US population (1988–2006) and recent modest increases (2007–2010). Although it is unclear to what extent supplement usage compared with different laboratory methods explain the increases in 25(OH)D, the use of higher vitamin D supplement dosages coincided with the increase. Marked race-ethnic differences in 25(OH)D concentrations were apparent. These data provide the first standardized information about temporal trends in the vitamin D status of the US population. *Am J Clin Nutr* 2016;104:454–61.

Keywords: standardization, survey, vitamin D, trend, NHANES, supplements

INTRODUCTION

The NHANESs track the health and nutrition status of the noninstitutionalized civilian US population. Before 1999,

NHANESs were conducted periodically. In 1999, the NHANES was redesigned to become a continuous survey; data are released for every 2-y survey cycle. Vitamin D status assessment has been included since NHANES III (1988–1994) (1). Serum concentrations of 25-hydroxyvitamin D [25(OH)D]⁷ metabolites, which are the primary biomarkers of status, are relatively long-lasting indicators of vitamin D intake from foods and supplements, and from endogenously produced vitamin D through the action of UV-B light on skin.

The CDC laboratory at the National Center for Environmental Health measured total 25(OH)D for NHANESs that were conducted from 1988 through 2006 by using a radioimmunoassay. Assay differences due to radioimmunoassay reformulation between NHANES III (the last periodic survey) and NHANES 2001–2002 [the first of the continuous 2-y surveys with complete 25(OH)D data] and assay drifts during testing of the 2003–2004 and 2005–2006 survey samples were detected, so a round table of experts was convened (2) and regression equations were developed to adjust the survey data for these radioimmunoassay differences. The adjustments allowed the comparison of harmonized 25(OH)D data from 1988 to 2006 (3, 4). Without these radioimmunoassay adjustments, population estimates for 25(OH)D suggested that vitamin D status had substantially

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² The findings and conclusions in this article are those of the authors and do not necessarily represent the official views or positions of the CDC/Agency for Toxic Substances and Disease Registry, the NIH, or the Department of Health and Human Services.

³ Supplemental Tables 1–3 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://ajcn.nutrition.org>.

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⁷ Abbreviations used: IOM, Institute of Medicine; LC-MS/MS, liquid chromatography–tandem mass spectrometry; NIST, National Institute of Standards and Technology; SRM, standard reference material; 25(OH)D, 25-hydroxyvitamin D; 25(OH)D₂, 25-hydroxyvitamin D₂; 25(OH)D₃, 25-hydroxyvitamin D₃.

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decreased since NHANES III (5). However, much of the apparent downward trend in 25(OH)D was mitigated by the adjustment equations (6–9). Documentation indicating the data user's need to harmonize NHANES III concentrations by using an equation and data files with radioimmunoassay-harmonized participant concentrations for NHANES 2003–2006 was released in November 2010 (3).

Reports in the literature of excessive method bias and imprecision in existing 25(OH)D assays (10) supported the rationale for the National Institute of Standards and Technology (NIST) to develop a reference measurement procedure on the basis of liquid chromatography–tandem mass spectrometry (LC-MS/MS) and standard reference materials (SRMs) for 25(OH)D measurements (11). Because of the difficulty in maintaining long-term stability with the use of an immunoassay, the CDC decided that an LC-MS/MS method traceable to NIST SRMs would be used to measure 25(OH)D in NHANES 2007 and beyond. An LC-MS/MS method affords better control of accuracy, specificity, and long-term stability than do other methods. By 2011, the CDC laboratory completed the development of a routine LC-MS/MS method (12) and applied this method to measure 25(OH)D in specimens from NHANES 2007–2010 (13), which had been stored until the new assay was validated. In addition, representative specimens from NHANESs between 1988 and 2006 were retested in a bridging (crossover) study that was designed to develop equations to standardize all of the original radioimmunoassay data to LC-MS/MS–equivalent data (14); the predicted LC-MS/MS–equivalent data were released on the NHANES website in October 2015 (15) and are the best data to use for the correct interpretation of trends. The primary objective of this study was to use the standardized NHANES 25(OH)D data to describe temporal trends in the vitamin D status of the US population between 1988 and 2010.

