National Estimates of Serum Total 25-Hydroxyvitamin D and Metabolite Concentrations Measured by Liquid Chromatography–Tandem Mass Spectrometry in the US Population during 2007–2010[1–4]

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Abstract

Background: The 2007–2010 NHANES provides the first US nationally representative serum 25-hydroxyvitamin D [25(OH)D] concentrations measured by standardized liquid chromatography–tandem mass spectrometry.

Objective: We describe patterns for total 25(OH)D and individual metabolites in persons aged ≥1 y stratified by race-ethnicity and grouped by demographic, intake, physiologic, and lifestyle variables.

Methods: We measured 25-hydroxycholecalciferol [$25(OH)D_3$], 25-hydroxyergocalciferol [$25(OH)D_2$], and C3-epimer of $25(OH)D_3$ [C3-epi-$25(OH)D_3$] in serum samples ($n = 15,652$) from the 2007–2010 cross-sectional NHANES [total 25(OH)D = $25(OH)D_3$ + $25(OH)D_2$].

Results: Concentrations (median, detection rate) of $25(OH)D_3$ (63.6 nmol/L, 100%) and C3-epi-$25(OH)D_3$ (3.40 nmol/L, 86%) were generally detectable; $25(OH)D_2$ was detectable in 19% of the population. Total 25(OH)D, $25(OH)D_3$, and C3-epi-$25(OH)D_3$ displayed similar demographic patterns and were strongly correlated (Spearman's $r > 0.70$). Concentrations of $25(OH)D_2$ (90th percentile) were much higher in persons aged ≥60 y (17.3 nmol/L) than in younger age groups (≤4.88 nmol/L). We noted significant race-ethnicity differences in mean total 25(OH)D [non-Hispanic blacks (NHBs), Hispanics, and non-Hispanic whites (NHWs): 46.6, 57.2, and 75.2 nmol/L, respectively] and in the prevalence of total 25(OH)D <30 nmol/L overall (24% of NHBs, 6.4% of Hispanics, and 2.3% of NHWs) as well as stratified by season (winter months: 30% of NHBs, 7.5% of Hispanics, and 3.8% of NHWs; summer months: 17% of NHBs, 4.4% of Hispanics, and 1.6% of NHWs). Persons with higher vitamin D intakes (diet, supplements, or both) and those examined during May–October had significantly higher total 25(OH)D. Significant race-ethnicity interactions in a multiple linear regression model confirmed the necessity of providing race-ethnicity–specific estimates of total 25(OH)D.

Conclusions: Race-ethnicity differences in the prevalence of low total 25(OH)D remained strong even after adjustment for season to account for the NHANES design imbalance between season, latitude, and race-ethnicity. The strong correlation between C3-epi-$25(OH)D_3$ and $25(OH)D_3$ may be because the epimer is a metabolite of $25(OH)D_3$. The presence of $25(OH)D_2$ mainly in older persons is likely a result of high-dose prescription vitamin D_2. *J Nutr* 2016;146:1051–61.

Keywords: NHANES, 25-hydroxyvitamin D_3, 25-hydroxyvitamin D_2, C3-epimer of 25-hydroxyvitamin D_3, LC-MS/MS

Introduction

Serum 25-hydroxyvitamin D [25(OH)D][9] is currently the biomarker of choice to assess vitamin D status, because it integrates dietary vitamin D intake from foods and supplements with endogenously produced cholecalciferol (also called vitamin D_3) by the action of solar UV-B radiation on 7-dehydrocholesterol. A

2013 systematic review found that worldwide population data on vitamin D status are limited, both overall and within key subgroups (1). Data from contemporary national surveys are even more limited (2–4). In 2015, the US Preventive Services Task Force published a new recommendation, which concluded that the current evidence is insufficient to assess the balance of benefits and harms of screening for vitamin D deficiency in asymptomatic adults (5). The inadequate quality of 25(OH)D testing methods was mentioned as a limiting factor.

Between 1988 and 2006, the NHANES produced nationally representative 25(OH)D data for the US population aged ≥12 y [1988–1994 (6)], ≥6 y [2001–2002 (7)], and ≥1 y [2003–2006 (7)] by using an RIA. Various reports documented method bias and imprecision problems with existing assays, particularly if they were immunoassay-based (8, 9). As technology advanced, LC coupled with tandem MS (LC-MS/MS) became more accessible for routine, high-volume analyses (10), reference methods and materials for 25(OH)D became available (11, 12), and the Vitamin D Standardization Program developed protocols for standardizing data from national surveys (13). A 2009 expert roundtable on 25(OH)D in NHANES recommended LC-MS/MS as a superior analytical technique going forward from 2007–2008 onward (14). The UK Food Standards Agency also recommended LC-MS/MS for their future National Diet and Nutrition Survey (15). Previously generated immunoassay data from the 2008–2010 Irish National Adult Nutrition Survey (NANS) were standardized to LC-MS/MS equivalents and results were compared with a full reanalysis of survey samples using LC-MS/MS (2).

Consistent with these other national surveys, the CDC laboratory began to measure 25(OH)D in NHANES 2007–2008 with the use of an accurate LC-MS/MS method standardized to international reference materials (16). The main objective of this article is to describe the latest vitamin D status of the US population aged ≥1 y participating in the NHANES 2007–2010 with these newly available 25(OH)D data generated by LC-MS/MS. We explored demographic patterns in total 25(OH)D and individual metabolites: 25-hydroxycholecalciferol [25(OH)D₃], 25-hydroxyergocalciferol [25(OH)D₂], and C3-epimer of 25(OH)D₃ [C3-epi-25(OH)D₃]. We also assessed the associations between total 25(OH)D and selected intake, physiologic, and lifestyle variables. Because of known race-ethnicity differences in 25(OH)D, we present data separately for Hispanics, non-Hispanic blacks (NHBs), and non-Hispanic whites (NHWs).

Methods

Study participants and study design. The NHANES, conducted by the National Center for Health Statistics at the CDC, collects cross-sectional data on the health and nutritional status of the civilian noninstitutionalized US population. The survey uses a stratified, multistage, probability-sample design. Survey participants are interviewed in their home, and sociodemographic, dietary supplement use, and health-related information is collected. The participants then visit a mobile examination center where they undergo various physical examinations, provide biological specimens, and complete a 24-h dietary recall (17). Although the sample design in 2007–2010 continued to oversample

various population groups, oversampling for Hispanic persons such that one can create estimates for Mexican Americans and all Hispanics (including Mexican Americans and other Hispanics) was new (18, 19). Of the 25,205 participants aged ≥1 y selected to participate in NHANES 2007–2010, 19,775 (78.5%) were interviewed, 19,142 (75.9%) agreed to the examination, and 15,652 (62.1%) provided serum samples for 25(OH)D testing (81.8% of those examined) (20). Estimated characteristics of the US population based on the study population are shown in **Supplemental Table 1**. All of the respondents gave their informed consent, and the NHANES protocol was reviewed and approved by the National Center for Health Statistics Research Ethics Review Board.

