Cross-sectional evaluation of the relationship between vitamin D status and supplement use across levels of kidney function in adults

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ABSTRACT

Objectives The objective of this study was to assess vitamin D status of US non-pregnant adults using a standardised assay across 15 mL/min/1.73 m² increments of kidney function, report the use of dietary supplements containing vitamin D and assess relationships between vitamin D and markers of bone resorption.

Design This study is a cross-sectional evaluation.

Setting The study is from the US National Health and Nutrition Examination Survey in 2001–2012.

Participants The participants were non-institutionalised, non-pregnant adults, age ≥20 years.

Primary and secondary outcome measures The primary outcome measure was serum 25OHD evaluated using liquid chromatography-tandem mass spectrometry traceable to international reference standards. Secondary outcome measures were use of dietary supplements containing vitamin D and the serum intact parathyroid hormone and bone-specific alkaline phosphatase in a subset of participants.

Results The median 25OHD concentration in 27 543 US non-pregnant adults was 25.7 ng/mL (range, 2.2–150.0 ng/mL). Vitamin D supplements were used by 38.0%; mean (SE)=757 (43) international units/day. The range of 250HD concentration across groups, stratified by kidney function, was 23.0–28.1 ng/mL. The lowest concentration of 250HD observed was in people with higher kidney function (23.0 ng/mL for estimated glomerular filtration rate >105 mL/min/1.73 m²). Only 24% of people not taking a dietary supplement had a 250HD concentration >30 ng/mL. Serum intact parathyroid hormone inversely correlated with 250HD within all kidney function groups. Bone-specific alkaline phosphatase was also negatively associated with 250HD concentration.

Conclusions These data indicate that 250HD concentrations and supplement use may be suboptimal in a significant proportion of the population, across all kidney function levels. The response of bone resorption markers further suggests that 250HD levels could be improved. Together, these data support a re-evaluation of the 250HD concentration associated with health in adults.

INTRODUCTION

Research over the past 15 years has highlighted the importance of vitamin D in multiple medical conditions and disease states, leading to a developing interest in basal serum vitamin D concentrations of adults, measured as 25-hydroxycholecalciferol (calcidiol or 25OHD). Season, latitude and ethnicity are important considerations when evaluating 25OHD status. A high prevalence of insufficiency has been demonstrated in adults around the world,1–3 including the USA.4

The interpretation of ‘deficiency’ in these reports1–4 is based on concentrations of 25OHD that are associated with a higher risk of bone fractures. The National Academy of Medicine (NAM, formerly the Institute of Medicine) has recommended allowances of vitamin D (from dietary intake or supplementation) for US adults at 600 international units (IU), or 800 IU for adults >70 years, based on the amount of vitamin D required to achieve a concentration of at least 20 ng/mL in almost everyone.5, 6 However, others have recommended concentrations of 25OHD >30 ng/mL to support the functions of vitamin D beyond bone health.7–10
Although the influence of vitamin D on bone is the main and most widely studied role, vitamin D also exhibits a variety of non-skeletal or non-calcemic functions. Low 25OHD concentrations are associated with increased risk of infection, autoimmune disease, cancer (especially colon cancer), muscle weakness, diabetes mellitus and cardiovascular disease. Stronger evidence is necessary to better understand these associations.

Both kidney disease and obesity/insulin resistance are associated with lower vitamin D concentration. Vitamin D metabolism is altered by kidney dysfunction, which interrupts the nephrological hydroxylation of 25OHD, resulting in lower levels of 1,25(OH)₂D₃, the active hormonal form of vitamin D. 25OHD is also rarely sufficient in patients with chronic kidney disease (CKD), particularly patients with severely depressed kidney function. The decline in 25OHD concentration appears to occur when glomerular filtration rate (GFR) falls below 80 mL/min/1.73m². Low serum 25OHD decreases the availability of 25OHD in the kidney for 1α-hydroxylase conversion to 1,25-(OH)₂D at any level of kidney function. Additionally, whether vitamin D sequosters in adipose tissue or is diluted in relationship to an increased tissue mass, serum 25OHD levels are markedly decreased in the setting of obesity. Thus, the high proportion of overweight and obesity in the USA contributes to the prevalence of deficiency and insufficiency of serum 25OHD.

Variability in assays used across previously published studies further complicates understanding of vitamin D in populations. A good assay of vitamin D should appropriately uncouple vitamin D from its binding proteins and overcome matrix effects of the sample environment (e.g., ureaemia, low vitamin D binding protein) for an accurate measurement. For example, the variability of vitamin D results across races have been associated with race-related differences in vitamin D binding protein. Immunoassays, like the radioimmunoassay (RIA) used in the National Health and Nutrition Examination Survey (NHANES) from 2001 to 2006, are less reliable for detecting the true vitamin D concentration when the matrix is compromised, as occurs in uraemia. The preferred vitamin D assay should be consistently accurate, precise and specific and use internationally approved reference standards. In 2010, a committee was formed to devise a solution to the vitamin D assay variability. The National Centre for Health Services Research Ethical Review Board of the US Department of Health and Human Services. All participants provided written informed consent prior to study procedures.

