Reproducibility of Serum 25-Hydroxyvitamin D and Vitamin D-Binding Protein Levels Over Time in a Prospective Cohort Study of Black and White Adults

Jennifer S. Sonderman*, Heather M. Munro, William J. Blot, and Lisa B. Signorello

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Prospective epidemiologic studies generally rely on 1 baseline biologic sample from participants for measurement of prediagnostic biomarkers, assuming that 1 measurement adequately represents the participant's "typical" level. The body of work assessing the reproducibility of circulating serum 25-hydroxyvitamin D (25(OH)D) levels over time focuses almost exclusively on populations of European descent, and data for vitamin D-binding protein (VDBP) are virtually nonexistent. Thus, the authors measured levels of serum 25(OH)D and VDBP twice in samples collected between 2005 and 2008 from 225 participants (155 black, 70 white) in the Southern Community Cohort Study. Reproducibility for 25(OH)D was uniformly high, with adjusted intraclass correlation coefficients (ICCs) of 0.84 (95% confidence interval (CI): 0.79, 0.88) for blacks and 0.92 (95% CI: 0.87, 0.95) for whites, and there was substantial agreement for assignment of 25(OH)D quartile ($\kappa$ = 0.83, 95% CI: 0.78, 0.87) and vitamin D adequacy status ($\kappa$ = 0.76, 95% CI: 0.69, 0.83). VDBP levels were highly stable over time, with adjusted ICCs of 0.97 (95% CI: 0.96, 0.98) for blacks and 0.96 (95% CI: 0.93, 0.97) for whites. These findings suggest that single, baseline 25(OH)D and VDBP serum measurements provide reasonably representative measures of these compounds for both white and black adults, demonstrating their utility as epidemiologic biomarkers in prospective studies.

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Prospective epidemiologic studies generally rely on 1 baseline biologic sample from participants for measurement of prediagnostic biomarkers, assuming that 1 measurement adequately represents the participant's "typical" level. The body of work assessing the reproducibility of circulating serum 25-hydroxyvitamin D (25(OH)D) levels over time focuses almost exclusively on populations of European descent, and data for vitamin D-binding protein (VDBP) are virtually nonexistent. Thus, the authors measured levels of serum 25(OH)D and VDBP twice in samples collected between 2005 and 2008 from 225 participants (155 black, 70 white) in the Southern Community Cohort Study. Reproducibility for 25(OH)D was uniformly high, with adjusted intraclass correlation coefficients (ICCs) of 0.84 (95% confidence interval (CI): 0.79, 0.88) for blacks and 0.92 (95% CI: 0.87, 0.95) for whites, and there was substantial agreement for assignment of 25(OH)D quartile ($\kappa$ = 0.83, 95% CI: 0.78, 0.87) and vitamin D adequacy status ($\kappa$ = 0.76, 95% CI: 0.69, 0.83). VDBP levels were highly stable over time, with adjusted ICCs of 0.97 (95% CI: 0.96, 0.98) for blacks and 0.96 (95% CI: 0.93, 0.97) for whites. These findings suggest that single, baseline 25(OH)D and VDBP serum measurements provide reasonably representative measures of these compounds for both white and black adults, demonstrating their utility as epidemiologic biomarkers in prospective studies.

Vitamin D has been of intense scientific interest over the past decade for its potential utility in preventing cardiovascular disease, certain cancers, and autoimmune diseases (1–3). In humans, the major source of vitamin D is sun exposure (ultraviolet B radiation); cholesterol compounds in the skin are converted to a preliminary form of vitamin D upon exposure to ultraviolet B radiation, which is then metabolized in the liver to 25-hydroxyvitamin D (25(OH)D), the major circulating metabolite, and then in the kidney and other organs to 1,25-dihydroxyvitamin D$_3$, the biologically active hormone calcitriol (1, 2, 4). Vitamin D-binding protein (VDBP) solubilizes and transports these compounds in sera, prolonging their half-life and providing some short-term protection against vitamin D deficiency (5).