Laboratory methods. Serum samples from participants aged ≥1 y (n = 15,652) were analyzed by the CDC laboratory for 25(OH)D metabolites by use of LC-MS/MS: 25(OH)D₃, n = 15,651; 25(OH)D₂, n = 15,651; and C3-epi-25(OH)D₃, n = 15,473 (16). Serum total 25(OH)D (n = 15,650) was calculated as the sum of 25(OH)D₃ and 25(OH)D₂, not including C3-epi-25(OH)D₃. The epimer was chromatographically resolved from 25(OH)D₃ to avoid misclassification bias. When metabolite results were below the limit of detection (LOD), the NHANES data release contained an imputed value (LOD divided by the square root of 2). We used the imputed value for 25(OH)D₂ in our total 25(OH)D calculation when 25(OH)D₂ was less than the LOD (2.05 nmol/L); no 25(OH)D₃ result was less than the LOD (2.23 nmol/L). Serum total 25(OH)D was missing if the CDC laboratory was unable to obtain a valid result for either 25(OH)D₃ or 25(OH)D₂. Westgard-type quality-control multi-rules were used to judge assay performance (21). Long-term CVs for 3 serum quality-control pools analyzed in every assay were <4% for 25(OH)D₃ (29.2–87.3 nmol/L), <6% for 25(OH)D₂ (14.9–62.6 nmol/L), and ≤9% for C3-epi-25(OH)D₃ (8.67–42.7 nmol/L) (**Supplemental Table 2**) (22, 23). Additional method performance information, such as bias relative to international reference materials, is also described in Supplemental Table 2.

Study variables. We categorized the demographic variables as follows: age group based on standard NHANES reporting protocol (1–5, 6–11, 12–19, 20–39, 40–59, and ≥60 y), sex (male and female), and race-ethnicity [Hispanic (Mexican American + other Hispanic), NHB, and NHW; other racial-ethnic groups were included in overall estimates but estimates were not shown for them separately]. We also reported separate estimates (means and selected percentiles) for Mexican Americans as part of the supplemental tables to allow comparison to previous reports. Race-ethnicity was self-reported by the survey respondent. We examined selected physiologic and lifestyle variables previously shown to be associated with serum total 25(OH)D (6, 24–26), namely the following: inflammation as assessed by using serum C-reactive protein measured by latex-enhanced nephelometry [<10 mg/L (no inflammation) and ≥10 mg/L (inflammation)] (27), BMI (in kg/m²) based on measured height and weight [<18.5 (underweight), 18.5 to <25 (normal-weight), 25 to <30 (overweight), and ≥30 (obese); limited to adults ≥20 y] (28), practice of sun protection behaviors (very likely, somewhat likely, and unlikely; limited to adults 20–59 y) (26), season of blood draw [winter months (November–April) and summer months (May–October)], and latitude [southern (25–34°N) and northern (≥35°N)] where the specimen was collected (6). Last, we examined selected intake variables: any dietary supplement use (yes or no), dietary intake of vitamin D from food (<200 or ≥200 IU/d), supplemental intake of vitamin D (none, <400 IU/d, or ≥400 IU/d), and total vitamin D intake from diet and supplements (<200 or ≥200 IU/d) (for details, see **Supplemental Methods**).

Statistical methods. We performed statistical analyses using SAS (version 9.3; SAS Institute) and SUDAAN (version 11.0.0; RTI) software to address the complex survey design and used the examination weights to account for differential nonresponse or noncoverage and to adjust for oversampling of some groups. We combined 2 periods (2007–2008 and 2009–2010) to provide estimates with greater statistical precision and to increase the sample size for multilevel stratifications. We used no exclusion criteria and pairwise deletion for missing values in bivariate analyses. The distributions of total 25(OH)D and 25(OH)D₃ were reasonably symmetric, but the 25(OH)D₂ and C3-epi-25(OH)D₃ and

distributions were right-skewed (**Supplemental Figure 1**). We calculated arithmetic or geometric means and selected percentiles (95% CIs) by demographic characteristics and calculated the contribution of $25(OH)D_3$ relative to total $25(OH)D$ and of C3-epi-$25(OH)D_3$ relative to $25(OH)D_3$. Spearman coefficients were used to assess pairwise correlations among $25(OH)D$ metabolites. We expressed the prevalence of total $25(OH)D$ by season, age group, and sex at cutoff values defined by the Institute of Medicine [<30, <40, <50, and >125 nmol/L (29)] or recommended by the Endocrine Society [<75 nmol/L (30)]. Bivariate associations were evaluated between mean total $25(OH)D$ and selected intake, physiologic, or lifestyle variables; and means were compared across the categories by using the Wald F-test. We used a 2-sided significance level of $\alpha = 0.05$ to indicate differences for statistical comparisons.

We developed a covariate-adjusted multiple linear regression model for total $25(OH)D$ using backward elimination. The initial model retained all study variables that had a P value < 0.25 for each of the bivariate associations with $25(OH)D$ for all the race-ethnicity groups. Variables that were significant ($P < 0.05$) remained in the model after each elimination step. Other variables were omitted if there was no evidence of data-based confounding (>30% change in the variable estimate). Pairwise interactions with race-ethnicity were explored in the final model, and we retained any significant interactions. To compare the fully adjusted model with the bivariate results, we constructed partially adjusted models for each of the covariates; these included race-ethnicity, a single variable (demographic, intake, life-style, or physiologic factor), and the corresponding pairwise interaction. To facilitate the interpretation of the race-ethnicity interactions, we held the level of race-ethnicity constant when reporting the β coefficients.

Results

Overall serum total 25(OH)D and metabolite concentrations and correlations. Mean total $25(OH)D$ concentrations for 2007–2008 and 2009–2010 were similar (67.9 and 68.1 nmol/L; $P = 0.89$). We therefore combined data from both survey periods. The concentration ranges of total $25(OH)D$, $25(OH)D_3$, $25(OH)D_2$, and C3-epi-$25(OH)D_3$ were 5.41–262, 3.02–260, <LOD (2.05)−183, and <LOD (1.64)−37.8 nmol/L, respectively. We found detectable $25(OH)D_3$ and C3-epi-$25(OH)D_3$ in 100% and 86% of the population, respectively, but detected $25(OH)D_2$ in only 19% of the population. We found strong positive and significant Spearman correlations between total $25(OH)D$ and $25(OH)D_3$ ($r = 0.95$, $P < 0.0001$), between total $25(OH)D$ and C3-epi-$25(OH)D_3$ ($r = 0.75$, $P < 0.0001$), and between $25(OH)D_3$ and C3-epi-$25(OH)D_3$ ($r = 0.77$, $P < 0.0001$). The positive correlation between total $25(OH)D$ and $25(OH)D_2$ ($r = 0.13$, $P < 0.0001$) was significant but weak. The correlation between $25(OH)D_3$ and $25(OH)D_2$ was not significant ($P = 0.0542$).

Serum total 25(OH)D and metabolite concentrations grouped by demographic variables. Visual inspection revealed similar demographic patterns of median total $25(OH)D$ (**Figure 1**A) and median $25(OH)D_3$ (Figure 1B), such as a U-shaped age pattern, similar concentrations in males and females, and pronounced race-ethnicity differences. Because $25(OH)D_2$ concentrations were detectable only in ~20% of the population, we evaluated demographic patterns at the 90th percentile (Figure 1C). Persons aged ≥ 60 y showed several-fold higher $25(OH)D_2$ (17.3 nmol/L) than did younger age groups [<LOD (1.64)−4.88 nmol/L], whereas race-ethnicity differences in $25(OH)D_2$ were less pronounced (2.10–5.02 nmol/L). The median concentration of C3-epi-$25(OH)D_3$ was small (3.40 nmol/L) compared with total $25(OH)D$ (66.7 nmol/L) or