MATERIALS AND METHODS

NHANES uses a complex, multistage probability sampling strategy to collect health data of US residents using 2-year sequences or cycles. Data from Southern residents are collected from November through April and from Northern residents from May through October. The data for this study were collected in six 2-year cycles (2001–2012) with vitamin D data released in October 2015 for the 2001–2010 cycles and in October 2017 for the 2011–2012 cycles. The study population consists of adults (age ≥20 years) who completed both the interview and the physical/laboratory examination components. Pregnant women were excluded. NHANES was approved by the National Centre for Health Services Research Ethical Review Board of the US Department of Health and Human Services. All participants provided written informed consent prior to study procedures.

Laboratory assessments

Serum creatinine was analysed by the isotope dilution mass spectrometry-traceable Synchron LX Creatinine Reagent kit (Beckman Coulter, Brea, California, USA; reference 0.7–1.3 mg/dL for men and 0.6–1.1 mg/dL for women) by Collaborative Laboratory Services, LLC (Ottumwa, Iowa, USA). In 2001–2006, the Diasorin RIA method was used to measure 25OHD. From 2007 to 2012, measurements of 25OHD were performed using the LC-MS/MS assay, traceable to international reference standards. A bridging study using stored specimens from 2001 to 2006, previously described, provided regression equations for applying the conversion of the RIA method to the LC-MS/MS assay for 2001–2006 (r=0.99). Vitamin D was measured as 25OHD (ng/mL) at the Fat Soluble Laboratories (CDC, Atlanta, Georgia, USA). The laboratory reference cut-points for the LC-MS/MS assay is <12 ng/mL, 12–20 ng/mL, >20 ng/mL and >50 ng/mL. Parathyroid hormone was measured as intact parathyroid hormone (iPTH, reference 18–74 pg/mL) using the ECL/Origen-electrochemiluminescence, Elecsys 1010 analyser (Roche, Basel, Switzerland) at Harborview Medical Centre, University of Washington (Seattle, Washington, USA). Bone-specific alkaline phosphatase (BAP) was analysed using the Access Octase assay (Beckman Coulter, Fullerton, California, USA), on the Beckman Access at University of Washington (Seattle, Washington, USA) (reference 3.7–20.0 µg/L for men and 2.9–14.5 µg/L for premenopausal women). No participants >49 years received BAP assessment.

Therefore, this study aimed to examine the 25OHD status of US non-pregnant adults measured by LC-MS/MS while accounting for the level of kidney function and dietary supplement use, and to characterise the relationship of vitamin D with other biological markers available in NHANES in 2001–2012.
Clinical assessments

Kidney function was assessed as estimated GFR (eGFR) using the Chronic Kidney Disease Epidemiology Consortium equation (CKD-Epi) and reported in increments of 15mL/min/1.73m² according to the recommendations of the Kidney Disease Improving Global Outcomes practice guidelines. NAM data set has only one value of serum creatinine rather than multiple values over 3 months; therefore, chronicity of kidney disease cannot be assumed from these data. However, even acute reductions in eGFR increase the risk for kidney disease, drug toxicities, metabolic complications, cardiovascular disease and mortality. Thus, the appropriate handling of an assessment of kidney function from cross-sectional data sets is to report the spectrum of kidney function without inferring chronicity.

For the purpose of this report, vitamin D sufficiency was defined as serum 25OHD concentration >30ng/mL, insufficiency as 20 to 30ng/mL and deficiency as <20ng/mL. The use of vitamin supplements during the previous 30 days containing vitamin D was examined. The type of supplement (single component, multivitamin with minerals) was recorded and the amount of the nutrient in the supplement was assessed.

Socioeconomic status was assessed as education level and family income level. Education was less than a high school diploma, high school graduate or general education development test equivalent, some college or Associate of Arts degree, or college graduate or above. Annual family income was <$US20,000; US$20,000 to <US$45,000; US$45,000 to <US$75,000 or ≥US$75,000.

iPTH was assessed only during 2003–2006. BAP was assessed in a subset of participants <50 years of age during 2001–2004. For comparisons with iPTH, only 2003–2004 data could be used. Demographic and clinical characteristics of the iPTH and BAP subsets are provided in online supplementary tables 1 and 2.

Patient involvement

NHANES is designed by the National Centre for Health Statistics of the CDC of the US Department of Health and Human Services. Participants are volunteers but are not invited to input study design. Participants are made aware of the study results through publication.

