



Practice of Epidemiology

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

* Correspondence to Jennifer S. Sonderman, International Epidemiology Institute, 1455 Research Blvd., Suite 550, Rockville, MD 20850 (e-mail: jennifer@iei.us).

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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.

African Americans; biological markers; prospective studies; reproducibility of results; vitamin D; vitamin D-binding protein

Abbreviations: CI, confidence interval; ICC, intraclass correlation coefficient; 25(OH)D, 25-hydroxyvitamin D; SCCS, Southern Community Cohort Study; VDBP, vitamin D-binding protein.

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 D₃, 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 (Nashville, Tennessee), where they were separated into components and frozen at -80°C within 24 hours.

From May to October 2008, we attempted to contact all SCCS participants from our study base who had enrolled at one of 9 community health centers either 12, 24, or 36 months prior (during 2007, 2006, or 2005, respectively; $n = 1,102$) with a written invitation to return to the community health center and provide a second blood sample. Among those assumed to have received the invitation ($n = 1,010$, based on undeliverable mail), 66% ($n = 662$) returned to provide the repeat blood sample and complete a short interview focused on potential changes in lifestyle factors (e.g., weight changes). The blood was collected and handled using procedures identical to those used for the baseline sample with regard to collection materials and methods, shipping, processing, and frozen storage. The distribution of time since enrollment for this group (1 year for 472 participants, 2 years for 152 participants, and 3 years for 38 participants) reflects the fact that community health centers were typically

only active enrollment sites for 1–2 years. For the present analysis, we selected a random sample of 100 participants whose blood samples were spaced 1 year apart, 100 whose samples were spaced 2 years apart, and all those ($n = 38$) whose samples were spaced 3 years apart.

The SCCS was approved by institutional review boards at 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. not), 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

Characteristic	Baseline Sample (2005–2007)				Repeat Sample (2008)			
	No.	%	Mean (SD)	Median (IQR ^a)	No.	%	Mean (SD)	Median (IQR)
Age, years			54.6 (8.6)				56.9 (8.6)	
Body mass index ^b			31.8 (8.1)				31.9 (7.8)	
Current smoker	75	33.3			78	34.7		
Vigorous activity, hours/week			0.5 (1.6)				1.1 (2.9)	
Month of serum collection								
May	66	29.3			49	21.8		
June	68	30.2			59	26.2		
July	38	16.9			36	16.0		
August	39	17.3			47	20.9		
September	12	5.3			31	13.8		
October	2	0.9			3	1.3		
Vitamin D status								
Deficient (<12 ng/mL)	39	17.3			45	20.0		
Inadequate (12–19.9 ng/mL)	86	38.2			65	28.9		
Adequate (≥20 ng/mL)	100	44.4			115	51.1		
25-Hydroxyvitamin D level, ng/mL								
Overall				18.8 (13.2–26.3)				20.2 (13.2–26.1)
Black participants ($n = 155$)				16.9 (12.7–23.7)				18.2 (12.0–24.0)
May				15.6 (10.8–22.5)				14.4 (11.0–21.8)
June–August				17.0 (12.9–24.0)				18.3 (12.7–23.5)
September–October				20.6 (13.9–29.6)				19.2 (12.5–25.9)
White participants ($n = 70$)				23.1 (18.0–30.2)				25.1 (17.5–32.4)
May				27.9 (15.7–35.2)				27.9 (18.5–35.5)
June–August				22.5 (18.7–29.5)				22.0 (17.3–28.6)
September–October				17.9 ^c				24.8 ^d (24.0–40.3)
Vitamin D-binding protein level, µg/mL				140.9 (74.9–284.7)				131.6 (70.3–279.7)

Abbreviations: IQR, interquartile range; SD, standard deviation.

^a Quartile 1–quartile 3.

^b Weight (kg)/height (m)².

^c Based on 1 participant.

^d Based on 4 participants.

Table 2. Comparison of Levels of 25-Hydroxyvitamin D and Vitamin D-Binding Protein in Repeat Versus Baseline Serum Samples for 225 Black and White Participants, Southern Community Cohort Study, 2005–2008