Melanin in the skin cells absorbs much of the ultraviolet B energy before it has an opportunity to produce vitamin D (4, 6). Therefore, the prevalence of vitamin D inadequacy in the United States is higher in blacks than in whites, even after accounting for differences in dietary intake (6–8). Consequently, vitamin D has come under scrutiny as a potential mediator of certain health disparities, and prospective studies using prediagnostic vitamin D measurements offer a promising avenue for testing associations between vitamin D status and health outcomes and for assessing the impact of low vitamin D levels on the health of blacks and nonblacks alike.

Serum VDBP concentrations are generally presumed to remain stable throughout adulthood (5), whereas serum 25(OH)D levels (used to assess clinical vitamin D status...
(9) are known to vary dramatically in response to ultraviolet B exposure (4, 6). Apart from expected seasonal fluctuation, it is important to document within-person variation in 25(OH)D given that prospective studies chiefly rely on a single blood sample to capture baseline levels. The underlying assumption is that this one measurement adequately represents the participant’s “typical” baseline level and that repeated measurements would not exhibit substantial variability. Several recent studies in populations of European descent have examined within-person variation in circulating 25(OH)D levels, with most investigators (10–14), but not all (15), concluding that a single measurement is sufficiently reliable for use in association studies. It is unclear how well these findings generalize to blacks, among whom there are currently no published data to address this issue. Our objective, therefore, was to evaluate within-person variation in serum 25(OH)D and VDBP levels among black and white participants in the Southern Community Cohort Study (SCCS), a prospective cohort study designed to evaluate cancer and other health disparities and within which vitamin D will be assessed as a risk factor for a number of health endpoints.

MATERIALS AND METHODS

Study population and blood collection

Overall SCCS methods have been described in detail elsewhere (16, 17). Of the approximately 86,000 SCCS cohort members enrolled from 2002 to 2009 in 12 southeastern US states, approximately 39,400 (46%) provided a baseline, non-fasting 20-mL venous blood sample at the time of their enrollment and completion of an in-person baseline interview. These predominantly low-income and low-education participants, all of whom were blacks and whites aged 40–79 years who had not been treated for cancer (except nonmelanoma skin cancer) during the year preceding baseline and who enrolled in the study at their local community health center, served as the study base for this analysis. Their blood samples were refrigerated at the community health centers and kept chilled during overnight shipment to Vanderbilt University and Meharry Medical College (Nashville, Tennessee). All subjects provided written informed consent both for the main study and for the collection of the second blood sample.

Serum 25(OH)D and VDBP measurement

Serum 25(OH)D levels were measured at Heartland Assays, Inc. (Ames, Iowa) using a Food and Drug Administration-approved direct, competitive chemiluminescence immunoassay, the DiaSorin LIAISON 25-OH Vitamin D Total Assay (DiaSorin, Inc., Stillwater, Minnesota), which is co-specific for 25-hydroxyvitamins D2 and D3 (18, 19). VDBP levels were also measured at Heartland Assays, Inc., using the R&D Systems Human Vitamin D Binding Protein Quantikine ELISA Kit (R&D Systems, Minneapolis, Minnesota), which employs the quantitative sandwich enzyme immunoassay technique. Sera from the baseline and repeat blood samples for each subject were analyzed within the same batch. Among 3 (blinded) triplicate sets of identical serum samples included for quality control, the average intraassay coefficient of variation was 2.0% for 25(OH)D and 4.7% for VDBP.

Statistical analysis

Measures of agreement were calculated for the entire sample as well as by race. Using recent, though still debated (20–25), guidelines proposed by the Institute of Medicine (20), vitamin D status was categorized as deficient (<12 ng/mL 25(OH)D), inadequate (12–19.9 ng/mL), or adequate (≥20 ng/mL). Weighted kappa coefficients, calculated with Fleiss-Cohen weights, were used to compare the levels of agreement for categorical assignment between baseline and repeat samples (26). Kappa values were interpreted according to the standard criteria of Landis and Koch (27), with agreement considered slight to fair for values less than 0.40, moderate for 0.41–0.60, substantial for 0.61–0.80, and almost perfect for 0.80–1.00.