Statistical analysis

Complex survey statistics were evaluated using SAS Survey (SAS V.9.2, SAS Institute, Cary, North Carolina, USA) with appropriate weighting. Least squares mean (SE) for vitamin D and iPHTH were determined across all kidney function groups and compared using one-way analysis of variance with Bonferroni correction. Wald log-linear χ² was used to compare categorical variables across kidney function groups. Non-linear regression demonstrated the relationship of 25OHD to iPHTH, by choosing a model showing the lowest Akaike information criterion and Bayesian information criterion (linear, logarithmic, power or S-curve). For comparisons using BAP, due to using only a single 2-year cycle in adults 20 to 49 years of age, a simple random sample analysis was conducted. No imputation of missing data was performed because the missing data represented <10% of the potential participants.

RESULTS

During the 2001–2012 period of the NHANES, a total of 61,951 US non-institutionalised residents were surveyed (see online supplementary figure 1). After excluding pregnancy, a total of 30,232 participants ≥20 years of age completed both the interview and medical examination components. Additional exclusions comprised 2689 who did not have a serum 25OHD level or a serum creatinine for kidney function assessment. A comparison of evaluable and non-evaluable cases demonstrated that 8.9% of US non-pregnant adults were non-evaluable for the question (see online supplementary table 3). Thus, the final sample population was 27,543 non-pregnant US adults (see online supplementary figure 1).

Demographic and clinical characteristics of the US non-pregnant adult population demonstrated the diverse socioeconomic strata and proportion of obesity, hypertension and hyperglycaemia (table 1) similar to what has been reported elsewhere. As expected, participants with low GFR were significantly more likely to be older, men, have hypertension and diabetes and to be of lower education level and socioeconomic status. The presence of obesity, however, was consistent across kidney function level groups.

Vitamin D concentration and supplement use

The mean (SE) 25OHD concentration was 26.2 (0.2) ng/mL and ranged from 2.2 to 105.2 ng/mL (table 2) with a median of 25.7 ng/mL (see online supplementary table 4); fewer than 30% had a 25OHD >30 ng/mL. NAM reference range of vitamin D of ≥20 ng/mL bone health was met by 72.1% of the participants (see online supplementary table 5) and 27.9% demonstrated vitamin D insufficiency (<20 ng/mL).

Vitamin D supplement use was reported by 38.0% of study participants (n=9522; table 2 and online supplementary table 5) with an overall mean (SE) of 757.9 (5.4) IU per day. Only 24.0% (n=3179) of participants not taking a vitamin D supplement had a 25OHD concentration >30 ng/mL while 36.1% (n=8194) had serum 25OHD 20 ng/mL and 8.8% (n=2239) had serum 25OHD <12 ng/mL (online supplementary table 5). Of those in the highest vitamin D supplement group (2800 IU/d), only 6.7% (n=197) had serum 25OHD <20 ng/mL.

Vitamin D concentration and supplement use stratified by kidney function

The mean 25OHD concentration among kidney function level groups ranged from 23.0 to 28.1 ng/mL, with no apparent relationship to the degree of kidney dysfunction.
Table 1  Demographic and clinical characteristics of adults ≥20 years of age in NHANES 2001–2012 by level of kidney function