$25(OH)D_3$ (63.6 nmol/L). Concentrations of C3-epi-$25(OH)D_3$ showed demographic patterns similar to $25(OH)D$ and $25(OH)D_3$, but age group differences were more pronounced (Figure 1D).
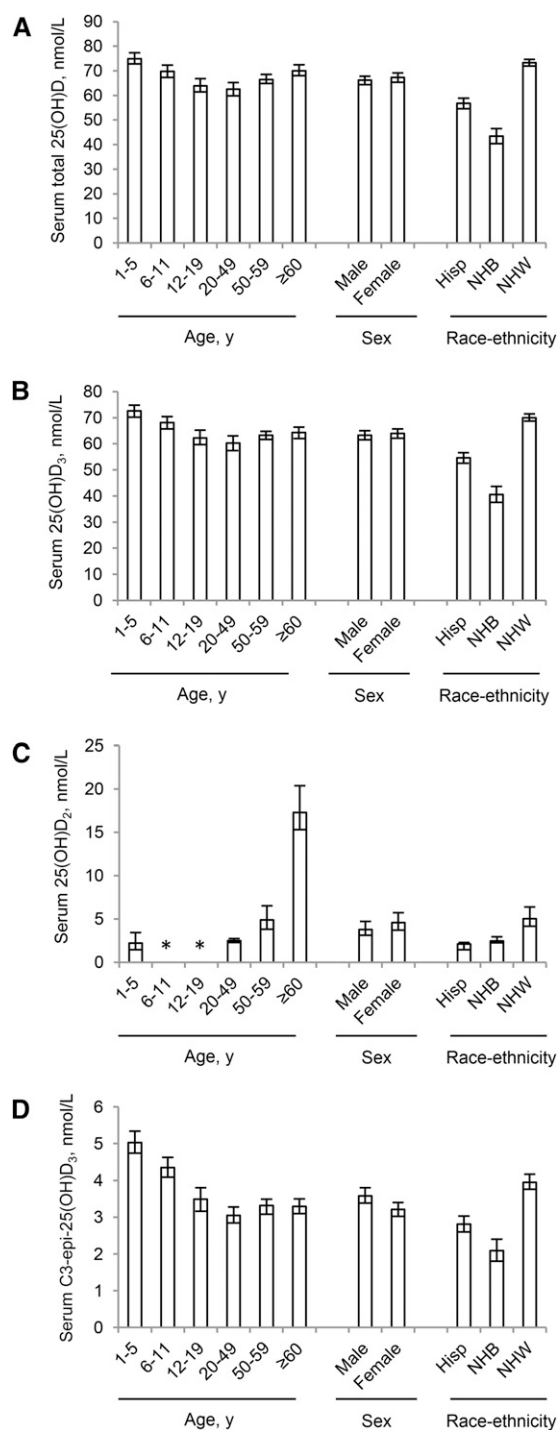
FIGURE 1 Demographic patterns of total $25(OH)D$ (A), $25(OH)D_3$ (B), $25(OH)D_2$ (C), and C3-epi-$25(OH)D_3$ (D) for persons aged ≥ 1 y: NHANES 2007–2010. Values are medians for total $25(OH)D$, $25(OH)D_3$, and C3-epi-$25(OH)D_3$ and 90th percentiles for $25(OH)D_2$; error bars denote 95% CIs. *$25(OH)D_2$ concentrations were less than the limit of detection (i.e., <2.05 nmol/L) for participants aged 6–11 and 12–19 y. C3-epi-$25(OH)D_3$, C3-epimer of 25-hydroxycholecalciferol; Hisp, Hispanic; NHB, non-Hispanic black; NHW, non-Hispanic white; $25(OH)D$, 25-hydroxyvitamin D; $25(OH)D_2$, 25-hydroxyergocalciferol; $25(OH)D_3$, 25-hydroxycholecalciferol.

Serum total 25(OH)D and metabolite concentrations stratified by race-ethnicity. Overall, we noted a U-shaped age pattern for mean total 25(OH)D, with children (1–5 y) having significantly higher and adolescents (12–19 y) and adults (20–59 y) having significantly lower concentrations than older adults (≥60 y) (**Table 1**). However, when stratified by race-ethnicity, the U-shaped age pattern was not apparent in NHWs for whom total 25(OH)D declined from childhood to a relatively flat level throughout adulthood. Overall, females had higher total 25(OH)D than did males, but the sex pattern was also different by race-ethnicity: Hispanic females had lower, NHW females had higher, and NHB females had similar total 25(OH)D compared with their male counterparts. All pairwise race-ethnicity comparisons within a category of age group or sex were significant, with NHBs having the lowest, Hispanics having intermediate, and NHWs having the highest total 25(OH)D concentrations.

The central 95% reference intervals (2.5th–97.5th percentile) for total 25(OH)D and individual metabolites showed distinct race-ethnicity differences (**Table 2**). NHBs had the lowest, Hispanics had intermediate, and NHWs had the highest reference intervals for total 25(OH)D, 25(OH)D_3, and C3-epi-25(OH)D_3. Hispanics had the lowest reference interval for 25(OH)D_2, whereas NHBs and NHWs had similar reference intervals. Detailed sets of percentiles categorized by age group and sex and stratified by race-ethnicity are available for total 25(OH)D, 25(OH)D_3, 25(OH)D_2, and C3-epi-25(OH)D_3 (**Supplemental Tables 3–6**, respectively).

Contribution of metabolites to serum total 25(OH)D concentrations. Serum 25(OH)D_3 made up ~95% of total 25(OH)D. Mean C3-epi-25(OH)D_3 increased significantly in each quartile of 25(OH)D_3, overall and by age group (1–19 y, ≥20 y) (**Supplemental Table 7**). The relative amount of C3-epi-25(OH)D_3 compared with 25(OH)D_3 was ~6–7%, with a significantly higher proportion in the highest quartile than in the other 3 quartiles. Whereas C3-epi-25(OH)D_3 was detected in the majority of the population, the detection frequency varied with age and race-ethnicity (**Table 3**). Children aged 1–5 y and 6–11 y showed the highest detection frequency (98% and 96%, respectively) compared with older age groups (83–88%). Among race-ethnicity groups, NHWs (91%) showed the highest and NHBs (64%) showed the lowest detection frequency.

Regardless of the demographic subgroup, vitamin D supplement users showed higher detection frequencies than did nonusers. We detected 25(OH)D_2 in <20% of the population overall, but the detection frequency varied with age (35% in persons ≥60 y, 21% in 20–39 y olds, and <15% in younger persons), race-ethnicity (22% in NHWs compared with 10–12% in Hispanics and NHBs), and supplement use (34% in users compared with 10% in nonusers) (Table 3).

Prevalence of serum total 25(OH)D at selected cutoff values stratified by race-ethnicity. When we applied the selected cutoff values, we found that overall, 5.9%, 13%, 24%, and 64% of the population had total 25(OH)D concentrations <30, <40, <50, and <75 nmol/L, respectively, whereas 2.3% had concentrations >125 nmol/L (**Table 4**). However, we observed a seasonal difference with prevalence estimates for low total 25(OH)D being higher during the winter months (November–April) than during the summer months (May–October). Twenty-four percent of NHBs (30% in winter compared with 17% in summer months) compared with only 6.4% of Hispanics (7.5% in winter compared with 4.4% in summer months) and 2.3% of NHWs (3.8% in winter compared with 1.6% in summer months) had total 25(OH)D concentrations <30 nmol/L. On the other hand, 3.4% of NHWs compared with <1% of Hispanics and <1% of NHBs had total 25(OH)D concentrations >125 nmol/L, with little difference between winter and summer months.