Comparison With Baseline	No.	%	Median (IQR)	κ	95% CI for κ	ICC	95% CI for ICC
<i>Overall (n = 225)</i>							
Difference in calendar day of serum collection, days			4 (–8 to 20)				
25(OH)D agreement							
Change in 25(OH)D level, ng/mL			0.5 (–2.1 to 2.9)				
Same 25(OH)D quartile	145	64.4		0.83	0.78, 0.87		
Same vitamin D status ^a	168	74.7		0.76	0.69, 0.83		
Crude ICC						0.88	0.85, 0.91
Adjusted ICC ^b						0.87	0.83, 0.89
VDBP agreement							
Change in VDBP level, $\mu\text{g/mL}$			–4.5 (–20.7 to 6.2)				
Same VDBP quartile	186	82.7		0.93	0.91, 0.95		
Crude ICC						0.98	0.97, 0.98
Adjusted ICC						0.97	0.96, 0.97
<i>Black Participants (n = 155)</i>							
Difference in calendar day of serum collection, days			11 (–4 to 28)				
25(OH)D agreement							
Change in 25(OH)D level, ng/mL			0.6 (–2.1 to 3.1)				
Same 25(OH)D quartile	100	64.5		0.81	0.75, 0.87		
Same vitamin D status	111	71.6		0.73	0.65, 0.82		
Crude ICC						0.84	0.79, 0.88
Adjusted ICC						0.84	0.79, 0.88
VDBP agreement							
Change in VDBP level, $\mu\text{g/mL}$			–4.3 (–14.9 to 4.7)				
Same VDBP quartile	128	82.6		0.91	0.88, 0.95		
Crude ICC						0.97	0.96, 0.98
Adjusted ICC						0.97	0.96, 0.98
<i>White Participants (n = 70)</i>							
Difference in calendar day of serum collection, days			–3 (–9 to 5)				
25(OH)D agreement							
Change in 25(OH)D level, ng/mL			0.3 (–2.2 to 2.6)				
Same 25(OH)D quartile	45	64.3		0.81	0.72, 0.89		
Same vitamin D status	57	81.4		0.77	0.64, 0.90		
Crude ICC						0.93	0.89, 0.96
Adjusted ICC						0.92	0.87, 0.95
VDBP agreement							
Change in VDBP level, $\mu\text{g/mL}$			–9.4 (–40.7 to 10.8)				
Same VDBP quartile	58	82.9		0.82	0.72, 0.92		
Crude ICC						0.94	0.91, 0.96
Adjusted ICC						0.96	0.93, 0.97

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 (\geq 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).

Table 3. Intraclass Correlation Coefficients for Serum 25-Hydroxyvitamin D Levels According to Number of Years Between Samples for 225 Black and White Participants, Southern Community Cohort Study, 2005–2008

No. of Years Between Samples	Black Participants (n = 155)				White Participants (n = 70)			
	No.	%	ICC	95% CI	No.	%	ICC	95% CI
1	49	31.6			47	67.1		
Crude ICC			0.90	0.83, 0.94			0.90	0.83, 0.94
Adjusted ICC ^a			0.89	0.81, 0.93			0.87	0.79, 0.93
2	71	45.8			23	32.9		
Crude ICC			0.88	0.82, 0.93			0.96	0.90, 0.98
Adjusted ICC ^a			0.90	0.84, 0.93			0.95	0.89, 0.98
3	35	22.6						
Crude ICC			0.68	0.46, 0.82				
Adjusted ICC ^a			0.65	0.41, 0.80				

Abbreviations: CI, confidence interval; ICC, intraclass correlation coefficient.

^a Adjusted for age, gender, body mass index, current smoking, hours per week of vigorous sports, absolute difference in calendar days between the baseline and repeat samples (days), and whether the calendar day of the repeat sample was earlier or later than the baseline day.

(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 ($\kappa = 0.83$). Similarly, three-quarters of participants were classified as having the same vitamin D status in both samples ($\kappa = 0.76$). 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 ($\kappa = 0.93$). 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 intraindividual 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 populations 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 measurements (15). We were also successful in controlling for seasonal 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 generalized 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 participant'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 compounds for both white and black adults, demonstrating their utility as epidemiologic biomarkers in prospective studies.

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Author affiliations: International Epidemiology Institute, Rockville, Maryland (Jennifer S. Sonderman, Heather M. Munro, William J. Blot, Lisa B. Signorello); Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt University, Nashville, Tennessee (William J. Blot, Lisa B. Signorello); and Vanderbilt-Ingram Cancer Center, Vanderbilt University, Nashville, Tennessee (William J. Blot, Lisa B. Signorello).