<table>
<thead>
<tr>
<th>Variable</th>
<th>All</th>
<th>&lt;30*</th>
<th>30-44*</th>
<th>45-59*</th>
<th>60-74*</th>
<th>75-89*</th>
<th>90-105*</th>
<th>&gt;105†</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>27543 (100)</td>
<td>444 (0.9)</td>
<td>1361 (3.5)</td>
<td>3401 (12.2)</td>
<td>4559 (19.3)</td>
<td>5071 (18.4)</td>
<td>5862 (22.4)</td>
<td>6845 (23.2)</td>
<td></td>
</tr>
<tr>
<td>Males, n (%)‡</td>
<td>13949 (49.4)</td>
<td>287 (59.4)</td>
<td>982 (68.9)</td>
<td>2464 (76.4)</td>
<td>2811 (66.1)</td>
<td>2520 (43.1)</td>
<td>2623 (42.2)</td>
<td>2262 (29.9)</td>
<td>&lt;0.001§</td>
</tr>
<tr>
<td>Females, n (%)‡</td>
<td>13594 (50.6)</td>
<td>157 (40.6)</td>
<td>379 (31.1)</td>
<td>937 (23.6)</td>
<td>1738 (33.9)</td>
<td>2551 (56.1)</td>
<td>3239 (57.8)</td>
<td>4583 (70.1)</td>
<td></td>
</tr>
<tr>
<td>Age, mean (SE) year</td>
<td>47.1 (0.3)</td>
<td>72.7 (0.7)</td>
<td>71.8 (0.5)</td>
<td>60.6 (0.4)</td>
<td>49.5 (0.3)</td>
<td>50.4 (0.4)</td>
<td>44.9 (0.2)</td>
<td>32.8 (0.2)</td>
<td>&lt;0.001¶</td>
</tr>
<tr>
<td>Race, non-Hispanic white, n (%)‡</td>
<td>13444 (71.1)</td>
<td>268 (75.2)</td>
<td>1011 (86.7)</td>
<td>2341 (86.1)</td>
<td>2777 (81.9)</td>
<td>2664 (76.9)</td>
<td>2427 (67.8)</td>
<td>1956 (50.5)</td>
<td>&lt;0.001§</td>
</tr>
<tr>
<td>BMI, mean (SE) kg/m²</td>
<td>26.4 (0.1)</td>
<td>28.8 (0.3)</td>
<td>29.2 (0.2)</td>
<td>28.6 (0.1)</td>
<td>28.7 (0.1)</td>
<td>28.4 (0.1)</td>
<td>28.6 (0.1)</td>
<td>28.1 (0.1)</td>
<td>&lt;0.001¶</td>
</tr>
<tr>
<td>Obesity, n (%)‡</td>
<td>9478 (33.5)</td>
<td>150 (35.4)</td>
<td>463 (35.3)</td>
<td>1102 (33.0)</td>
<td>1588 (34.4)</td>
<td>1687 (32.6)</td>
<td>2110 (34.4)</td>
<td>2378 (32.7)</td>
<td>0.07§</td>
</tr>
<tr>
<td>Hypertension, n (%)‡</td>
<td>10913 (33.9)</td>
<td>400 (92.5)</td>
<td>1129 (82.5)</td>
<td>2190 (56.3)</td>
<td>2038 (36.7)</td>
<td>2288 (38.4)</td>
<td>1893 (28.7)</td>
<td>975 (12.0)</td>
<td>&lt;0.001§</td>
</tr>
<tr>
<td>Hyperglycaemia, n (%)‡</td>
<td>3433 (8.8)</td>
<td>153 (34.1)</td>
<td>365 (26.4)</td>
<td>615 (12.6)</td>
<td>491 (6.9)</td>
<td>692 (9.6)</td>
<td>636 (7.7)</td>
<td>481 (5.1)</td>
<td>&lt;0.001¶</td>
</tr>
<tr>
<td>ACR, mean (SE) mg/g</td>
<td>30.7 (1.6)</td>
<td>657.6 (76.6)</td>
<td>124.0 (18.6)</td>
<td>31.7 (3.4)</td>
<td>19.8 (2.4)</td>
<td>20.3 (1.6)</td>
<td>18.3 (1.3)</td>
<td>19.0 (1.4)</td>
<td>&lt;0.001¶</td>
</tr>
<tr>
<td>ACR &lt;30, n(%)</td>
<td>23757 (90.9)</td>
<td>145 (35.9)</td>
<td>870 (68.5)</td>
<td>2748 (87.6)</td>
<td>4040 (93.0)</td>
<td>4450 (91.7)</td>
<td>5293 (93.4)</td>
<td>6211 (93.0)</td>
<td>&lt;0.001§</td>
</tr>
<tr>
<td>Education, n (%)‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001§</td>
</tr>
<tr>
<td>&lt;HS diploma</td>
<td>7775 (18.3)</td>
<td>182 (35.1)</td>
<td>480 (26.6)</td>
<td>963 (17.3)</td>
<td>1083 (14.1)</td>
<td>1427 (17.0)</td>
<td>1640 (17.3)</td>
<td>2000 (22.1)</td>
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</tr>
<tr>
<td>HS diploma or GED</td>
<td>6461 (24.1)</td>
<td>100 (27.6)</td>
<td>329 (26.0)</td>
<td>808 (24.1)</td>
<td>1158 (25.5)</td>
<td>1121 (23.3)</td>
<td>1324 (23.7)</td>
<td>1621 (23.7)</td>
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</tr>
<tr>
<td>Some college</td>
<td>7604 (30.8)</td>
<td>95 (22.1)</td>
<td>277 (22.7)</td>
<td>824 (28.0)</td>
<td>1236 (29.6)</td>
<td>1427 (32.0)</td>
<td>1670 (31.3)</td>
<td>2075 (33.1)</td>
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</tr>
<tr>
<td>College degree or higher</td>
<td>5668 (26.9)</td>
<td>64 (15.0)</td>
<td>272 (24.7)</td>
<td>801 (30.7)</td>
<td>1075 (30.8)</td>
<td>1087 (27.7)</td>
<td>1223 (27.7)</td>
<td>1146 (21.1)</td>
<td></td>
</tr>
<tr>
<td>Income, n (%)‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001§</td>
</tr>
<tr>
<td>&lt;US$20 000</td>
<td>7358 (19.9)</td>
<td>150 (31.6)</td>
<td>452 (25.3)</td>
<td>881 (16.3)</td>
<td>1099 (15.7)</td>
<td>1334 (19.0)</td>
<td>1445 (18.2)</td>
<td>1997 (26.0)</td>
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</tr>
<tr>
<td>$US20 000 to &lt;$US45 000</td>
<td>8995 (30.2)</td>
<td>177 (42.6)</td>
<td>474 (36.0)</td>
<td>1105 (29.8)</td>
<td>1423 (28.5)</td>
<td>1631 (30.5)</td>
<td>1826 (27.7)</td>
<td>2359 (32.4)</td>
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</tr>
<tr>
<td>$US45 000 to &lt;$US75 000</td>
<td>4869 (21.5)</td>
<td>58 (14.5)</td>
<td>235 (21.1)</td>
<td>613 (22.0)</td>
<td>836 (22.5)</td>
<td>873 (21.1)</td>
<td>1106 (23.3)</td>
<td>1148 (19.2)</td>
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<td>≥$US75 000</td>
<td>5354 (28.5)</td>
<td>37 (10.2)</td>
<td>151 (16.7)</td>
<td>705 (32.3)</td>
<td>1056 (33.7)</td>
<td>1050 (29.3)</td>
<td>1280 (31.2)</td>
<td>1075 (21.7)</td>
<td></td>
</tr>
</tbody>
</table>