Associations between serum total 25(OH)D and selected intake, physiologic, or lifestyle variables stratified by race-ethnicity. Persons with higher intakes of vitamin D (diet, supplements, or both) and those who reported consuming any supplements had significantly higher total 25(OH)D (**Table 5**) and C3-epi-25(OH)D_3 (**Supplemental Table 8**), overall and within each race-ethnicity subgroup. All pairwise race-ethnicity comparisons within a category of intake were significant. We observed inverse associations between total 25(OH)D or C3-epi-25(OH)D_3 and inflammation as measured by elevated serum C-reactive protein concentrations and between total 25(OH)D or C3-epi-25(OH)D_3 and BMI, both overall and in each race-ethnicity subgroup. Persons who were examined during May–October had significantly higher total 25(OH)D or

TABLE 1 Serum total 25(OH)D stratified by race-ethnicity and grouped by age or sex for persons aged ≥1 y: NHANES 2007–2010[1]

Group	Total[2]		Hispanic[3]		Non-Hispanic black		Non-Hispanic white	
	n	nmol/L	n	nmol/L	n	nmol/L	n	nmol/L
All	15,650	68.0 (66.2, 69.8)	5138	57.2 (55.4, 59.0)	2997	46.6 (43.8, 49.3)	6711	75.2 (73.6, 76.8)
Age, y								
1–5	1349	76.5* (74.4, 78.7)	550	70.6* (68.8, 72.3)	277	63.3* (60.2, 66.3)	438	84.4* (81.1, 87.8)
6–11	1855	72.2 (69.6, 74.7)	782	63.0* (61.0, 65.1)	409	57.4* (55.1, 59.6)	558	80.3* (76.4, 84.3)
12–19	2118	65.8* (63.1, 68.4)	837	54.3* (51.8, 56.7)	479	42.3* (39.1, 45.4)	683	76.5 (73.2, 79.8)
20–39	3349	64.6* (62.2, 66.9)	1104	53.6* (51.2, 56.0)	602	40.6* (37.1, 44.1)	1433	74.4 (72.3, 76.6)
40–59	3377	67.9* (66.0, 69.9)	1043	56.4* (54.2, 58.7)	625	45.4* (41.6, 49.2)	1549	74.1 (72.1, 76.1)
≥60	3602	71.0[ref] (68.9, 73.0)	822	59.2[ref] (57.3, 61.2)	605	52.0[ref] (48.6, 55.5)	2050	74.4[ref] (72.2, 76.6)
Sex								
Male	7802	66.8[ref] (65.0, 68.7)	2499	58.7[ref] (56.9, 60.5)	1486	47.0[ref] (44.1, 49.9)	3411	73.0[ref] (71.2, 74.9)
Female	7848	69.1* (67.3, 71.0)	2639	55.6* (53.6, 57.6)	1511	46.2 (43.4, 49.0)	3300	77.3* (75.5, 79.2)

[1] Values are arithmetic means (95% CIs) unless otherwise indicated. All pairwise race-ethnicity comparisons within a category of age or sex differ, $P < 0.0001$ (t test). *Different from reference group for age or sex comparison within race-ethnicity subgroup, $P < 0.05$ (Wald F-test). ref, reference group for age or sex comparison within race-ethnicity subgroup; 25(OH)D, 25-hydroxyvitamin D.

[2] Estimates for "other" race-ethnicity group (persons with multiethnic background) are not shown but are included in total estimates.

[3] The Hispanic subgroup represents the sum of Mexican-American and other Hispanic ethnicities.

TABLE 2 Central 95% reference intervals for serum concentrations of total 25(OH)D and metabolites stratified by race-ethnicity for persons aged ≥1 y: NHANES 2007–2010[1]

Analyte	Total,[2] nmol/L		Hispanic,[3] nmol/L		Non-Hispanic black, nmol/L		Non-Hispanic white, nmol/L	
	2.5th	97.5th	2.5th	97.5th	2.5th	97.5th	2.5th	97.5th
Total 25(OH)D	23.5 (21.8, 25.1)	124 (120, 128)	24.2 (21.8, 25.6)	95.1 (93.5, 98.0)	15.4 (13.5, 17.0)	94.6 (90.1, 99.8)	30.6 (28.7, 32.3)	130 (126, 135)
25(OH)D$_3$	20.7 (19.4, 22.1)	120 (117, 124)	22.3 (19.8, 24.0)	92.9 (91.3, 96.2)	13.6 (11.5, 14.9)	89.6 (86.3, 94.2)	26.2 (24.8, 27.6)	126 (123, 131)
25(OH)D$_2$	<LOD	21.0 (19.0, 24.2)	<LOD	9.15 (7.55, 12.4)	<LOD	20.4 (16.7, 22.9)	<LOD	22.6 (20.2, 27.6)
C3-epi-25(OH)D$_3$	<LOD	10.7 (10.2, 11.7)	<LOD	7.36 (6.91, 7.98)	<LOD	6.45 (5.59, 7.83)	<LOD	11.9 (10.8, 13.1)

[1] Values are percentiles (95% CIs); for sample sizes, see Supplemental Tables 3–6. LOD [2.05 nmol/L for 25(OH)D$_2$ and 1.64 nmol/L for C3-epi-25(OH)D$_3$]; total 25(OH)D, total 25-hydroxyvitamin D [sum of 25(OH)D$_3$ and 25(OH)D$_2$]. C3-epi-25(OH)D$_3$, C3-epimer of 25-hydroxycholecalciferol; LOD, limit of detection; 25(OH)D$_2$, 25-hydroxyergocalciferol; 25(OH)D$_3$, 25-hydroxycholecalciferol.
[2] Estimates for "other" race-ethnicity group (persons with multiethnic background) are not shown but are included in total estimates.
[3] The Hispanic subgroup represents the sum of Mexican-American and other Hispanic ethnicities.

C3-epi-25(OH)D$_3$ than did those examined during November–April, both overall and in each race-ethnicity subgroup. We did not find significant differences in total 25(OH)D when participants were grouped by latitude (except for NHWs) or by practice of sun protection behaviors (except for Hispanics) [not tested for C3-epi-25(OH)D$_3$]. All pairwise race-ethnicity comparisons within a physiologic or lifestyle variable category were significant.

Covariate-adjusted serum total 25(OH)D concentrations. We developed a regression model for total 25(OH)D and examined pairwise interactions with race-ethnicity to assess the necessity of providing race-ethnicity–specific estimates and to assess the robustness of any conclusions drawn from the stratified results presented in Tables 1 and 5. The final fully adjusted regression model included age, sex, race-ethnicity, total vitamin D intake (from food and supplements), any supplement use, season, and BMI as well as significant interactions between race-ethnicity and age, sex, total vitamin D intake, or BMI (**Table 6**). When we compared this model with partially adjusted models that corresponded to stratified bivariate results, we noted that both models arrived at similar conclusions for sex,

total vitamin D intake, any supplement use, season, and BMI, although inflammation was no longer significantly associated with total 25(OH)D. However, the impact of the association of age with total 25(OH)D within each race-ethnicity group was different after simultaneously controlling for total vitamin D intake (dietary and supplements at 200 IU), any supplement use (no use), season (May–October), and BMI (25 kg/m^2) (Table 6, **Supplemental Figure 2**). We found no significant interaction with race-ethnicity for the effects of any supplement use or season in the fully adjusted model, suggesting that these effects were not modified by race-ethnicity, although the effect of any supplement use was attenuated by at least 50% after controlling for the other variables.