METHODS

Study population

The NHANESs have been periodically (e.g., NHANES III, a 6-y survey equally divided into 2 phases) or continuously (1999–present, multiple 2-y surveys) conducted by the National Center for Health Statistics, CDC, to assess the health and nutritional status of the noninstitutionalized US population by using a complex, multistage probability sample (16, 17). Note that no specimens were collected for 25(OH)D measurements in 1999, and year 2000 data are not publicly available to protect against inadvertent disclosure of confidential information from a smaller data set; thus, the 2000 survey data were not included in this analysis. Because 25(OH)D was measured in different age groups in different surveys—namely, 1988–1994 (≥ 12 y old), 2001–2002 (≥ 6 y old), and 2003–2010 (≥ 1 y old)—we made comparisons between those aged ≥ 12 y in the present trend analysis. Sample sizes for NHANES participants with 25(OH)D data (measured or predicted) are shown in **Table 1**. A total of 75,280 persons, aged ≥ 12 y, were selected to participate in the 1988–2010 NHANESs (excluding the year 2000). Of these, 59,505 (79%) agreed to be interviewed and 51,146 (68%) agreed to be examined and have their 25(OH)D measured. Although, overall, 68% of those in this age range who were selected for participation had their 25(OH)D measured, the range of values for the individual

surveys was between 58% and 70%. All of the NHANES participants provided written informed consent, and all procedures were approved by the National Center for Health Statistics Research Ethics Review Board (18).

Laboratory measurements of 25(OH)D with the use of radioimmunoassay and LC-MS/MS

For the NHANESs conducted between 1988 and 2006, radioimmunoassay (DiaSorin) was used to measure serum total 25(OH)D (in duplicate) (19). A fully validated LC-MS/MS method (12), traceable to NIST reference materials, was used to measure 25-hydroxyvitamin D₃ [25(OH)D₃], 25-hydroxyvitamin D₂ [25(OH)D₂], and the C3 epimer of 25(OH)D₃ for all eligible participants in NHANES 2007–2010 (individually) and for selected stored specimens for those NHANES participants between 1988 and 2006 with available radioimmunoassay data for the bridging study (in duplicate). For the LC-MS/MS method, total 25(OH)D was defined as the sum of 25(OH)D₃ and 25(OH)D₂, excluding the C3 epimer of 25(OH)D₃ about which less is known. The bias of the LC-MS/MS method relative to NIST SRMs during the course of the bridging study and NHANES 2007–2010 testing was minimal ($\leq 1\%$) for 25(OH)D₃ or for 25(OH)D₂ at concentrations > 2 nmol/L. The mean bias of this method in the NIST/NIH Vitamin D Metabolites Quality Assurance Program was $< 3\%$ for total 25(OH)D during this same period of time. Furthermore, the mean 25(OH)D bias of the CDC's LC-MS/MS method for a set of 50 individual donor serum samples from the first Interlaboratory Comparison Study sponsored by the Vitamin D Standardization Program (20) relative to 2 independent reference measurement procedures carried out by the NIST and the University of Ghent (21, 22) was 1.4% (95% CI: 0.9%, 1.8%).

Data on supplement use

Supplement usage information was obtained from the dietary supplement questionnaire, which was used to collect information on the participant's use of vitamins, minerals, herbal supplements, and other supplements over the 30 d preceding the household interview. Information on type, frequency, duration, serving size, quantity, and dose taken was collected for each reported dietary supplement product. Any participant who had non-missing information about these features and reported taking a supplement that contained vitamin D was considered a vitamin D supplement user. Mean vitamin D dosage over the course of 30 d was calculated separately for each product and then summed across all such products reported by a participant. Information on prescribed vitamin D₂, which is the only prescribed form currently available in the United States, was also obtained from the dietary supplement questionnaire.

Statistical analyses

For analysis of temporal trends in the US population, we calculated the weighted arithmetic mean and weighted prevalence of LC-MS/MS–equivalent total 25(OH)D above or below certain cutoff concentrations for each NHANES. Specifically, we examined the prevalence of concentrations < 30 , < 40 , < 50 , and < 75 nmol/L because consensus about optimal thresholds for 25(OH)D is currently lacking. The Institute of Medicine

TABLE 1

Sample sizes for persons aged ≥ 12 y who were screened, interviewed, examined, and had serum 25(OH)D measured, grouped by demographic variables or vitamin D supplement use and stratified by survey: NHANESs 1988–2010¹

Group	NHANES, <i>n</i>					
	1988–1994	2001–2002	2003–2004	2005–2006	2007–2008	2009–2010
Screened	27,145	9710	9565	9408	9530	9922
Interviewed	22,266	7898	7344	7267	7173	7557
Examined and 25(OH)D measured ²	18,851	6816	6553	6480	5536	6910
Age, y						
12–19	2950	2167	2057	1985	937	1181
20–39	6447	1691	1558	1703	1432	1917
40–59	4271	1449	1277	1382	1456	1921
≥ 60	5181	1509	1661	1410	1711	1891
Sex						
Male	8823	3285	3228	3149	2751	3400
Female	10,028	3531	3325	3331	2785	3510
Race-ethnicity ³						
Mexican American	5293	1678	1533	1569	1032	1388
Non-Hispanic black	5350	1487	1604	1677	1082	1229
Non-Hispanic white	7420	3122	2949	2776	2541	3174
Supplement use ⁴						
No	14,554	4695	4530	4469	3859	4696
Yes	4001	1915	1979	1937	1639	2164

¹25(OH)D, 25-hydroxyvitamin D.