*CKD-Epi, mL/min/1.73 m².
†Reference group.
‡Population or weighted proportion according to US population estimates for 2001 to 2012 for totals; group proportion for CKD-Epi groups.
§ Adjusted Wald log-linear χ². 
¶ One-way analysis of variance with Bonferroni-adjusted significance set at p < 0.00714.
ACR, albumin-to-creatinine ratio, mg/g; BMI, body mass index, kg/m²; CKD-EPI, Chronic Kidney Disease-Epidemiology Collaboration estimation formula for glomerular filtration rate, mL/min/1.73 m²; GED, general educational development test; HS, high school; NHANES, National Health and Nutrition Examination Survey.
The lowest mean value of serum 25OHD was noted among participants with eGFR >105 mL/min/1.73 m². Overall, the lowest 25OHD concentration ≥75 mL/min/1.73 m², representing 64% of the US non-pregnant adult population. In all kidney function groups, participants taking vitamin D supplements had significantly higher vitamin D concentration than those not taking supplements (p<0.001), but none demonstrated mean vitamin D levels within the sufficient range (>30 ng/mL) without oral supplementation, at any level of kidney function (see online supplementary table 6). The lowest proportion of participants considered vitamin D sufficient had eGFR >105 mL/min/1.73 m² (figure 1 and online supplementary table 7) followed by participants with eGFR 90–105 mL/min/1.73 m².

**Relationship of serum iPTH to serum vitamin D**

In US non-pregnant adults evaluated in NHANES 2003–2006, iPTH was highest in participants with a vitamin D concentration <20 ng/mL and was lowest in those with vitamin D concentrations >30 ng/mL (figure 2 and online supplementary table 7). This observation was consistent at each kidney function level. The iPTH concentration progressively increased as the measured 25OHD concentration decreased. The non-linear relationship (figure 3 and online supplementary table 8) of 25OHD and iPTH demonstrates an increased iPTH at 25OHD concentrations <40 ng/mL at each kidney function level.

### Table 2: Mean vitamin D concentration and proportion of participants taking vitamin D supplements according to kidney function level and dietary vitamin D supplement groups in NHANES 2001–2012