Discussion

To our knowledge, this article presents the first and most current US national data for total 25(OH)D obtained by a standardized LC-MS/MS method. Previous reports from NHANES relied on data from an RIA and focused on earlier time periods. To our knowledge, the present study also provides the first opportunity

TABLE 3 Prevalence of detectable serum C3-epi-25(OH)D$_3$ and serum 25(OH)D$_2$ concentrations stratified by vitamin D–containing dietary supplement use and grouped by age, sex, or race-ethnicity for persons aged ≥1 y: NHANES 2007–2010[1]

Group	C3-epi-25(OH)D$_3$, %			25(OH)D$_2$, %		
		Vitamin D supplements			Vitamin D supplements	
	Overall	Nonusers	Users	Overall	Nonusers	Users
All	85.6* (82.8, 88.1)	82.7 (79.3, 85.7)	91.1 (89.1, 92.7)	18.9* (16.8, 21.1)	10.5 (8.7, 12.6)	34.4 (31.5, 37.4)
Age, y						
1–5	98.1* (96.6, 98.9)	97.4 (95.5, 98.6)	99.4 (97.8, 99.8)	11.0* (8.7, 13.7)	8.3 (6.3, 10.9)	16.4 (11.8, 22.4)
6–11	96.3* (94.5, 97.5)	95.2 (93.0, 96.8)	98.6 (96.9, 99.4)	9.9* (7.3, 13.4)	6.9 (4.1, 11.3)	16.2 (12.5, 20.7)
12–19	87.8* (84.3, 90.6)	86.4 (82.5, 89.6)	93.7 (90.0, 96.1)	8.2* (5.5, 11.9)	6.2 (4.0, 9.4)	16.8 (11.1, 24.6)
20–39	82.7* (78.8, 86.0)	80.6 (76.2, 84.4)	88.4 (84.1, 91.6)	13.6* (11.9, 15.6)	8.4 (6.7, 10.4)	27.8 (23.9, 32.0)
40–59	84.7* (81.5, 87.4)	81.1 (77.2, 84.5)	90.4 (87.5, 92.6)	21.1* (17.9, 24.6)	12.1 (9.6, 15.3)	35.3 (31.0, 39.9)
≥60	83.0* (80.1, 85.8)	75.4 (70.4, 79.7)	90.3 (88.1, 92.1)	34.5* (31.4, 37.8)	18.6 (16.2, 21.3)	49.0 (44.7, 53.2)
Sex						
Male	88.6* (85.6, 91.0)	87.0 (83.4, 89.9)	92.2 (90.0, 94.0)	18.2* (15.7, 20.9)	10.0 (8.1, 12.3)	36.9 (32.5, 41.5)
Female	82.7* (79.8, 85.4)	78.0 (74.3, 81.2)	90.2 (88.0, 92.0)	19.5* (17.5, 21.7)	11.0 (9.1, 13.2)	32.6 (29.6, 35.6)
Race-ethnicity						
Hispanic[2]	82.4* (77.8, 86.2)	81.0 (76.3, 85.0)	87.8 (82.7, 91.5)	10.2* (9.2, 11.4)	6.4 (5.3, 7.6)	25.1 (21.9, 28.6)
Non-Hispanic black	64.2* (56.0, 71.7)	61.7 (53.1, 69.6)	72.4 (63.2, 80.0)	12.2* (11.1, 13.5)	7.5 (5.9, 9.6)	27.4 (23.4, 31.8)
Non-Hispanic white	91.0* (89.5, 92.2)	89.1 (87.3, 90.7)	93.7 (92.3, 94.9)	21.7* (19.1, 24.6)	12.0 (9.5, 15.0)	36.1 (32.8, 39.5)

[1] Estimates are weighted percentages (95% CIs). *Supplement users and nonusers differ, $P < 0.05$ (Wald F-test). C3-epi-25(OH)D$_3$, C3-epimer of 25-hydroxycholecalciferol; 25(OH)D$_2$, 25-hydroxyergocalciferol.
[2] The Hispanic subgroup represents the sum of Mexican-American and other Hispanic ethnicities.

TABLE 4 Prevalence of serum total 25(OH)D concentrations at various cutoff values stratified by race-ethnicity and grouped by season, age, or sex for persons aged ≥1 y: NHANES 2007–2010[1]

Cutoff value and group	Total[2]	Hispanic[3]	Non-Hispanic black	Non-Hispanic white
Less than 30 nmol/L				
All	5.9 (4.9, 7.1)	6.4 (5.1, 8.2)	24 (19, 29)	2.3 (1.7, 2.9)
Winter months[4]	9.9 (8.3, 12)	7.5 (5.6, 10)	30 (24, 36)	3.8 (2.7, 5.4)
Summer months[5]	3.3 (2.8, 3.9)	4.4 (3.2, 6.1)	17 (12, 23)	1.6 (1.2, 2.2)
Age, y				
1–11	0.7 (0.5, 1.1)	0.8 (0.4, 1.6)[6]	3.2 (2.2, 4.7)	0.0 (0.0, 0.3)[7]
12–19	5.9 (4.4, 7.7)	6.7 (4.2, 10)	25 (19, 32)	1.0 (0.5, 1.9)[6]
20–39	8.2 (6.6, 10)	9.7 (7.3, 13)	32 (26, 40)	1.7 (1.0, 2.9)
40–59	5.9 (4.7, 7.6)	6.3 (4.4, 8.9)	26 (19, 34)	2.5 (1.8, 3.6)
≥60	5.7 (4.6, 7.1)	5.6 (4.1, 7.6)	22 (17, 28)	3.9 (2.8, 5.5)
Sex				
Male	4.9 (3.9, 6.2)	4.6 (3.4, 6.1)	21 (16, 27)	1.7 (1.1, 2.4)
Female	6.9 (5.7, 8.2)	8.4 (6.5, 11)	26 (21, 31)	2.8 (2.2, 3.6)
Less than 40 nmol/L				
All	13 (11, 15)	18 (15, 22)	43 (38, 49)	5.8 (4.9, 7.0)
Winter months[4]	20 (18, 23)	21 (18, 25)	49 (44, 55)	9.3 (7.1, 12)
Summer months[5]	8.6 (7.5, 9.8)	13 (9.5, 18)	36 (29, 43)	4.4 (3.7, 5.3)
Age, y				
1–11	3.0 (2.2, 4.1)	3.2 (2.2, 4.6)	12 (8.2, 16)	0.5 (0.2, 1.6)[7]
12–19	14 (11, 17)	20 (15, 27)	47 (39, 56)	2.8 (1.6, 4.7)
20–39	18 (15, 20)	24 (20, 29)	56 (48, 63)	5.9 (4.4, 8.0)
40–59	13 (11, 16)	19 (16, 23)	47 (40, 55)	5.9 (4.5, 7.9)
≥60	13 (11, 15)	19 (16, 24)	39 (33, 45)	9.1 (7.5, 11)
Sex				
Male	11 (9.4, 14)	14 (12, 17)	41 (34, 48)	4.5 (3.4, 5.9)
Female	15 (13, 17)	23 (19, 27)	45 (40, 50)	7.2 (5.8, 8.8)
Less than 50 nmol/L				
All	24 (21, 27)	36 (32, 40)	62 (56, 67)	13 (12, 15)
Winter months[4]	35 (31, 38)	40 (36, 44)	68 (64, 71)	19 (17, 23)
Summer months[5]	17 (15, 19)	29 (23, 36)	55 (46, 63)	11 (9.2, 13)
Age, y				
1–11	9.7 (7.8, 12)	13 (10, 16)	29 (24, 34)	2.8 (1.8, 4.3)
12–19	25 (21, 30)	41 (34, 47)	70 (63, 76)	7.7 (5.4, 11)
20–39	30 (26, 34)	45 (39, 51)	75 (66, 82)	14 (12, 18)
40–59	24 (22, 28)	38 (33, 42)	65 (57, 72)	15 (12, 18)
≥60	22 (20, 24)	35 (31, 40)	53 (47, 59)	17 (15, 19)
Sex				
Male	22 (19, 25)	31 (27, 36)	60 (53, 66)	12 (9.9, 14)
Female	26 (23, 29)	41 (36, 45)	64 (58, 69)	15 (13, 17)
Less than 75 nmol/L				
All	64 (61, 66)	83 (81, 86)	91 (89, 93)	53 (50, 55)
Winter months[4]	75 (72, 78)	86 (83, 88)	93 (91, 95)	61 (58, 65)
Summer months[5]	57 (54, 59)	79 (75, 83)	88 (85, 91)	49 (46, 52)
Age, y				
1–11	56 (52, 61)	73 (69, 77)	83 (77, 87)	40 (32, 47)
12–19	70 (66, 73)	89 (85, 92)	97 (94, 99)	54 (48, 60)
20–39	69 (66, 73)	87 (84, 90)	96 (93, 97)	56 (52, 60)
40–59	64 (61, 66)	84 (81, 87)	92 (87, 94)	54 (52, 57)
≥60	56 (53, 60)	79 (76, 82)	82 (78, 86)	51 (47, 55)
Sex				
Male	66 (63, 69)	82 (79, 85)	92 (89, 94)	57 (54, 60)
Female	61 (59, 64)	84 (82, 87)	90 (88, 92)	49 (46, 51)
Greater than 125 nmol/L				
All	2.3 (1.9, 2.9)	0.1 (0.1, 0.3)[6]	0.4 (0.2, 0.7)[6]	3.4 (2.7, 4.2)
Winter months[4]	1.5 (1.0, 2.4)	<1[7]	0.4 (0.2, 0.9)[6]	2.9 (1.8, 4.7)
Summer months[5]	2.9 (2.2, 3.7)	<1[7]	<1[7]	3.5 (2.7, 4.6)