The intraclass correlation coefficient (ICC), the proportion of the total variance in 25(OH)D and VDBP levels explained by the between-subject variance, was calculated using variance components from a random-effects model (28). ICCs are presented crudely and adjusted for characteristics typically associated with 25(OH)D levels (1, 2), including baseline age (years; continuous), race (overall estimates only), gender, body mass index category (weight (kg)/height (m)2; <25.0, 25.0–29.9, or ≥30.0), smoking status (current smoker vs. non-smoker), hours spent per week in vigorous sports (e.g., jogging, aerobics, bicycling), and number of years between blood sampling (1, 2, or 3). To further account for differences in potential sun exposure between the two time points, we also adjusted for the absolute value of the difference (in days) between the calendar dates of the baseline sample and the repeat sample, accompanied by a
categorical variable indicating whether the repeat sample was collected earlier or later in calendar time (e.g., a baseline sample from August 1, 2007, and a repeat sample from August 8, 2008, equals 7 days later). The continuous difference in calendar dates could not be utilized in the model because of a nonlinear relation with 25(OH)D and VDBP. Use of vitamin D supplements was not assessed on the baseline questionnaire. Data on 25(OH)D and VDBP levels were log-transformed in ICC calculations to improve normality.

RESULTS

Ten of the randomly selected serum samples were not assayed, and 3 had undergone hemolysis, leaving 225 of the 238 serum samples (94.5%) available for analysis. Ninety-six repeat samples were drawn 1 year postbaseline, 94 were drawn 2 years postbaseline, and 35 (consisting entirely of samples from black participants) were drawn 3 years postbaseline. Approximately 69% \((n = 155)\) of the participants were black, 57% \((n = 129)\) were female, 64% \((n = 143)\) had at least a high school diploma, and 74% \((n = 166)\) reported a household income of less than $25,000 per year. Average body mass index, vigorous sports activity, and smoking status were similar at the time of the baseline and repeat samples (Table 1). Median 25(OH)D concentrations increased monotonically in black participants from spring (May) to summer (June–August) to fall (September–October). Among whites, this pattern was reversed, with 25(OH)D values falling from spring to summer and with too few subjects to evaluate levels in the fall. Data on factors that may play a role in these trends (e.g., sunscreen use) were not collected. As expected

Table 1. Characteristics of the 225 Black and White Participants With Baseline and Repeat Serum Samples, Southern Community Cohort Study, 2005–2008

<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>No. % Mean (SD)</td>
<td>Median (IQR)</td>
<td>No. % Mean (SD)</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>Age, years</td>
<td>54.6 (8.6)</td>
<td>56.9 (8.6)</td>
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<tr>
<td>Body mass index (\text{a})</td>
<td>31.8 (8.1)</td>
<td>31.9 (7.8)</td>
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<tr>
<td>Current smoker</td>
<td>75 33.3</td>
<td>78 34.7</td>
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<tr>
<td>Vigorous activity, hours/week</td>
<td>0.5 (1.6)</td>
<td>1.1 (2.9)</td>
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<tr>
<td>Month of serum collection</td>
<td></td>
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<tr>
<td>May</td>
<td>66 29.3</td>
<td>49 21.8</td>
<td></td>
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<tr>
<td>June</td>
<td>68 30.2</td>
<td>59 26.2</td>
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<tr>
<td>July</td>
<td>38 16.9</td>
<td>36 16.0</td>
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<tr>
<td>August</td>
<td>39 17.3</td>
<td>47 20.9</td>
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<tr>
<td>September</td>
<td>12 5.3</td>
<td>31 13.8</td>
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<tr>
<td>October</td>
<td>2 0.9</td>
<td>3 1.3</td>
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<tr>
<td>Vitamin D status</td>
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<td></td>
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<tr>
<td>Deficient (&lt;12 ng/mL)</td>
<td>39 17.3</td>
<td>45 20.0</td>
<td></td>
<td></td>
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<tr>
<td>Inadequate (12–19.9 ng/mL)</td>
<td>86 38.2</td>
<td>65 28.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adequate (≥20 ng/mL)</td>
<td>100 44.4</td>
<td>115 51.1</td>
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</tr>
<tr>
<td>25-Hydroxyvitamin D level, ng/mL</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Overall</td>
<td>18.8 (13.2–26.3)</td>
<td>20.2 (13.2–26.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black participants ((n = 155))</td>
<td>16.9 (12.7–23.7)</td>
<td>18.2 (12.0–24.0)</td>
<td></td>
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<tr>
<td>May</td>
<td>15.6 (10.8–22.5)</td>
<td>14.4 (11.0–21.8)</td>
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<tr>
<td>June–August</td>
<td>17.0 (12.9–24.0)</td>
<td>18.3 (12.7–23.5)</td>
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<tr>
<td>September–October</td>
<td>20.6 (13.9–29.6)</td>
<td>19.2 (12.5–25.9)</td>
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<tr>
<td>White participants ((n = 70))</td>
<td>23.1 (18.0–30.2)</td>
<td>25.1 (17.5–32.4)</td>
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<tr>
<td>May</td>
<td>27.9 (15.7–35.2)</td>
<td>27.9 (18.5–35.5)</td>
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<tr>
<td>June–August</td>
<td>22.5 (18.7–29.5)</td>
<td>22.0 (17.3–28.6)</td>
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<tr>
<td>September–October</td>
<td>17.9(\text{d})</td>
<td>24.8(\text{d}) (24.0–40.3)</td>
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<tr>
<td>Vitamin D-binding protein level, µg/mL</td>
<td>140.9 (74.9–284.7)</td>
<td>131.6 (70.3–279.7)</td>
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</tbody>
</table>

