Estimating mean annual 25-hydroxyvitamin D concentrations from single measurements: the Multi-Ethnic Study of Atherosclerosis[1–3]

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ABSTRACT

Background: The seasonal variation in circulating 25-hydroxyvitamin D [25(OH)D] concentrations is large relative to mean values. Single measurements may misclassify annual exposure, which may lead to bias in research and complicate clinical decision making.

Objective: We aimed to develop and validate a model for adjusting a single measurement of a serum 25(OH)D concentration to the time of year it was measured.

Design: We measured serum 25(OH)D concentrations by using mass spectrometry in 6476 participants from the Multi-Ethnic Study of Atherosclerosis at baseline and again in a subset of 368 participants at a median of 17 mo later. We estimated a cosinor model to describe the seasonal variability in 25(OH)D concentrations and evaluated this model by using follow-up 25(OH)D measurements.

Results: The mean age of subjects was 62.1 y, 61.2% of participants were nonwhite, and 53.3% of participants were women. The cosinor model predicted follow-up 25(OH)D concentrations better than a single measurement [difference in root mean squared error (RMSE): 1.3 ng/mL; $P < 0.001$]. The cosinor model also better predicted the measured annual mean 25(OH)D concentration (difference in RMSE: 1.0 ng/mL; $P < 0.001$). Annual mean 25(OH)D concentrations estimated from the cosinor model reclassified 7.1% of participants with regard to 25(OH)D deficiency, which was defined as <20 ng/mL. An estimated annual mean 25(OH)D concentration <20 ng/mL was significantly associated with lower bone mineral density, whereas an untransformed 25(OH)D concentration <20 ng/mL was not.

Conclusions: Cross-sectional data can be used to estimate subject-specific mean annual 25(OH)D concentrations from single values by using a cosinor model. The tool we developed by using this approach may assist research and clinical care of adults in North America by reducing the misclassification of 25(OH)D deficiency. *Am J Clin Nutr* 2013;97:1243–51.

INTRODUCTION

Vitamin D deficiency is commonly defined by a single measurement of the circulating 25-hydroxyvitamin D [25(OH)D][4] concentration (1). Vitamin D deficiency is a known risk factor for low bone mineral density (BMD) and fracture (2–4). In addition, vitamin D deficiency may promote a number of highly prevalent chronic diseases, including cancer, cardiovascular disease, and diabetes (5, 6). As a result, 25(OH)D concentrations are frequently obtained to guide vitamin D supplementation, which effectively raises 25(OH)D (7).

However, the seasonal variation in circulating 25(OH)D concentrations is large relative to mean values (8–11). As a result, a single 25(OH)D measurement may misclassify year-long 25(OH)D status and potentially lead to bias and imprecision in research studies and inappropriate clinical decisions regarding vitamin D supplementation (11–13). Serum 25(OH)D concentrations generally follow a sinusoidal pattern throughout the calendar year, probably because of the corresponding sinusoidal variation in solar irradiation at most latitudes (8, 9, 11). Other studies have previously shown that a cosinor model approximates seasonal differences in group mean 25(OH)D concentrations (8, 9, 11, 14, 15).

In this study, our goal was to develop and validate a tool to estimate mean annual 25(OH)D concentrations from single measurements in a large, diverse population, building on previous studies that showed the utility of the cosinor model. An accurate estimated mean annual 25(OH)D concentration could facilitate a more accurate classification of vitamin D deficiency for research and clinical care. We use cross-sectional measurements of 25(OH)D from a large, multiethnic cohort to build a cosinor model for seasonal variability in 25(OH)D concentrations and validated this model by using longitudinal 25(OH)D measurements from a subset of participants that were measured a median of 17 mo (range: 16–19 mo) later.

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[4] Abbreviations used: BMD, bone mineral density; MESA, Multi-Ethnic Study of Atherosclerosis; RMSE, root mean squared error; 25(OH)D, 25-hydroxyvitamin D.

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SUBJECTS AND METHODS

Study participants

The Multi-Ethnic Study of Atherosclerosis (MESA), which is a prospective, community-living, cohort study, was designed to examine risk factors for the development and progression of subclinical cardiovascular disease and progression to clinical cardiovascular disease (16). Subjects aged 45–84 y were recruited from the following 6 communities in the United States: Forsyth County, NC; New York, NY; Baltimore, MD; Saint Paul, MN; Chicago, IL; and Los Angeles County, CA. For this study, baseline serum samples drawn in 2000–2002 were available for 6564 of 6814 MESA participants (96%). The 25(OH)D assay failed for 88 participants who did not differ substantially with respect to demographic characteristics. This study included 6476 participants who had successful 25(OH)D measurements. Repeat 25(OH)D measurements at the 2002–2004 study visit were made for a subset of 368 participants who were randomly selected from strata defined by ethnicity and study site. Of the repeat subset, 135 participants had a pair of 25(OH)D measurements that occurred 18 calendar months apart. Because of privacy concerns, the month of the 25(OH)D measurement was the most granular information available about the timing of measurements.