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REFERENCES

- Garland CF, Garland FC, Gorham ED, et al. The role of vitamin D in cancer prevention. *Am J Public Health*. 2006;96(2):252–261.
- Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357(3):266–281.
- Giovannucci E. Vitamin D and cancer incidence in the Harvard cohorts. *Ann Epidemiol*. 2009;19(2):84–88.
- Clemens TL, Adams JS, Henderson SL, et al. Increased skin pigment reduces the capacity of skin to synthesise vitamin D₃. *Lancet*. 1982;1(8263):74–76.
- White P, Cooke N. The multifunctional properties and characteristics of vitamin D-binding protein. *Trends Endocrinol Metab*. 2000;11(8):320–327.
- Harris SS, Dawson-Hughes B. Seasonal changes in plasma 25-hydroxyvitamin D concentrations of young American black and white women. *Am J Clin Nutr*. 1998;67(6):1232–1236.
- Nesby-O'Dell S, Scanlon KS, Cogswell ME, et al. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: Third National Health and Nutrition Examination Survey, 1988–1994. *Am J Clin Nutr*. 2002;76(1):187–192.
- Looker AC, Pfeiffer CM, Lacher DA, et al. Serum 25-hydroxyvitamin D status of the US population: 1988–1994 compared with 2000–2004. *Am J Clin Nutr*. 2008;88(6):1519–1527.
- Holick MF. Vitamin D status: measurement, interpretation, and clinical application. *Ann Epidemiol*. 2009;19(2):73–78.
- Jorde R, Sneve M, Hutchinson M, et al. Tracking of serum 25-hydroxyvitamin D levels during 14 years in a population-based study and during 12 months in an intervention study. *Am J Epidemiol*. 2010;171(8):903–908.
- Al-Delaimy WK, Jansen EH, Peeters PH, et al. Reliability of biomarkers of iron status, blood lipids, oxidative stress, vitamin D, C-reactive protein and fructosamine in two Dutch cohorts. *Biomarkers*. 2006;11(4):370–382.
- Hofmann JN, Yu K, Horst RL, et al. Long-term variation in serum 25-hydroxyvitamin D concentration among participants in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *Cancer Epidemiol Biomarkers Prev*. 2010;19(4):927–931.
- Platz EA, Leitzmann MF, Hollis BW, et al. Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and subsequent risk of prostate cancer. *Cancer Causes Control*. 2004;15(3):255–265.
- Kotsopoulos J, Tworoger SS, Campos H, et al. Reproducibility of plasma and urine biomarkers among premenopausal and postmenopausal women from the Nurses' Health Studies. *Cancer Epidemiol Biomarkers Prev*. 2010;19(4):938–946.
- Rejman L, Lauridsen AL, Brot C, et al. Vitamin D and its binding protein Gc: long-term variability in peri- and postmenopausal women with and without hormone replacement therapy. *Scand J Clin Lab Invest*. 2006;66(3):227–238.
- Signorello LB, Hargreaves MK, Steinwandel MD, et al. Southern Community Cohort Study: establishing a cohort to investigate health disparities. *J Natl Med Assoc*. 2005;97(7):972–979.
- Signorello LB, Hargreaves MK, Blot WJ. The Southern Community Cohort Study: investigating health disparities. *J Health Care Poor Underserved*. 2010;21(1 suppl):26–37.
- Ersfeld DL, Rao DS, Body JJ, et al. Analytical and clinical validation of the 25 OH vitamin D assay for the LIAISON automated analyzer. *Clin Biochem*. 2004;37(10):867–874.
- Wagner D, Hanwell HE, Vieth R. An evaluation of automated methods for measurement of serum 25-hydroxyvitamin D. *Clin Biochem*. 2009;42(15):1549–1556.
- Food and Nutrition Board, Institute of Medicine, National Academy of Sciences. *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, DC: National Academy Press; 2010.

21. Maxmen A. Nutrition advice: the vitamin D-lemma. *Nature*. 2011;475(7354):23–25.
22. Yngve A, Tseng M, Haapala I, et al. Vitamin D—the big D-bate [editorial]. *Public Health Nutr*. 2011;14(4):565.
23. Shapses SA, Manson JE. Vitamin D and prevention of cardiovascular disease and diabetes: why the evidence falls short. *JAMA*. 2011;305(24):2565–2566.
24. Manson JE, Mayne ST, Clinton SK. Vitamin D and prevention of cancer—ready for prime time? *N Engl J Med*. 2011;364(15):1385–1387.
25. Dawson-Hughes B, Mithal A, Bonjour JP, et al. IOF position statement: vitamin D recommendations for older adults. *Osteoporos Int*. 2010;21(7):1151–1154.
26. Fleiss JL, Cohen J. The equivalence of weighted kappa and the intraclass correlation coefficient as measures of reliability. *Educ Psychol Meas*. 1973;33:613–619.
27. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics*. 1977;33(1):159–174.
28. Rosner B. *Fundamentals of Biostatistics*. 6th ed. Belmont, CA: Duxbury Press; 2006.
29. Haddad JG Jr, Walgate J. Radioimmunoassay of the binding protein for vitamin D and its metabolites in human serum: concentrations in normal subjects and patients with disorders of mineral homeostasis. *J Clin Invest*. 1976; 58(5):1217–1222.
30. Bouillon R, van Baelen H, de Moor P. The measurement of the vitamin D-binding protein in human serum. *J Clin Endocrinol Metab*. 1977;45(2):225–231.
31. Winters SJ, Chennubhatla R, Wang C, et al. Influence of obesity on vitamin D-binding protein and 25-hydroxy vitamin D levels in African American and white women. *Metabolism*. 2009;58(4):438–442.
32. Blanton D, Han Z, Bierschenk L, et al. Reduced serum vitamin D-binding protein levels are associated with type 1 diabetes. *Diabetes*. 2011;60(10): 2566–2570.