²The 1988–1994 data set contains 32 participants fewer than the public release file (no valid radioimmunoassay analysis date); the 2003–2004 data set contains 4 participants fewer than the public release file (no valid radioimmunoassay result).

³An “Other” race-ethnic group is not shown but is included in total estimates.

⁴The use of any vitamin D-containing supplements in the month preceding the household interview.

(IOM) defined <30 nmol/L as indicating risk of deficiency, <40 nmol/L as the concentration that meets the needs of half the population, and <50 nmol/L as indicating a risk of insufficiency in individuals (23). We focused on <40 nmol/L because this concentration is consistent with an intake equivalent to the Estimated Average Requirement, and by using mean or median “requirements” (e.g., <40 nmol/L) rather than tails of requirement distributions (e.g., <50 nmol/L) to assess the prevalence of inadequacy in groups, more accurate prevalence estimates are obtained (24). It is generally accepted that the proportion of individuals with intakes below the Estimated Average Requirement provides a reasonable estimate of the expected prevalence of inadequate intakes for populations and that there are classification errors for individuals but the false positives and false negatives tend to cancel out. We also assessed the risk of excess by estimating prevalences of those with 25(OH)D >125 nmol/L. Wald-type CIs for the prevalence estimates were computed by using a logit transformation. The Wald *F* test from either linear or logistic regression was used to obtain a linear test of trend across consecutive surveys for the weighted means and prevalences, respectively. The weighted means for 25(OH)D and the weighted prevalence for vitamin D supplement intake were age-standardized to the 2000 US Census population by using the following groups: 12–19, 20–29, 30–39, 40–49, 50–59, 60–69, 70–79, and ≥ 80 y (25). To make pairwise comparisons between surveys for demographic groups with the use of age-standardized 25(OH)D means, we grouped the data into 3 time periods and recalculated the sample weights. These 3 time periods were based on the assay features—those with predictions from the original radioimmunoassay (1988–1994), those

with predictions from the reformulated radioimmunoassay (2001–2006), and those with direct LC-MS/MS measurements (2007–2010)—after confirming that there were no significant differences between the 2-y survey cycles being combined (data not shown).

Statistical analyses were performed by using SAS (version 9.3; SAS Institute) and SUDAAN (version 11.0.1; RTI) software to account for the complex survey design by incorporating the examination weights and by using Taylor series linearization to calculate variance estimates. We used pairwise deletion when there were missing or incomplete self-reported vitamin D supplement use data; $<2\%$ of the sample had missing vitamin D supplement usage data.

RESULTS

Temporal trends in 25(OH)D in 1988–2010 NHANESs

With the use of LC-MS/MS-standardized data, the overall mean predicted LC-MS/MS concentrations of 25(OH)D did not vary much during the period between 1988 and 2006 (Table 2). Mean differences between adjacent surveys were $<3\%$. However, mean 25(OH)D concentrations in the NHANES 2007–2010, based on directly measured LC-MS/MS values, were $\sim 8\%$ higher than in the preceding surveys. Linear trends for the 22-y period were evident for certain demographic groups, namely, those ≥ 40 y of age, females, non-Hispanic blacks, non-Hispanic whites, and vitamin D supplement users (Table 2).

Similarly, age-standardized mean 25(OH)D concentrations in the overall US population in the surveys from 1988 to 2006 were

TABLE 2

LC-MS/MS–equivalent serum 25(OH)D concentrations for persons aged ≥ 12 y stratified by NHANES and grouped by demographic variables: NHANESs 1988–2010¹