<table>
<thead>
<tr>
<th>Variable</th>
<th>All</th>
<th>&lt;30*</th>
<th>30-44*</th>
<th>45-59*</th>
<th>60-74*</th>
<th>75-89*</th>
<th>90-105*</th>
<th>&gt;105†</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>27,543 (100)</td>
<td>444 (0.9)</td>
<td>1,361 (3.5)</td>
<td>3,401 (12.2)</td>
<td>4,559 (19.3)</td>
<td>5,071 (18.4)</td>
<td>5,862 (22.4)</td>
<td>6,845 (23.2)</td>
</tr>
<tr>
<td>25OHD ng/mL, mean (SE)†</td>
<td>26.2 (0.2)</td>
<td>27.0 (0.8)</td>
<td>28.1 (0.5)</td>
<td>28.0 (0.3)</td>
<td>27.6 (0.3)</td>
<td>26.3 (0.3)</td>
<td>26.1 (0.3)</td>
<td>23.0 (0.3)</td>
</tr>
<tr>
<td>Taking ≥800 IU/day, n (%)‡§</td>
<td>2,351 (9.8)</td>
<td>50 (12.8)</td>
<td>144 (13.4)</td>
<td>354 (12.0)</td>
<td>448 (10.0)</td>
<td>525 (12.0)</td>
<td>512 (10.5)</td>
<td>318 (5.5)</td>
</tr>
<tr>
<td>Taking 400 to &lt;800 IU/day, n (%)§¶</td>
<td>4,391 (16.9)</td>
<td>97 (21.8)</td>
<td>340 (25.9)</td>
<td>779 (23.2)</td>
<td>70 (17.6)</td>
<td>900 (18.3)</td>
<td>867 (16.7)</td>
<td>648 (10.6)</td>
</tr>
<tr>
<td>Taking 1 to &lt;400 IU/day, n (%)§¶</td>
<td>2,780 (11.3)</td>
<td>19 (5.6)</td>
<td>91 (7.2)</td>
<td>317 (9.6)</td>
<td>511 (12.3)</td>
<td>534 (11.5)</td>
<td>649 (12.4)</td>
<td>659 (11.0)</td>
</tr>
<tr>
<td>Taking no vitamin D, n (%)§</td>
<td>18,021 (62.0)</td>
<td>278 (59.8)</td>
<td>786 (53.5)</td>
<td>1,951 (55.2)</td>
<td>2,840 (60.1)</td>
<td>3,112 (58.2)</td>
<td>3,834 (60.4)</td>
<td>5,220 (72.9)</td>
</tr>
<tr>
<td>25OHD ng/mL, mean (SE)§</td>
<td>34.8 (0.4)</td>
<td>42.3 (2.3)</td>
<td>37.9 (1.4)</td>
<td>35.6 (0.7)</td>
<td>35.1 (0.5)</td>
<td>35.9 (0.7)</td>
<td>34.1 (0.9)</td>
<td>31.0 (0.8)</td>
</tr>
<tr>
<td>Taking ≥800 IU/day‡</td>
<td>28.9 (0.2)</td>
<td>31.1 (1.4)</td>
<td>29.9 (0.6)</td>
<td>29.5 (0.4)</td>
<td>29.3 (0.4)</td>
<td>29.9 (0.4)</td>
<td>28.3 (0.4)</td>
<td>26.6 (0.4)</td>
</tr>
<tr>
<td>Taking 1 to &lt;400 IU/day‡</td>
<td>26.6 (0.3)</td>
<td>24.7 (2.6)</td>
<td>27.7 (0.9)</td>
<td>28.4 (0.7)</td>
<td>27.7 (0.5)</td>
<td>27.6 (0.4)</td>
<td>26.4 (0.5)</td>
<td>24.2 (0.4)</td>
</tr>
<tr>
<td>Taking no vitamin D‡</td>
<td>24.0 (0.2)</td>
<td>22.5 (0.8)</td>
<td>24.9 (0.6)</td>
<td>25.6 (0.4)</td>
<td>25.8 (0.3)</td>
<td>24.6 (0.3)</td>
<td>24.1 (0.3)</td>
<td>21.7 (0.3)</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.329</td>
<td>0.491</td>
<td>0.406</td>
<td>0.325</td>
<td>0.290</td>
<td>0.359</td>
<td>0.299</td>
<td>0.263</td>
</tr>
<tr>
<td>Parameter estimate (SE)</td>
<td>3.146 (0.10)</td>
<td>5.73 (0.66)</td>
<td>3.63 (0.38)</td>
<td>2.77 (0.21)</td>
<td>2.55 (0.16)</td>
<td>3.38 (0.19)</td>
<td>2.90 (0.22)</td>
<td>2.81 (0.17)</td>
</tr>
</tbody>
</table>

SI conversion factors: to convert 25OHD to nmol/L, multiply values by 2.5.

*CKD-Epi, mL/min/1.73 m².
†Reference group.
‡Wald log-linear χ², p<0.001.
§Population or weighted proportion according to US population estimates for 2001 to 2012 for totals, group proportion for CKD-Epi groups.
¶Domain regression analysis (vitamin D supplement group) for serum 25OHD concentration (dependent variable) and kidney function level group (independent variable), all P-values<0.001.
**Reference group.
CKD-Epi, Chronic Kidney Disease-Epidemiology Collaboration estimation formula for glomerular filtration rate; IU, international units; NHANES, National Health and Nutrition Examination Survey.
relationship rises more steeply for CKD-Epi \( \geq 75 \text{mL/min/1.73 m}^2 \) at 25OHD<30 ng/mL.