Measurement of 25(OH)D

The serum concentration of total 25(OH)D [sum of $25(OH)D_2$ and $25(OH)D_3$] was measured by using high-performance HPLC–tandem mass spectrometry with internal standards at the University of Washington (17–19). The reproducibility over the course of the measurement was ensured by using 2 complementary approaches. First, aliquots of 2 serum samples were run with every plate of MESA samples. Second, a larger panel of aliquots from 20 normal control subjects was run on 9 occasions. Over the course of the measurement, we identified one shift in the $25(OH)D_3$ concentration. We used quality-control measurements to recalibrate values obtained after the shift to those obtained before the shift. Interassay CVs that were calculated by using 81 repeat measurements of quality-control specimens placed in each plate of MESA samples were 8.5% at 24.8 ng/mL for $25(OH)D_3$ and 11.8% at 7.0 ng/mL for $25(OH)D_2$. Calibration was verified by using standard reference material 972 from the National Institute of Standards and Technology (20) with an accuracy of 91–95% for $25(OH)D_3$ and 100–116% for $25(OH)D_2$.

Clinical characteristics

Age, sex, and ethnicity were defined by self-report. Medication inventories were completed by MESA staff with the use of medication bottles of participants. Total hours per week of moderate and vigorous physical activity were measured by using a detailed, semiquantitative questionnaire adapted from the Cross-Cultural Activity Participation Study. Total reported hours were categorized into quartiles, and the middle 2 quartiles were combined because of the form of observed associations with 25(OH)D. Diabetes was defined as a fasting serum glucose concentration $\geq$126 mg/dL or the use of glucose-lowering medications (21). BMI (in kg/m^2) was calculated as weight divided by the square of height. The glomerular filtration rate was estimated from serum creatinine and demographics by using the Chronic Kidney Disease Epidemiology Collaboration equation (22). The vertebral BMD at L3 was measured by using computed tomography 1–4 y after the baseline MESA study visit in a subset of 2032 individuals at the second or third MESA examination, $\sim$18 or 36 mo after the baseline examination and 25(OH)D measurements (23). In participants with baseline 25(OH)D measurements, 1898 of 6476 subjects (29%) had BMD measurements. The serum parathyroid hormone concentration was measured by using the Beckman-Coulter DxI automated immunoassay (Beckman-Coulter) (19).

Statistical methods

On the basis of biological plausibility, published reports that 25(OH)D follows a sinusoidal pattern (8, 9, 11, 14, 15), and observed data, we fit a cosinor model to the cross-sectional measurements made at the baseline MESA study visit. In the cosinor model, 25(OH)D follows a sine wave over time characterized by a phase shift (location of the peak and trough along the time axis), height (vertical shift of the sine wave), and amplitude (maximum variation of the sine wave from its mean height).

In the cosinor model, the time variable t (month) is transformed as

$$r = \cos[(2\pi \div 12) \times t] \quad (1)$$

and

$$s = \sin[(2\pi \div 12) \times t] \quad (2)$$

which are then fit as predictors of 25(OH)D in a linear model. The coefficients β_r and β_s of the r and s predictors are transformed to give the amplitude

$$\text{Amplitude} = \sqrt{\beta_r^2 + \beta_s^2} \quad (3)$$

and phase shift

$$\text{Shift} = \arctan(\beta_r \div \beta_s) \quad (4)$$

of the sine curve. The annual mean is the intercept term of the model.

The addition of main effects of covariates to the model allows the annual mean 25(OH)D concentration to vary on the basis of the covariates, which corresponds to shifting the sine curve up or down. The addition of interaction terms between the covariates and r and s allows the amplitude and shift of the sine curve to differ on the basis of covariate values. In adjusted models, we considered associations with age, sex, study site, ethnicity, BMI, and self-reported hours per week of moderate to vigorous physical activity (in tertiles). SEs of these estimated differences were estimated by using the Δ method (24).

Improvement in the model fit between nested models was assessed by using likelihood ratio tests and adjusted R^2 terms.

Multivariate Wald tests were used to test the null hypothesis that amplitudes do not differ by the covariate. Model-based SEs for regression variables were used, which assumed that errors were constant as a function of the covariates, independent, and identically distributed. We did not detect any noticeable departures from this assumption.

For a given participant, we predicted their 25(OH)D values at a particular time during the year from the cosinor model. The amplitude and phase shift of a participant-specific sine curve were determined from the fitted sine curve determined by the participant's covariate values. The height of the participant-specific sine curve was determined from the observed value at baseline. This method was equivalent to assuming that an individual's residual departure from the curve estimated by the covariates was constant at both time points. Although the measurements were 16–19 mo apart (median: 17 mo), we did not assume that a person's sine curve would shift up or down over the time between measurements.

To assess the performance of cosinor models, we first compared predicted to measured follow-up 25(OH)D values. For each of the 368 participants whose 25(OH)D was measured at exam 2, we obtained a predicted value from the cosinor model for the month of the exam 2 measurement. In addition, for participants (n = 135) whose pair of 25(OH)D measurements were measured 18 mo apart (ie, participants who had 2 measurements at opposite sides of the sine curve), we estimated their annual mean 25(OH)D exposure by averaging the 2 values. This mean was considered to be the gold-standard estimate of the annual mean exposure that we compared with fitted values (intercepts) from the cosinor model. We compared the cosinor model to carrying forward the measured value, which is the most common method in clinical practice, and to the model with dummy variables for 3 of 4 seasons, which is the most common method in epidemiologic research. Comparisons in prediction performance, as measured by squared error, between models were tested by using the paired t test.