(Continued)

TABLE 4 *Continued*

Cutoff value and group	Total[2]	Hispanic[3]	Non-Hispanic black	Non-Hispanic white
Age, y				
1–11	1.2 (0.6, 2.7)[6]	<1[7]	<1[7]	2.3 (1.1, 5.0)[6]
12–19	2.3 (1.4, 3.8)	<1[7]	<1[7]	3.8 (2.3, 6.2)
20–39	2.9 (2.1, 4.0)	<1[7]	<1[7]	4.6 (3.2, 6.6)
40–59	2.1 (1.4, 3.1)	<1[7]	<1[7]	2.7 (1.9, 4.0)
≥60	2.6 (1.7, 4.0)	<1[7]	2.1 (1.1, 4.0)[6]	3.0 (2.0, 4.6)
Sex				
Male	1.0 (0.6, 1.7)	<1[7]	<1[7]	1.4 (0.8, 2.5)
Female	3.6 (3.0, 4.4)	0.2 (0.1, 0.4)[6]	0.6 (0.3, 1.2)[6]	5.2 (4.3, 6.4)

[1] Estimates are weighted percentages (95% CIs). The Institute of Medicine Dietary Reference Intake Report defines cutoff values for 25(OH)D as follows: <30 nmol/L, at risk for deficiency; 30 to <50 nmol/L, at risk for inadequacy; ≥50, sufficient; >125 nmol/L, may be reason for concern; and 40 nmol/L, targeted level for median dietary requirements (29). The Endocrine Society recommends concentrations of at least 75 nmol/L (30). 25(OH)D, 25-hydroxyvitamin D.

[2] Estimates for ''other'' race-ethnicity group (persons with multiethnic background) are not shown but are included in total estimates.

[3] The Hispanic subgroup represents sum of Mexican-American and other Hispanic ethnicities.

[4] Participants sampled from 1 November through 30 April.

[5] Participants sampled from 1 May through 31 October.

[6] 30% ≤ relative SE < 40%.

[7] Estimate suppressed, relative SE ≥40%.

to examine 25(OH)D metabolites, including C3-epi-25(OH)D$_3$, in a representative sample of the US population aged ≥1 y. Although the interest in this epimer has grown considerably, its biological significance is not yet clear. Some data suggest that the activated form, C3-epi-1,25-dihydroxyvitamin D$_3$, has less effect on vitamin D–related gene activation and calcemic activity than 1,25-dihydroxyvitamin D$_3$ (31). Other data suggest that the C3-epimer form potently suppresses parathyroid hormone secretion (32) and inhibits keratinocyte growth (31).

Previous information on individual metabolites of 25(OH)D came from studies that used convenience samples and were mostly small in size. However, 2 large cross-sectional population studies, the US Atherosclerosis Risk in Communities Study [ARIC; n = 13,108 (33)] and the Irish NANS [n = 1122 (34, 35)], recently published vitamin D metabolite data. We found a strong correlation between 25(OH)D$_3$ and C3-epi-25(OH)D$_3$ consistent with previous findings (34, 36, 37), although some studies, including ARIC, showed weaker (33, 38, 39) or no correlation (40). The metabolites 25(OH)D$_3$ and C3-epi-25(OH)D$_3$ showed similar demographic patterns compared with total 25(OH)D. Concentrations of C3-epi-25(OH)D$_3$ were detected in 86% of the population, but its detection frequency and concentrations were higher in children than in older age groups. With the exception of ARIC (33), other studies reported that the majority of adults and children showed detectable C3-epi-25(OH)D$_3$ (31, 41–43) and concentrations were usually higher in children than in adults (31, 44, 45). Similar to the Irish NANS (34), we found that the C3-epi-25(OH)D$_3$ proportion was ~6% of 25(OH)D$_3$.

We found the same significant associations between C3-epi-25(OH)D$_3$ or total 25(OH)D and intake or physiologic variables overall and within each race-ethnicity group—namely, positive associations with vitamin D intake, inverse associations with inflammation and BMI, and higher concentrations for persons examined during May–October. Cashman et al. (34) in the NANS also reported similar variables to be predictive of C3-epi-25(OH)D$_3$. Cholecalciferol from foods, supplements, and endogenous production in the skin are likely the common origins of 25(OH)D$_3$ and C3-epi-25(OH)D$_3$. Dose-response studies showed that C3-epi-25(OH)D$_3$ increased with dietary or supplemental cholecalciferol (46, 47). Skin cells showed the

C3-epimerization pathway (48), and the skin of mice fed diets containing cholecalciferol contained up to 20% of 25(OH)D$_3$ as the C3-epimer (49). To date, little to no C3-epimer has been found in vitamin D supplements (36, 39, 45), and C3-epimers of cholecalciferol or its metabolites have not been reported in foods.

If C3-epi-25(OH)D$_3$ is captured as part of 25(OH)D$_3$, participants could be misclassified as not being deficient. The misclassification bias was reported to be small (<5%) in adults (33, 34, 50). We also found a small misclassification bias at total 25(OH)D concentrations <30 nmol/L [prevalence of 5.0% (included) compared with 5.9% (not included)] and <40 nmol/L [prevalence of 12.0% (included) compared with 13% (not included)] (data not shown). In our experience, C3-epi-25(OH)D$_3$ more often than the other metabolites shows mass spectrometric interference and is measured with less precision. For the most accurate assessment, this metabolite should be chromatographically separated from 25(OH)D$_3$.

We detected 25(OH)D$_2$ in <20% of the population, but the detection frequency varied with age and supplement use. In fact, 49% of persons aged ≥60 y who reported taking vitamin D supplements had detectable 25(OH)D$_2$ compared with only 6–8% of children and young adults who were non–supplement users. Approximately one-fifth of adults aged ≥20 y (22%) with detectable 25(OH)D$_2$ reported taking vitamin D supplements at an average dose of 517 IU/d (median: 344 IU/d) (data not shown). This dose was higher than the average dose of supplements in participants with 25(OH)D$_2$ less than the LOD (220 IU/d; median: 0 IU/d; P = 0.0013), suggesting that the presence of 25(OH)D$_2$ was likely a result of high-dose prescription ergocalciferol. Furthermore, 25% of those with high total 25(OH)D (>125 nmol/L) had detectable 25(OH)D$_2$, suggesting supplementation with ergocalciferol (data not shown). However, we could not ascertain whether participants were using ergocalciferol because the NHANES dietary supplements files do not distinguish between cholecalciferol and ergocalciferol. Cashman et al. (34) reported age and vitamin D supplement use to be positive predictors of 25(OH)D$_2$. The 90th percentile of 25(OH)D$_2$ for Irish adults aged ≥18 y was 6.36 nmol/L (35). However, due to the lower LOD (0.44 nmol/L) compared with NHANES (2.05 nmol/L), 99% of Irish survey participants had detectable 25(OH)D$_2$ (34).