**Relationship of serum iPTH and serum vitamin D to serum bone-specific alkaline phosphatase**

Subgroup analysis of BAP demonstrated significant correlations with serum iPTH and 25OHD concentrations. For iPTH, a positive relationship with BAP occurred in participants with eGFR \( \geq 60 \text{mL/min/1.73 m}^2 \) \((r=0.16, \text{Parameter estimate (PE)}=0.53 \text{ (0.001), } p<0.001) \) but was stronger in those with eGFR <60 mL/min/1.73 m\(^2\) \((r=0.49, \text{PE}=4.00 \text{ (0.003), } p<0.001) \). In contrast, the relationship between BAP and 25OHD was negative at eGFR \( \geq 60 \text{mL/min/1.73 m}^2 \), \(r=-0.14, \text{PE}=-0.235 \text{ (0.001), } p<0.001\); and stronger at eGFR <60 mL/min/1.73 m\(^2\), \(r=-0.29, \text{PE}=-0.535 \text{ (0.001), } p<0.001\).

**DISCUSSION**

Due to variations between previously used immunological assays for serum vitamin D, large-scale cross-sectional analysis has not been able to be reliably performed. The present study used normalised data derived from the large cohort of NHANES participants collected from 2001 to 2012. During these years, the 25OHD status of US adults was measured by LC-MS/MS (either prospectively or retrospectively). The data presented here represent the 25OHD status of the US non-pregnant adult population determined by this gold standard assay methodology for vitamin D. Prior issues with assay performance have influenced the ability to determine vitamin D status due to immunoassay instability, variability in the assay types used across studies and performance of vitamin D assays under special circumstances. To our knowledge, the current study represents the first attempt to report these data across all levels of kidney function using the gold standard assay.

The present study confirms previous reports of a low prevalence of vitamin D sufficiency, when sufficiency is defined as >30 ng/mL, both in the general population and advanced kidney dysfunction. Despite the high prevalence of vitamin D insufficiency and deficiency, less than 40% of participants in the current study were taking vitamin D supplements. Even in those participants taking vitamin D supplements, the majority did not reach a 25OHD>30ng/mL, suggesting that improved supplemental vitamin D dosing strategies may be necessary to increase sufficiency.

In contrast to the variation of 25OHD with worsening kidney function noted in prior studies, and similar to cohort studies of kidney disease, this analysis did not demonstrate a correlation of 25OHD deficiency with kidney function. Instead, a mild inverted U-shape was observed. In fact, only 19.9% of participants with eGFR >105 mL/min/1.73 m\(^2\) were vitamin D sufficient, which was the lowest proportion of any of the kidney function groups examined. Similarly, only 31.9% of participants with the worst eGFR (<30 mL/min/1.73 m\(^2\)) were vitamin D sufficient. In this lowest eGFR group, the lower proportion of vitamin D sufficiency may be explained by known increases in 24-hydroxylase related to elevated fibroblast growth factor (FGF)-23 that would lead to increased catabolism and elimination of vitamin D. The low eGFR group also had the highest percent of lowest-income participants (31.6%) and the highest percent with less than high school education (35.1%), which might limit access to adequate diet and nutritional supplements. Perhaps contributing to the low prevalence of vitamin D sufficiency in participants with eGFR >105 mL/min/1.73 m\(^2\) may be the fact that fewer subjects in this group were taking supplemental vitamin D (only 27.1%) in comparison to any other group. Factors contributing to low prevalence of vitamin D sufficiency in this group may be the highest percent women (70.1%), younger age (mean age, 32.8 years) and the highest percent of non-Caucasians. Whereas vitamin D is an immune modulator and levels may be altered by inflammation (eg, an increased presence of FGF-23 activates 24-hydroxylase, thereby increasing the elimination of vitamin D), it may suppress inflammation (interleukin (IL)-2) and caspase 8. Further studies are needed to determine the effect on vitamin D sufficiency, which may be important in a large adult population.
promote anti-inflammatory cytokines (IL-10), it is not possible to explain the lower concentration of 25OHD in this highest eGFR group since FGF-23 or other markers of inflammation or anti-inflammatory markers were not available to evaluate.

An important observation in this study is the progressive increase in serum iPTH with decreasing 25OHD. This phenomenon was observed at each strata of kidney function, from normal to severely impaired. Also interesting was the further augmentation in the elevation of iPTH with decreasing vitamin D levels as kidney function declined. Similar observations were reported by Taal et al in a substudy of the Renal Risk in Derby study. Using the LC-MS/MS assay for 25OHD, investigators in this study reported an independent, negative relationship between vitamin D concentration and iPTH. In contrast to the present study, however, the population in the Taal report included only those with reduced kidney function. The reasonable conclusion is that 25OHD concentration must always be considered when evaluating iPTH in patients with impaired kidney function and people with normal kidney function. Furthermore, both iPTH and BAP are biomarkers of bone resorption and negatively correlate with vitamin D concentration. In the current study, a higher iPTH was demonstrated with a vitamin D concentration <40 ng/mL, regardless of kidney function level. This suggests possible bone resorption occurring at vitamin D concentrations within the range advocated by the NAM as being consistent with bone health. These early biomarkers of bone health (iPTH and BAP) offer further indication that a higher than currently recommended vitamin D concentration may be warranted.