Group	NHANES, nmol/L						<i>P</i> ²
	1988–1994	2001–2002	2003–2004	2005–2006	2007–2008	2009–2010	
All	62.3 (61.1, 63.5)	62.2 (60.4, 64.1)	62.7 (59.3, 66.2)	61.0 (58.6, 63.4)	67.1 (64.8, 69.4)	67.4 (64.6, 70.2)	<0.0001
Age, y							
12–19	66.2 (64.1, 68.4)	63.0 (60.8, 65.2)	63.9 (59.4, 68.4)	61.9 (58.5, 65.4)	66.6 (62.3, 70.9)	65.0 (61.5, 68.6)	0.8117
20–39	64.4 (62.8, 66.0)	62.8 (60.6, 64.9)	62.9 (59.0, 66.9)	62.5 (59.5, 65.6)	66.0 (62.4, 69.6)	63.4 (60.0, 66.7)	0.7915
40–59	60.1 (58.7, 61.5)	62.4 (59.9, 64.8)	62.2 (58.0, 66.4)	60.1 (57.7, 62.6)	67.0 (64.2, 69.8)	68.7 (65.8, 71.5)	<0.0001
≥ 60	58.4 (57.4, 59.5)	60.4 (58.0, 62.9)	62.5 (60.0, 65.0)	59.4 (57.0, 61.9)	69.0 (66.7, 71.2)	72.6 (69.2, 76.0)	<0.0001
Sex							
Male	65.6 (64.3, 66.9)	63.2 (61.3, 65.1)	63.1 (59.4, 66.7)	61.1 (58.9, 63.2)	65.8 (63.1, 68.4)	65.5 (62.7, 68.3)	0.4978
Female	59.2 (57.9, 60.6)	61.3 (59.1, 63.5)	62.4 (59.0, 65.8)	60.9 (58.2, 63.7)	68.3 (66.0, 70.6)	69.1 (66.0, 72.3)	<0.0001
Race-ethnicity							
Mexican American	54.7 (53.3, 56.2)	55.0 (51.9, 58.2)	54.2 (50.7, 57.7)	51.2 (47.2, 55.1)	53.9 (49.7, 58.0)	53.9 (52.2, 55.5)	0.3357
Non-Hispanic black	42.8 (41.1, 44.6)	39.3 (38.2, 40.5)	40.9 (37.7, 44.1)	41.7 (39.5, 43.9)	42.0 (39.0, 45.0)	46.0 (41.6, 50.5)	0.0443
Non-Hispanic white	66.7 (65.4, 68.0)	67.3 (65.2, 69.4)	68.4 (64.9, 71.9)	66.2 (64.1, 68.3)	74.1 (72.1, 76.1)	75.0 (72.5, 77.4)	<0.0001

¹Values are weighted arithmetic means (95% CIs); sample sizes are shown in Table 1. NHANES 2007–2010 concentrations were measured directly by using LC-MS/MS. NHANES 1988–2006 concentrations were standardized from the original RIA measurements to LC-MS/MS equivalents as follows (in nmol/L units)—1988–1994: if $RIA_{original} \leq 102$ then $LC-MS/MS_{equivalent} = 1.57548 + 0.8429 \times RIA_{original}$; 1988–1994: if $RIA_{original} > 102$ then $LC-MS/MS_{equivalent} = 59.2296 + 0.2788 \times RIA_{original}$; 2001–2002: $LC-MS/MS_{equivalent} = 6.43435 + 0.95212 \times RIA_{original}$; 2003–2004: $LC-MS/MS_{equivalent} = 1.72786 + 0.98284 \times RIA_{original}$; 2005–2006: $LC-MS/MS_{equivalent} = 8.36753 + 0.97012 \times RIA_{original}$. LC-MS/MS, liquid chromatography–tandem mass spectrometry; RIA, radioimmunoassay; 25(OH)D, 25-hydroxyvitamin D.

²Linear trend based on Wald *F* test.

not significantly different, but a significant 5.01-nmol/L (95% CI: 2.95, 7.08 nmol/L; $P < 0.0001$) mean increase was apparent in the 2007–2010 surveys (**Figure 1**). This increase appeared to be related to vitamin D supplement use, at least to some extent. Compared with surveys conducted during 1988–2006, the mean 25(OH)D in 2007–2010 was significantly higher in vitamin D supplement users by 8.66 nmol/L (95% CI: 6.68, 10.6 nmol/L; $P < 0.0001$) but not in nonusers (2.11 nmol/L; 95% CI: -0.33 , 4.55 nmol/L; $P = 0.09$).

We noted temporal changes in age-standardized usage of vitamin D–containing supplements during the NHANESs conducted

between 1988 and 2010 (**Figure 2**). After an 8% increase in vitamin D supplement usage between NHANES III and NHANES 2001–2002 (from 27% to 35%), vitamin D supplement usage was relatively stable from 2001 to 2010 between 34% and 38% for those aged ≥ 12 y. Although the median amount taken on a daily basis did not increase from the 2001–2002 to the 2009–2010 surveys (399 compared with 398 IU/d in supplement users), proportionately more people were taking ≥ 600 IU/d over time (**Figure 2**). The age-standardized proportion of supplement users taking ≥ 600 IU/d during the 3 time periods increased from 2% in 1988–1994, to 3.5% in 2001–2006, to 10% in 2007–2010,