Abbreviations: IQR, interquartile range; SD, standard deviation.
\(\text{a}\) Quartile 1–quartile 3.
\(\text{b}\) Weight (kg)/height (m\(^2\)).
\(\text{c}\) Based on 1 participant.
\(\text{d}\) Based on 4 participants.
<table>
<thead>
<tr>
<th>Comparison With Baseline</th>
<th>No.</th>
<th>%</th>
<th>Median (IQR)</th>
<th>95% CI for κ</th>
<th>ICC</th>
<th>95% CI for ICC</th>
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<tr>
<td>Overall (n = 225)</td>
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<td>Difference in calendar day of serum collection, days</td>
<td>4 (−8 to 20)</td>
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<tr>
<td>25(OH)D agreement</td>
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<tr>
<td>Change in 25(OH)D level, ng/mL</td>
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<tr>
<td>Same 25(OH)D quartile</td>
<td>145</td>
<td>64.4</td>
<td>0.83</td>
<td>0.78, 0.87</td>
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<tr>
<td>Same vitamin D status</td>
<td>168</td>
<td>74.7</td>
<td>0.76</td>
<td>0.69, 0.83</td>
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<td>0.85, 0.91</td>
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<tr>
<td>Adjusted ICC</td>
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<td></td>
<td>0.87</td>
<td>0.83, 0.89</td>
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<tr>
<td>VDBP agreement</td>
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<tr>
<td>Change in VDBP level, µg/mL</td>
<td></td>
<td></td>
<td>−4.5 (−20.7 to 6.2)</td>
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<tr>
<td>Same VDBP quartile</td>
<td>186</td>
<td>82.7</td>
<td>0.93</td>
<td>0.91, 0.95</td>
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<td>Crude ICC</td>
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<td></td>
<td>0.98</td>
<td>0.97, 0.98</td>
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<tr>
<td>Adjusted ICC</td>
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<td>0.97</td>
<td>0.96, 0.97</td>
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<td>Black Participants (n = 155)</td>
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<td>11 (−4 to 28)</td>
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<td>25(OH)D agreement</td>
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<tr>
<td>Change in 25(OH)D level, ng/mL</td>
<td></td>
<td></td>
<td>0.6 (−2.1 to 3.1)</td>
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<tr>
<td>Same 25(OH)D quartile</td>
<td>100</td>
<td>64.5</td>
<td>0.81</td>
<td>0.75, 0.87</td>
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<tr>
<td>Same vitamin D status</td>
<td>111</td>
<td>71.6</td>
<td>0.73</td>
<td>0.65, 0.82</td>
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<tr>
<td>Crude ICC</td>
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<td>0.84</td>
<td>0.79, 0.88</td>
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<tr>
<td>Adjusted ICC</td>
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<td>0.84</td>
<td>0.79, 0.88</td>
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<tr>
<td>VDBP agreement</td>
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<tr>
<td>Change in VDBP level, µg/mL</td>
<td></td>
<td></td>
<td>−4.3 (−14.9 to 4.7)</td>
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<tr>
<td>Same VDBP quartile</td>
<td>128</td>
<td>82.6</td>
<td>0.91</td>
<td>0.88, 0.95</td>
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<td>Crude ICC</td>
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<td>0.97</td>
<td>0.96, 0.98</td>
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<tr>
<td>Adjusted ICC</td>
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<td>0.97</td>
<td>0.96, 0.98</td>
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<td>White Participants (n = 70)</td>
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<tr>
<td>25(OH)D agreement</td>
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<tr>
<td>Change in 25(OH)D level, ng/mL</td>
<td></td>
<td></td>
<td>0.3 (−2.2 to 2.6)</td>
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<tr>
<td>Same 25(OH)D quartile</td>
<td>45</td>
<td>64.3</td>
<td>0.81</td>
<td>0.72, 0.89</td>
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<tr>
<td>Same vitamin D status</td>
<td>57</td>
<td>81.4</td>
<td>0.77</td>
<td>0.64, 0.90</td>
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<tr>
<td>Crude ICC</td>
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<td></td>
<td>0.93</td>
<td>0.89, 0.96</td>
</tr>
<tr>
<td>Adjusted ICC</td>
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<td></td>
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<td></td>
<td>0.92</td>
<td>0.87, 0.95</td>
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<td>VDBP agreement</td>
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</tr>
<tr>
<td>Change in VDBP level, µg/mL</td>
<td></td>
<td></td>
<td>−9.4 (−40.7 to 10.8)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Same VDBP quartile</td>
<td>58</td>
<td>82.9</td>
<td>0.82</td>
<td>0.72, 0.92</td>
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<td></td>
<td>0.94</td>
<td>0.91, 0.96</td>
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<tr>
<td>Adjusted ICC</td>
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<td></td>
<td></td>
<td></td>
<td>0.96</td>
<td>0.93, 0.97</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; ICC, intraclass correlation coefficient; IQR, interquartile range; 25(OH)D, 25-hydroxyvitamin D; VDBP, vitamin D-binding protein.