TABLE 5 Concentrations of serum total 25(OH)D stratified by race-ethnicity and grouped by selected intake, physiologic, or lifestyle variables for persons aged ≥1 y: NHANES 2007–2010[1]

	Total[2]		Hispanic[3]		Non-Hispanic black		Non-Hispanic white	
Variable	n	nmol/L	n	nmol/L	n	nmol/L	n	nmol/L
Dietary intake,[4] IU/d								
<200	9304	66.2 (64.4, 68.0)	2903	54.9 (52.7, 57.1)	2008	44.3 (41.6, 47.0)	3929	74.0 (72.6, 75.5)
≥200	5723	71.4 (69.6, 73.2)	2016	60.6 (59.1, 62.2)	877	52.2 (48.8, 55.6)	2551	77.3 (75.5, 79.2)
P		<0.0001		<0.0001		<0.0001		<0.0001
Supplemental intake, IU/d								
0	10,926	62.5 (60.4, 64.5)	4001	55.2 (53.3, 57.0)	2305	42.9 (40.0, 45.8)	4078	70.1 (68.1, 72.1)
<400	1889	72.0 (70.2, 73.7)	534	61.6 (59.9, 63.2)	288	51.2 (47.6, 54.8)	954	77.0 (75.0, 79.1)
≥400	2726	82.0 (80.2, 83.7)	559	67.7 (65.1, 70.3)	382	63.3 (59.8, 66.7)	1644	85.7 (84.1, 87.4)
P		<0.0001		<0.0001		<0.0001		<0.0001
Total intake,[5] IU/d								
<200	7001	60.8 (58.7, 63.0)	2418	53.1 (50.9, 55.2)	1629	40.9 (37.9, 43.9)	2608	68.9 (66.9, 71.0)
≥200	8026	73.9 (72.3, 75.5)	2501	61.5 (59.8, 63.2)	1256	54.1 (51.2, 57.1)	3872	79.6 (78.2, 81.1)
P		<0.0001		<0.0001		<0.0001		<0.0001
Any supplement use								
No	9413	62.1 (59.8, 64.3)	3549	55.2 (53.3, 57.1)	2042	42.8 (39.8, 45.9)	3366	70.1 (67.8, 72.3)
Yes	6228	75.1 (73.5, 76.6)	1586	61.8 (60.0, 63.7)	954	54.0 (51.4, 56.7)	3343	80.1 (78.6, 81.6)
P		<0.0001		<0.0001		<0.0001		<0.0001
Infection/ inflammation[6]								
CRP <10 mg/L	12,978	68.2 (66.4, 69.9)	4134	56.7 (54.8, 58.6)	2376	46.1 (43.3, 48.9)	5767	75.3 (73.8, 76.9)
CRP ≥10 mg/L	1190	62.4 (59.1, 65.6)	392	52.4 (50.2, 54.7)	278	41.6 (37.1, 46.2)	494	70.6 (66.5, 74.7)
P		<0.0001		0.0001		0.0184		0.0153
BMI[7,8]								
Underweight	163	72.2 (66.0, 78.5)	15	—[9]	39	50.3 (28.8, 71.8)	92	79.8 (72.6, 87.1)
Normal-weight	2711	73.6 (71.4, 75.8)	611	59.3 (56.5, 62.1)	416	46.4 (43.7, 49.0)	1465	81.2 (79.4, 83.0)
Overweight	3502	69.2 (67.3, 71.0)	1143	56.6 (54.7, 58.5)	525	46.4 (42.8, 50.1)	1687	75.7 (73.7, 77.6)
Obese	3831	60.6 (58.7, 62.5)	1173	51.8 (49.8, 53.7)	832	42.5 (39.4, 45.7)	1718	66.8 (65.0, 68.6)
P		<0.0001		<0.0001		0.0006		<0.0001
Sun protection behavior[10]								
Very likely	3153	65.6 (63.5, 67.7)	1024	53.9 (51.7, 56.1)	567	42.7 (39.2, 46.2)	1362	73.8 (71.8, 75.8)
Somewhat likely	2120	67.6 (65.4, 69.7)	528	57.5 (55.0, 60.0)	424	43.2 (39.7, 46.7)	1054	74.2 (72.2, 76.1)
Unlikely	1135	68.5 (65.6, 71.3)	355	56.7 (53.6, 59.8)	196	44.0 (40.1, 48.0)	542	75.8 (72.8, 78.9)
P		0.22		0.011		0.68		0.43
Season								
Winter months[11]	7261	60.6 (58.5, 62.7)	3371	55.4 (53.5, 57.3)	1597	43.3 (41.0, 45.6)	1865	70.1 (67.8, 72.5)
Summer months[12]	8389	72.8 (71.2, 74.3)	1767	60.5 (58.0, 62.9)	1400	50.5 (46.6, 54.3)	4846	77.3 (75.4, 79.2)
P		<0.0001		0.0016		0.0007		<0.0001
Latitude								
Southern[13]	5049	60.5 (57.5, 63.5)	2905	56.3 (53.9, 58.7)	946	45.3 (41.2, 49.4)	981	70.4 (66.7, 74.2)
Northern[14]	10,600	70.5 (68.7, 72.3)	2232	58.4 (56.1, 60.7)	2051	47.2 (43.5, 50.9)	5730	76.1 (74.5, 77.8)
P		0.59		0.20		0.51		0.008

[1] Values are arithmetic means (95% CIs). P values are based on the overall Wald F-test, which tests whether at least 1 of the means among the categories is significantly different; all pairwise comparisons across race-ethnic groups within the same category of each variable were significant at $P < 0.0001$. CRP, C-reactive protein; 25(OH)D, 25-hydroxyvitamin D.
[2] Estimates for ''other'' race-ethnicity group (persons with multiethnic background) are not shown but are included in total estimates.
[3] The Hispanic subgroup represents the sum of Mexican-American and other Hispanic ethnicities.
[4] 1 μg cholecalciferol = 40 IUs.
[5] Total intake represents the sum of dietary and supplemental vitamin D intake.
[6] CRP concentrations in serum were used to assess inflammation status (<10 mg/L, no inflammation; ≥10 mg/L, inflammation).
[7] BMI definitions (in kg/m²): <18.5 (underweight), 18.5 to <25 (normal-weight), 25 to <30 (overweight), and ≥30 (obese).
[8] Limited to adults aged ≥20 y.
[9] Estimate suppressed because considered statistically unreliable ($n < 30$).
[10] Limited to adults aged 20–59 y.
[11] Participants sampled from 1 November through 30 April.
[12] Participants sampled from 1 May through 31 October.
[13] Participants sampled at latitudes between 25°N and 34°N.
[14] Participants sampled at latitudes ≥35°N.