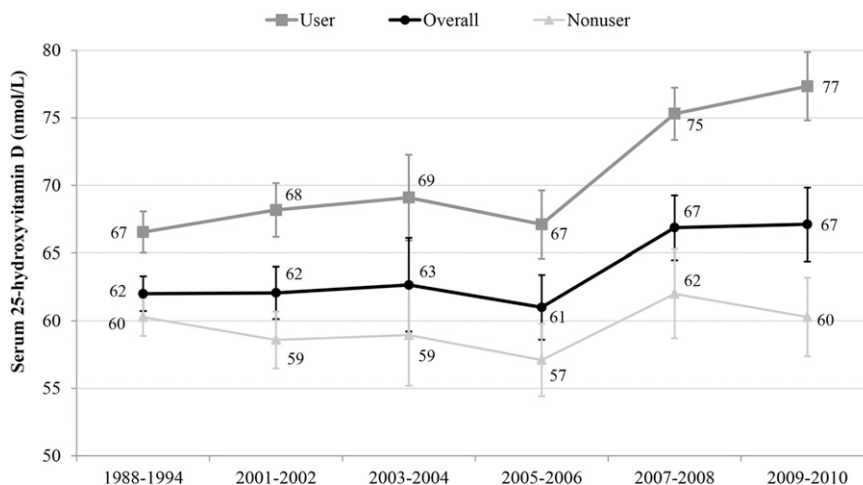


FIGURE 1 Trends in age-adjusted mean concentrations of serum 25-hydroxyvitamin D, stratified by vitamin D supplement usage, for persons aged ≥ 12 y: NHANESs 1988–2010. Values are weighted arithmetic means (95% CIs). Data were age-standardized by using the 2000 US Census as the standard population. NHANES 1988–2006 data represent predicted LC-MS/MS–equivalent concentrations; NHANES 2007–2010 data represent measured LC-MS/MS concentrations. The use of any vitamin D–containing supplements during the 30 d preceding the household interview was assessed and used to categorize participants as users or nonusers. Linear trend based on Wald *F* test: user, $P < 0.0001$; overall, $P < 0.0001$; and nonuser, $P = 0.5615$. LC-MS/MS, liquid chromatography–tandem mass spectrometry.

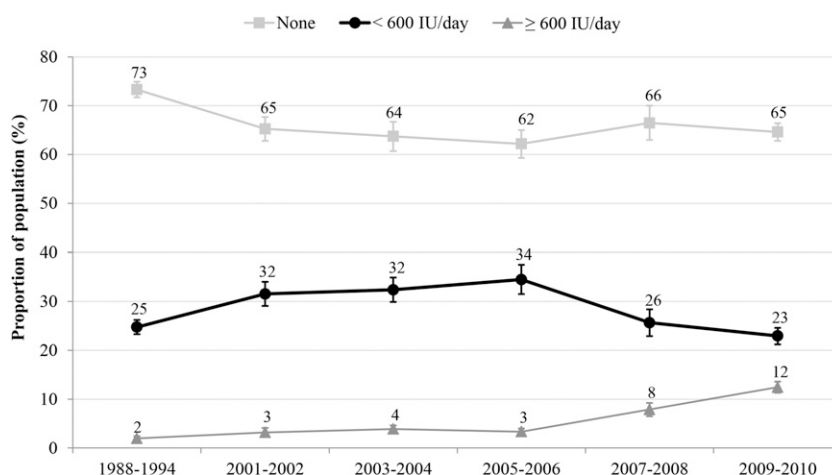


FIGURE 2 Trends in age-adjusted proportions of the population either using or not using vitamin D supplements, stratified by daily dose, for persons aged ≥ 12 y: NHANESs 1988–2010. Values are weighted proportions (95% CIs). Data were age-standardized by using the 2000 US Census as the standard population. The use of any vitamin D-containing supplements during the 30 d preceding the household interview was used to categorize participants on the basis of mean daily dose.

which represents a 3-fold increase (95% CI: 2.6-fold, 3.6-fold; $P < 0.0001$). A breakdown (not age-standardized) of the usage of vitamin D-containing supplements by demographic group and dose showed that, in general, younger participants (12–19 y of age) were least likely of all age groups to take vitamin D supplements (**Supplemental Table 1**). Older persons (≥ 40 y of age), females, and non-Hispanic whites were significantly more likely to take the higher-dose supplements (≥ 600 IU/d) than other demographic groups.