Sources and methods of exposure to vitamin D are multifactorial. Using serum 25OHD concentration as a biomarker of vitamin D status has some benefit over reliance on dietary intake estimates. Similar to therapeutic drug monitoring, assessing blood levels has been used to indicate biological processes. Assignment of cutpoints to categorise sufficiency of the vitamin has recently gained attention as a biomarker of the pathogenic process of bone disease. By NHANES. However, since 1,25(OH)₂D is also dependent on physiological functions (eg, decreased synthesis of 7-dehydrocholesterol in skin, decreased circulating 25OHD, decreased renal functional mass, increased FGF-23), it cannot be assumed that 25OHD concentrations directly correlate with 1,25(OH)₂D concentrations. Regardless, a critical issue in understanding the implications of a deficient 25OHD concentration must include the downstream effect of such a deficiency on the concentration of 1,25(OH)₂D.

The non-skeletal related functions of vitamin D should also be addressed in relationship to levels of kidney function. For example, inflammation may provoke an increase in factors, such as FGF-23, that activate the degradation of vitamin D and, thereby, contribute to lower vitamin D concentration. Increased inflammation and decreased immune response often accompany decreased kidney function. Furthermore, one of the hallmarks of kidney dysfunction, hypertension, has been shown to have a relationship with vitamin D concentration wherein randomised, placebo-controlled trials demonstrated reductions in systolic blood pressure with vitamin D supplementation. Similarly, raising the 25OHD concentration through ultraviolet B exposure has also demonstrated a reduction in systolic blood pressure. These studies suggest additional reasons to maintain higher levels of vitamin D. Whereas a therapeutic dose of vitamin D that would be most beneficial has not been established for these conditions, recommendations for levels >30 ng/mL have been suggested. The vitamin D level considered to be normal for the general population may not be sufficient if additional functions of vitamin D (eg, non-skeletal vs skeletal) are to be considered. Even in participants with the best kidney function, this study demonstrated that iPTH negatively associated with vitamin D concentration.

The cross-sectional design of this study limits the ability to infer causation and we are unable to test the role that other compounds (eg, FGF-23 and 24-hydroxylase or the measurement of 1,25(OH)₂D) or environmental conditions contribute to the 25OHD and iPTH concentrations observed. Whereas participants were assessed at the time of year when sun exposure was lowest in the Southern
USA and highest in the Northern USA, an approach by NHANES to normalise the level of vitamin D exposure represented by the participants, it is still possible that usual vitamin D levels were not demonstrated in this cross-sectional study. Another consideration is that while NHANES performs a very thorough study of participants’ health, it is not possible to measure every factor of interest; assessment of vitamin D receptor activity would perhaps provide greater insight into the associations seen in this study. Additionally, the single-point serum creatinine in NHANES does not allow for assigning chronicity (or stages) to the kidney function level which limits the opportunity to confirm that the findings reflect steady-state metabolism or would endure longitudinally. We did not assess the total dietary intake of vitamin D in this cohort because the nutrient data tables did not include dietary vitamin D in 2001–2006. Further evaluation of dietary vitamin D intake in the kidney function level groups is recommended.

The mechanisms influencing 25OHD and iPTH concentrations are complex and the current study further corroborates the complexity. Research incorporating prospective longitudinal studies could improve our understanding of these phenomena and should account for both kidney function and vitamin D status and should use international standards and accurate assays when assessing 25OHD concentration.

CONCLUSIONS

25OHD concentration should be measured, using accurate assay methodology, when assessing iPTH or other early biomarkers of bone health, and account for kidney function level. Vitamin D supplementation results in increased 25OHD concentrations at each level of kidney function. Optimal 25OHD concentrations are associated with lower iPTH concentration at all levels of kidney function. Together, these data support a re-evaluation of the range of 25OHD concentration associated with health in the US non-pregnant adult population.

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Contributors

UWM and WNS designed research; UWM conducted research, performed statistical analysis and had primary responsibility for final content; UWM, WNS, KEL, OMS, RJK and AOG wrote the paper. All authors have read and approved the final manuscript.

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Disclaimer

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None declared.

Patient consent for publication

Not required.

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The National Center for Health Services Research Ethical Review Board of the United States Department of Health and Human Services.

Provenance and peer review

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Data sharing statement

No additional data are available from the authors. The data used for this study was compiled from public use files accessible to anyone from the US Centers for Disease Control and Prevention at https://www.cdc.gov/nchs/nhanes/.

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