Older data from surveys that used immunoassays showed a positive relation of total 25(OH)D with sun exposure (3, 4, 51–54) and vitamin D intake from diet (52) or supplement use (3, 26, 51, 52) and a negative relation with BMI (3, 26, 52, 53) and inflammation (26). Our new LC-MS/MS data on total 25(OH)D showed similar associations. We confirmed that age, sex, race-ethnicity, total vitamin intake, any supplement use, season, and BMI were determinants of vitamin D status before

TABLE 6 Race-ethnicity–specific β coefficients from covariate-adjusted multiple linear regression models for serum total 25(OH)D grouped by selected demographic, intake, physiologic, or lifestyle variables for persons aged ≥1 y: NHANES 2007–2010[1]

	Partially adjusted model			Fully adjusted model		
	Hispanic[2]	Non-Hispanic black	Non-Hispanic white	Hispanic[2]	Non-Hispanic black	Non-Hispanic white
Age, y						
1–19	1.77 (−0.39, 3.94)	−0.69 (−4.07, 2.69)	4.66* (0.65, 8.67)	−4.41* (−6.9, −1.92)	−3.28* (−6.33, −0.23)	0.44 (−2.94, 3.82)
20–59	−4.53* (−6.51, −2.54)	−9.09* (−12.2, −6.04)	−0.18 (−2.10, 1.75)	−4.65* (−6.37, −2.94)	−7.18* (−9.85, −4.51)	2.68* (0.88, 4.48)
≥60	Ref	Ref	Ref	Ref	Ref	Ref
Sex						
Male	3.10* (1.75, 4.46)	0.81 (−0.64, 2.27)	−4.28* (−6.16, −2.41)	3.19* (1.93, 4.44)	0.05 (−1.28, 1.39)	−3.34* (−4.91, −1.78)
Female	Ref	Ref	Ref	Ref	Ref	Ref
Log$_{10}$ total intake[3]	14.2* (12.1, 16.3)	20.9* (17.7, 24.0)	17.0* (15.2, 18.9)	10.5* (8.31, 12.7)	17.7* (14.4, 20.9)	14.9* (13.0, 16.8)
Any supplement use						
No	−6.62* (−8.22, −5.01)	−11.2* (−13.8, −8.55)	−10.1* (−12.1, −8)	−3.59* (−5.04, −2.15)	−3.59* (−5.04, −2.15)	−3.59* (−5.04, −2.15)
Yes	Ref	Ref	Ref	Ref	Ref	Ref
Season						
Winter months[4]	−5.04* (−8.03, −2.06)	−7.2* (−11.1, −3.27)	−7.19* (−10.3, −4.09)	−6.91* (−9.26, −4.56)	−6.91* (−9.26, −4.56)	−6.91* (−9.26, −4.56)
Summer months[5]	Ref	Ref	Ref	Ref	Ref	Ref
Log$_{10}$ BMI	−41.6* (−45.5, −37.8)	−37.6* (−43.7, −31.5)	−53.8* (−62.9, −44.6)	−38.7* (−43.4, −33.9)	−30.6* (−38.7, −22.5)	−54.6* (−62.1, −47.1)
Inflammation[6]						
CRP <10 mg/L	4.27* (2.28, 6.27)	4.47* (0.80, 8.13)	4.72* (0.97, 8.48)	NS	NS	NS
CRP ≥10 mg/L	Ref	Ref	Ref	Ref	Ref	Ref

[1] Values are β coefficients (95% CIs). Each partially adjusted model includes race-ethnicity, a single variable (demographic, intake, lifestyle, or physiologic factor), and the corresponding pairwise interaction (race-ethnicity × age, race-ethnicity × sex, etc.); all interactions with race-ethnicity in the partially adjusted model were significant ($P < 0.05$, Satterthwaite F-test), except for inflammation (race-ethnicity adjusted β = 4.6) and season (race-ethnicity adjusted β = −6.81). The fully adjusted model was derived from backward elimination; variables in Tables 1 and 5 with a P value <0.25 for all race-ethnicity groups were included and all significant pairwise interactions with race-ethnicity were retained; persons who reported to be of other or multiple race-ethnicity groups were omitted from this analysis. To facilitate the interpretation of the race-ethnicity interactions, we held the level of race-ethnicity constant when reporting the β coefficients. *Significant β coefficient, $P < 0.05$ (Satterthwaite F-test). CRP, C-reactive protein; Ref, reference group; 25(OH)D, 25-hydroxyvitamin D.

[2] The Hispanic subgroup represents the sum of Mexican-American and other Hispanic ethnicities.

[3] Total intake represents the sum of dietary and supplemental vitamin D intake.

[4] Participants sampled from 1 November through 30 April.

[5] Participants sampled from 1 May through 31 October.

[6] CRP concentrations in serum were used to assess inflammation status (<10 mg/L, no inflammation; ≥10 mg/L, inflammation).

and after covariate adjustment, whereas inflammation was no longer a determinant after controlling for the other covariates examined in the present study. This is likely due to the known correlation between BMI and inflammation (55). The relation between latitude and total 25(OH)D is confounded by the NHANES seasonal design of sampling the lower latitudes during the winter months and the higher latitudes during the summer months (6). With the use of the results from the bivariate analyses for season or latitude stratified by race-ethnicity, we concluded that the season variable (which includes the latitude imbalance and is provided in the publicly released data) was a better predictor of 25(OH)D across race-ethnicity groups than the latitude variable (which includes the season imbalance but can only be derived by using the CDC Research Data Center).

The race-ethnicity patterns of total 25(OH)D seen in previous survey periods in which the RIA was used were similar to current periods in which the LC-MS/MS was used: NHWs > Hispanics > NHBs. We observed for each race-ethnicity group higher prevalence estimates of low total 25(OH)D during winter compared with summer months. However, even after we adjusted for season through stratification, the race-ethnicity differences in prevalence remained. This emphasizes the importance of reporting race-specific estimates of total 25(OH)D, which is still the accepted biomarker for vitamin D status. Studies investigating whether black Americans have lower vitamin D–binding protein concentrations than whites arrived at discordant results (56, 57), possibly due to technical issues with immuno-

assays. The ratio of 24,25-dihydroxyvitamin D to total 25(OH)D was shown to be positively associated with 25(OH)D$_3$, negatively correlated with parathyroid hormone, and to be similar in blacks and whites, suggesting that the ratio may have utility as biomarker for vitamin D status (58, 59).

Our study has limitations. The vitamin D intake data are likely underestimated because of underreporting of total intakes, underestimation of fortificant amounts in fortified foods and supplements as compared with label values, and failure of composition databases to account for naturally occurring food content of 25(OH)D (59–62). On the other hand, vitamin D status may be slightly overestimated because, by design, the NHANES visits northern states in the summer and southern states in the winter. Thus, stratifying by season in addition to race-ethnicity provides improved prevalence estimates to assess the current vitamin D status in the US population. We did not estimate usual intake, which adjusts for within-person variability and is preferred to describe the proportion of the population with intakes above or below threshold values. We instead assessed mean intake data collected at the time of the blood draw. However, measures of central tendency are generally not affected by within-person variability (63). The appropriateness of using RIA-derived cutoff values for total 25(OH)D data generated by LC-MS/MS has been questioned (64), but this issue cannot be addressed in the current study. Investigating various dietary sources of vitamin D and their relations with vitamin D metabolite concentrations was outside the scope of our analysis. Assessing long-term (1988–2010) temporal trends in vitamin D status of the

US population aged ≥ 12 y is the focus of a separate report that used RIA data to predict standardized LC-MS/MS equivalents for NHANES 1988–2006 (unpublished results, 2016).

The strengths of our study include the use of data from 2 survey periods measured with a new standardized LC-MS/MS method. This helps ensure the accuracy of the measurements as well as allowing comparability with other studies that use standardized laboratory methods. The high quality of the laboratory measurements was ensured by adhering to multiple levels of quality assurance and by following a strict traceability chain to international reference materials. To our knowledge, this study also presents the first nationally representative data on vitamin D metabolites for the US population aged ≥ 1 y and for individual race-ethnicity groups. In summary, we confirmed that race-ethnicity patterns of total 25(OH)D observed earlier and with a less specific laboratory method are still present. The similar patterns of C3-epi-25(OH)D$_3$ and 25(OH)D$_3$ relative to selected demographic, intake, physiologic, or lifestyle variables are not surprising given the probable common origin of these 2 compounds. The presence of 25(OH)D$_2$ mainly in older supplement users is likely a result of high-dose prescription vitamin D$_2$.

Acknowledgments

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