In addition to looking at consecutive survey linear trends in 25(OH)D concentrations, we performed comparisons with the use of longer periods by grouping the data into 3 categories on the basis of assay features: 1988–1994 (original radioimmunoassay), 2001–2006 (reformulated radioimmunoassay), and 2007–2010 (LC-MS/MS). Findings for the comparisons of age-standardized means between these 3 categories were similar to those described above for linear trends in non-age-standardized 25(OH)D means. Specifically, there were significant increases (6.39–12.6 nmol/L) in age-standardized means between the earlier periods (1988–1994 or 2001–2006) and 2007–2010 for the following groups: persons ≥ 40 y of age, females, non-Hispanic whites, and vitamin D supplement users (**Supplemental Table 2**). For non-Hispanic blacks, there was a significant pairwise increase in mean 25(OH)D between 2001–2006 and 2007–2010 (3.7 nmol/L) but no such difference between 1988–1994 and 2007–2010.

We also examined trends in the prevalence of 25(OH)D below or above various cutoffs [**Table 3** (multiple cutoffs for the overall population and < 40 nmol/L by demographic group) and **Supplemental Table 3** (multiple cutoffs by demographic group)]. The prevalence of 25(OH)D either < 30 or < 40 nmol/L between 1988 and 2010 remained stable for the overall population. Within demographic groups, the prevalence of concentrations < 40 nmol/L was much higher in non-Hispanic blacks, at 46–60%, and much lower in non-Hispanic whites, at 6–10% (prevalence of all non-Hispanic blacks compared with non-Hispanic whites with concentrations < 40 nmol/L; all pairwise differences $P < 0.001$). Several demographic groups, including

those ≥ 40 y of age, females, non-Hispanic whites, and vitamin D supplement users, showed decreasing linear trends in the prevalence of 25(OH)D < 40 nmol/L during the period 1988–2010. Although non-Hispanic blacks did not show a significant linear trend for 25(OH)D < 40 nmol/L, similar trends in this group were significant at the < 50 -nmol/L and < 75 -nmol/L cutoffs (**Supplemental Table 3**). Overall, the prevalence of 25(OH)D concentrations < 50 and < 75 nmol/L showed a significant linear decrease over the period 1988–2010, and fewer persons who were ≥ 40 y of age, female, non-Hispanic white, and vitamin D supplement users had 25(OH)D concentrations < 50 or < 75 nmol/L over time. Last, the prevalence of 25(OH)D > 125 nmol/L showed significant positive linear trends for all of the demographic groups except for Mexican Americans.

DISCUSSION

By using the predicted LC-MS/MS-equivalent concentrations to replace the historical NHANES 25(OH)D radioimmunoassay data, overall, we found no trends in the 25(OH)D survey means during the 18 y between 1988 and 2006. These findings are not consistent with reports of increasing vitamin D deficiency in the US population (6–9). Earlier, the CDC harmonized the 25(OH)D radioimmunoassay results for NHANES III and NHANES 2003–2006 (3, 4) in an attempt to provide comparable data for the correct interpretation of trends, which were overestimating vitamin D deficiency (5). The harmonization corrections mitigated the declines in 25(OH)D, but the results of the present study indicate that these corrections were only partially successful because they continued to suggest that the US population had become more vitamin D deficient between 1988 and 2006 (6–9). This was not found to be true with the use of standardized LC-MS/MS data.

The importance of reference material-traceable standardization to data quality and interpretation of the findings is clearly apparent in the present study. Other studies (26–28) noted confounded results when unstandardized immunoassay methods were used to measure 25(OH)D, either over- or underestimating vitamin D deficiency. However, it is worth mentioning that 25(OH)D assays have improved over the past few years as a result of

TABLE 3

Prevalence of LC-MS/MS–equivalent serum 25(OH)D concentrations below or above various cutoffs for persons aged ≥ 12 y, stratified by survey and grouped by demographic variables or vitamin D supplement use: NHANESs 1988–2010¹