a Categorized as deficient (<12 ng/mL), inadequate (12–19.9 ng/mL), or adequate (≥20 ng/mL).
b Adjusted for age, race (overall estimates only), gender, body mass index, current smoking, hours per week of vigorous sports, absolute difference in calendar days between the baseline and repeat samples (days), whether the calendar day of the repeat sample was earlier or later than the baseline day, and number of years between samples (1, 2, or 3).
(29–32), we observed no significant correlation between 25(OH)D and VDBP levels at baseline or in repeat measurements for either blacks or whites (not shown).

Measures of agreement between the baseline and repeat biomarker levels are shown in Table 2. Participants were successful in returning to provide the repeat sample close to the calendar date of their original sample, with the date intervals being more dispersed for blacks than for whites. The change in 25(OH)D level from baseline to the repeat sample was ±3 ng/mL for the majority (60%) of participants. Nearly two-thirds (64%) of participants were classified in the same 25(OH)D quartile as at baseline for each sample, with virtually all of the remainder (33%) classified in an adjacent quartile (κ = 0.83). Similarly, three-quarters of participants were classified as having the same vitamin D status in both samples (κ = 0.76). These measures were very similar for blacks and whites.

The adjusted 25(OH)D ICCs, although uniformly high, were somewhat lower for blacks than for whites (ICC = 0.84 and ICC = 0.92, respectively) (Table 2). ICCs were also slightly lower for females (ICC = 0.86, 95% confidence interval (CI): 0.80, 0.90) than for males (ICC = 0.89, 95% CI: 0.84, 0.92) and lower for never smokers (ICC = 0.82, 95% CI: 0.74, 0.88) than for ever smokers (ICC = 0.89, 95% CI: 0.85, 0.92). Black females had the lowest adjusted ICC among the race and gender subgroups (ICC = 0.82, 95% CI: 0.74, 0.88), while white males (ICC = 0.96, 95% CI: 0.91, 0.98) had the highest; the ICC was 0.87 (95% CI: 0.81, 0.92) for black males and 0.91 (95% CI: 0.84, 0.95) for white females.