25(OH)D cutoff group	NHANES						<i>P</i> ²
	1988–1994	2001–2002	2003–2004	2005–2006	2007–2008	2009–2010	
<30 nmol/L	6.0 (5.2, 6.9)	5.4 (4.1, 7.0)	7.5 (5.3, 10)	5.2 (3.8, 6.9)	6.4 (4.8, 8.6)	6.7 (5.2, 8.7)	0.46
<40 nmol/L	16 (15, 18)	17 (14, 20)	17 (13, 22)	18 (14, 22)	14 (11, 18)	15 (12, 18)	0.20
<50 nmol/L	30 (28, 32)	29 (26, 33)	30 (24, 37)	32 (27, 37)	26 (22, 30)	26 (22, 30)	0.0315
<75 nmol/L	70 (68, 73)	74 (70, 77)	71 (66, 76)	77 (73, 80)	65 (62, 68)	64 (60, 68)	0.0002
>125 nmol/L	0.0 (0.0, 0.1)	0.9 (0.6, 1.3)	1.5 (0.9, 2.4)	0.9 (0.6, 1.2)	2.4 (1.7, 3.3)	2.6 (1.8, 3.6)	<0.0001
<40 nmol/L ³							
Age, y							
12–19	10 (8.9, 12)	15 (11, 20)	14 (10, 19)	16 (12, 22)	14 (9.7, 20)	14 (10, 18)	0.34
20–39	15 (13, 17)	17 (14, 21)	18 (14, 24)	18 (14, 23)	17 (13, 22)	18 (15, 22)	0.28
40–59	18 (16, 20)	17 (14, 20)	17 (12, 23)	19 (15, 25)	13 (10, 16)	13 (10, 17)	0.0130
≥ 60	19 (18, 21)	18 (14, 23)	15 (12, 19)	17 (14, 20)	13 (10, 16)	13 (10, 16)	<0.0001
Sex							
Male	11 (10, 12)	14 (12, 16)	14 (10, 19)	16 (13, 20)	12 (9.1, 17)	13 (10, 16)	0.59
Female	21 (19, 23)	20 (17, 24)	20 (15, 25)	20 (16, 25)	16 (13, 19)	17 (14, 20)	0.0108
Race-ethnicity							
Mexican American	22 (19, 26)	21 (17, 26)	24 (18, 32)	28 (20, 37)	24 (17, 32)	23 (19, 27)	0.53
Non-Hispanic Black	51 (46, 55)	60 (57, 64)	53 (45, 61)	56 (49, 64)	52 (45, 60)	46 (37, 55)	0.09
Non-Hispanic White	10 (8.6, 11)	9.4 (7.6, 12)	9.1 (6.5, 12)	9.4 (7.1, 12)	6.2 (5.0, 7.8)	6.6 (4.9, 8.8)	0.0007
Supplement use ⁴							
No	19 (17, 20)	23 (19, 26)	22 (17, 28)	24 (19, 29)	19 (15, 24)	20 (16, 24)	0.91
Yes	9.1 (7.7, 11)	7.2 (5.6, 9.2)	7.9 (5.6, 11)	8.5 (6.3, 12)	5.1 (3.7, 7.0)	5.8 (4.3, 7.7)	0.0030

¹Values are weighted proportions (95% CIs). Data for NHANES 1988–2006 were standardized to LC-MS/MS equivalents; data for NHANES 2007–2010 were generated by using LC-MS/MS; sample sizes are shown in Table 1. LC-MS/MS, liquid chromatography–tandem mass spectrometry; 25(OH)D, 25-hydroxyvitamin D

²Linear trend based on Wald *F* test.

³40 nmol/L is the concentration consistent with an intake equivalent to the Estimated Average Requirement, which is useful for evaluating the possible adequacy of nutrient intakes of population groups.

⁴The use of any vitamin D–containing supplements in the month preceding the household interview.

standardization efforts conducted by the NIST, NIH, and CDC. At this time, there should be fewer problems with lot-to-lot variability in commercial or laboratory-developed tests for 25(OH)D.

Of particular interest is the significant increase in 25(OH)D in the population after 2006. It is difficult to know exactly when the interest in the potential healthful effects of vitamin D filtered down to the average American, but the increase in 25(OH)D in NHANES 2007–2010 appears to be consistent with several temporal trends. Toward the end of the decade, surges in 25(OH)D testing (29, 30) and vitamin D deficiency diagnoses (31) were reported, health care providers increasingly recommended vitamin D supplements at doses higher than the 1997 Dietary Reference Intake recommendations (32–36), and a growing number of foods were fortified with vitamin D (37, 38). Consumer spending on vitamin D supplements increased by >10-fold between 2001 and 2009 (39). Together, these data support the upward shift in the vitamin D status of the population at the end of the past decade, particularly in those taking vitamin D supplements.

Recent results from a racially diverse, multicenter cohort of women whose 25(OH)D measured in 1998–2000 and again in 2009–2011 (in a single-batch LC-MS/MS) showed a 16-nmol/L increase after adjustment for age, BMI, menopause status, location, and season; the adjusted increase in 25(OH)D was significantly higher in supplement users than in nonusers at 25 compared with 8 nmol/L, and the magnitude of the increase was similar across race-ethnic groups (40). A retrospective

population-based study showed a >20-fold increase in patients with 25(OH)D >125 nmol/L from 2002 to 2011; patients with high 25(OH)D concentrations were predominantly women, aged ≥ 50 y, and white (41). Incomplete data prevented conclusive evidence, but the increasing usage of prescription and over-the-counter supplements was suggested as the likely explanation for the increase in high concentrations of 25(OH)D (41). An observational study in men and women ≥ 40 y of age with the use of National Ambulatory Medical Care Survey data showed that the prevalence of visits involving the prescription of calcium and vitamin D steadily increased from 2000 to 2009, particularly for those aged ≥ 70 y, women, and whites (42). When we looked at supplement usage with the use of the NHANES data, we saw that, overall, the use of vitamin D–containing supplements has been stable for several decades. However, more persons in selected groups, in particular those persons ≥ 40 y old, females, and non-Hispanic whites, used higher-dose supplements in 2007–2010 (Figure 2). With regard to trends in other influential variables, a decline in BMI might positively affect 25(OH)D, but this has not been evident in recent NHANESs. During the period 2003–2010, the prevalence of obesity in the US population has shown little change in adults (43) and BMI trended upward in adolescent males aged 12–19 y during 1999–2010 (44). Other lifestyle factors that might explain the increase in 25(OH)D during 2007–2010 are milk consumption, sun exposure, and physical activity. We found that per capita consumption of fluid

milk decreased by 4% between 2006 and 2010 (45) and the percentage of adults who practice sun-protection behaviors (seeking shade, using protective clothing, or sunscreen) increased by ~5% between 2005 and 2010 (46). Neither of these changes would be expected to increase vitamin D status. However, the number of adults who engaged in no leisure-time physical activity declined by 6% between 2007 and 2010, whereas the number of adults who engaged in regular physical activity increased by 4% between 2008 and 2010 (47). If these activities occurred at least partly outdoors, these changes could be associated with greater sun exposure and increased endogenous synthesis of vitamin D₃ during 2007–2010.

The population reference ranges of 25(OH)D used by many clinical laboratories are based primarily on the DiaSorin radioimmunoassay (48, 49). Hollis argued that unless LC-MS methods are calibrated against DiaSorin methods, the DiaSorin reference range should not be used as a standard. By analogy, we considered whether to use the method comparison equations to adjust the IOM cutoff for risk of deficiency (30 nmol/L) to one that is standardized to the LC-MS/MS method. However, we cannot propose a single cutoff to correspond to the IOM cutoff because there are separate models for each survey cycle. Depending on the survey, the regression models predict LC-MS/MS-equivalent concentrations from 27 to 37 nmol/L for the 30-nmol/L cutoff. More important, there is currently a lack of agreement with regard to the threshold to define optimal 25(OH)D status, which further complicates efforts to standardize cutoffs. In particular, organizations such as the IOM (23), the Endocrine Society (50), the American Association of Clinical Endocrinologists/American College of Endocrinology/Obesity Society (51), Osteoporosis Canada (52), and the National Osteoporosis Foundation (53) have proposed different thresholds to define vitamin D insufficiency, ranging from 50 to 75 nmol/L. More complete information on the relations between health outcomes and vitamin D from several large randomized clinical trials (54, 55) that will become available within the next few years may provide more clear guidance with regard to the appropriate thresholds for 25(OH)D.

A major strength of the present study was the use of standardized 25(OH)D data to estimate the vitamin D status of the US population. The LC-MS/MS method that was used to standardize the results is state-of-the-art for measuring 25(OH)D metabolites, fully separating the C3 epimers from 25(OH)D₃ and 25(OH)D₂ and assessing other interferences by using ion ratios. A weakness of the present study is that regression is designed to predict the mean and is not optimal for predictions at the tails of the distribution, which includes those at risk of deficiency or excess. On the basis of regression, the variability in the LC-MS/MS-predicted data for 1988–2006 is expected to be underestimated, and thus inferences may fail to account for the true uncertainty of the predicted values. Furthermore, immunoassays are subject to nonspecific interferences; thus, even though the new method is accurate, the radioimmunoassay data underlying the predicted values are less than ideal. Finally, the occurrence of the significant increase in 25(OH)D in 2007–2010 corresponded to the period in which the assay method was changed from radioimmunoassay to LC-MS/MS. Although the radioimmunoassay original data were standardized to be LC-MS/MS equivalent to address this issue, we cannot completely rule out a possible role for the method change or prediction equation deficiencies in the observed increase in 25(OH)D in the population.

In conclusion, when 25(OH)D was expressed in LC-MS/MS equivalents, the vitamin D status of the US population aged ≥ 12 y was stable between 1988 and 2006 and then showed modest increases in 2007–2010. The increase in 25(OH)D at the end of the past decade corresponded in time with an increase in the use of supplements containing higher amounts of vitamin D. In addition to clarifying secular trends in vitamin D status of the US population, the present study highlights the importance of standardizing 25(OH)D measurements by using assay methods that are traceable to international reference materials.

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