Because the slightly lower ICCs observed for blacks could have been influenced by the fact that only blacks supplied samples 3 years apart, we also stratified the ICC analyses by the number of years between samples (Table 3). For both blacks and whites, the adjusted ICCs for samples taken 1 or 2 years apart were very high (ICC = 0.87–0.95), with blacks having slightly higher ICCs for the 1-year group and whites having higher ICCs for the 2-year group. There was more within-person variation apparent for the 35 sets of samples from black participants collected 3 years apart (ICC = 0.65). Excluding these subjects from the total, the adjusted ICC for blacks in our study (ICC = 0.89) was much closer to that for whites (ICC = 0.92).

As expected, VDBP was more stable than 25(OH)D over time (Table 2). Eighty-three percent of participants were in the same quartile and 100% were in the same quartile or an adjacent quartile using the baseline and repeat samples (κ = 0.93). We observed almost no within-person variation, with an adjusted ICC of 0.97 (95% CI: 0.96, 0.97) overall, and the ICCs for whites (ICC = 0.96) and blacks (ICC = 0.97) were nearly identical.

**DISCUSSION**

To our knowledge, this report is the first detailed examination of intrapersonal variation in serum 25(OH)D and VDBP levels over time in both black and white adults. The large majority (84%) of the dual blood collections occurred over a 2-year time span, and within this period we found serum 25(OH)D levels to be highly reproducible, with ICCs for blacks and whites near 0.90. We observed very little change in ICCs after adjustment for factors associated with 25(OH)D levels, as was the case in the only previous study to report both crude and adjusted ICCs (11). Importantly for many health studies, agreement between the serial measurements with regard to 25(OH)D quartile classification and vitamin D status (i.e., deficient, inadequate, or adequate) was substantial. VDBP concentrations were also very stable over time for both races, consistent with results obtained over a 5-year period in the non-hormone replacement therapy arm of the Danish Osteoporosis Prevention Study, which to our knowledge is the only other study to have reported on VDBP reproducibility (15).

Reproducibility was lower for black adults with serum samples taken 3 years apart than for those with samples taken 1 or 2 years apart. Baseline characteristics, including vitamin D status, were similar between these two groups, as was the change in these characteristics from baseline to follow-up, so an explanation based on participant characteristics is not
readily apparent. These findings may therefore indicate a true
decline in the ability of a baseline sample to be predictive of
25(OH)D levels over the long term; however, this finding was
based on small numbers (n = 35) and should be interpreted
with caution, especially given that other studies have found
higher agreement using samples spaced 3–5 years apart (with
reported correlation coefficients near 0.70, though in popula-
tions of European descent (10–14)). To date, only the Danish
Osteoporosis Prevention Study (15) has questioned the use of
a single 25(OH)D measurement to predict vitamin D status
over this length of time, despite coefficients of variation
within the generally acceptable range (<20%) (28).

Our study had several unique strengths. In addition to
being the first to present 25(OH)D reproducibility measures
for black adults, we are aware of only 1 other prospective
health study that considered the stability of VDBP measure-
ments (15). We were also successful in controlling for sea-
sonal variation by matching closely on the original
calendar day of the baseline sample collection. A limitation
of our analysis is that the blood samples were not spaced
longer than 3 years apart; thus, our findings cannot be gen-
eralized to longer time periods with confidence. However,
baseline measures are not expected to remain static over
very long periods; the goal is that they represent a partici-
 pant’s typical state within the baseline time frame, and for
this, our results are very reassuring.

The present findings suggest that single, prediagnostic
measurements of serum 25(OH)D and VDBP levels provide
a reasonably representative baseline measure of these com-
 pounds for both white and black adults, demonstrating their
utility as epidemiologic biomarkers in prospective studies.

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