Analyses were done with R version 2.13.1 software (R Foundation for Statistical Computing). No adjustment to CIs or P values was made for multiple comparisons.

RESULTS

Participant characteristics

At baseline, the mean age of participants was 62.1 y (**Table 1**). Of the 6476 participants included in all analyses, 61.2% of subjects were

TABLE 1
Baseline characteristics of the study population[1]

Baseline measurements	All participants (n = 6476)	Participants with follow-up measurements (n = 368)
Age (y)	62.1 ± 10.3[2]	62.5 ± 9.2
Age categories [n (%)]		
45–54 y	1855 (28.6)	88 (23.9)
55–64 y	1779 (27.5)	107 (29.1)
65–74 y	1917 (29.6)	135 (36.7)
75–84 y	925 (14.3)	38 (10.3)
Ethnicity [n (%)]		
White	2512 (38.8)	110 (29.9)
Chinese	787 (12.2)	74 (20.1)
Black	1763 (27.2)	107 (29.1)
Hispanic	1414 (21.8)	77 (20.9)
Sex (F) [n (%)]	3451 (53.3)	185 (50.2)
Sites [n (%)]		
Forsyth County, NC	1031 (15.9)	58 (15.8)
New York, NY	1142 (17.6)	75 (20.4)
Baltimore, MD	1017 (15.7)	57 (15.5)
Saint Paul, MN	1040 (16.1)	50 (13.6)
Chicago, IL	971 (15)	57 (15.5)
Los Angeles County, CA	1275 (19.7)	71 (19.3)
BMI [n (%)]		
<25 kg/m^2	1867 (28.8)	110 (29.9)
25 to <30 kg/m^2	2554 (39.4)	138 (37.5)
30 to <40 kg/m^2	1822 (28.1)	105 (28.5)
≥ 40 kg/m^2	233 (3.6)	15 (4.1)
eGFR <60 mL · min^{-1} · 1.73 m^{-2} [n (%)]	606 (9.4)	39 (10.6)
Diabetes [n (%)]	805 (12.4)	50 (13.6)
Physical activity [n (%)]		
Light	1615 (24.9)	95 (25.8)
Moderate	3283 (50.7)	189 (51.4)
High	1561 (24.1)	83 (22.6)
25(OH)D (ng/mL)	25.4 ± 11.5	23.5 ± 11
PTH (pg/mL)	44.8 ± 21.8	47.4 ± 28.1

[1] eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.
[2] Mean ± SD (all such values).

nonwhite, and 53.3% of subjects were women. Of 368 participants with repeat 25(OH)D measurements at the second MESA study visit, 70.1% of subjects were nonwhite, and 50.2% of subjects were women. Of 135 participants measured at discordant seasons, 72.1% of subjects were nonwhite, and 59.3% of subjects were women.

Cross-sectional models

Population mean 25(OH)D concentrations were lowest in January and February and highest in August and September (**Figure 1**). The cosinor model fit observed baseline data significantly better than did a mean model, which assumed a constant mean 25(OH)D concentration over the course of the year (likelihood ratio test $P < 0.0001$).

In the unadjusted cosinor model, the estimated mean 25(OH)D concentration over the calendar year was 25.7 ng/mL (95% CI: 25.4, 26 ng/mL). The estimated peak-trough difference in the 25(OH)D concentration was 7.2 ng/mL (28% of the mean; 95% CI: 6.4, 7.9 ng/mL), where the estimated peak occurred in August, and the estimated trough occurred in February.

Older age was associated with a higher mean 25(OH)D concentration but smaller amplitude in the seasonal 25(OH)D variation (adjusted $P < 0.001$) (**Table 2**). We showed no evidence to suggest that associations with age were nonlinear. Compared with participants of white ethnicity, nonwhite participants had a substantially lower mean 25(OH)D concentration but little or no difference in 25(OH)D amplitude, particularly after adjustment for age, sex, and site (*P*-heterogeneity in amplitude by ethnicity = 0.07 in the adjusted model). The mean 25(OH)D concentration did not differ by sex, but men tended to have a larger amplitude in both unadjusted and adjusted models. In unadjusted models, the mean 25(OH)D concentration and 25(OH)D amplitude appeared to differ by study site. However, after adjustment for demographics, only the New York study site appeared to have a significantly smaller amplitude (*P*-heterogeneity in amplitude by study site = 0.18 in the adjusted model). In adjusted models, a higher BMI was associated with a lower mean 25(OH)D concentration but no difference in amplitude. Diabetes was associated with a lower mean 25(OH)D concentration and smaller amplitude. In adjusted models, high physical activity (highest tertile) was associated with a higher mean 25(OH)D concentration and larger amplitude, whereas in unadjusted models, only the amplitude was significantly larger.

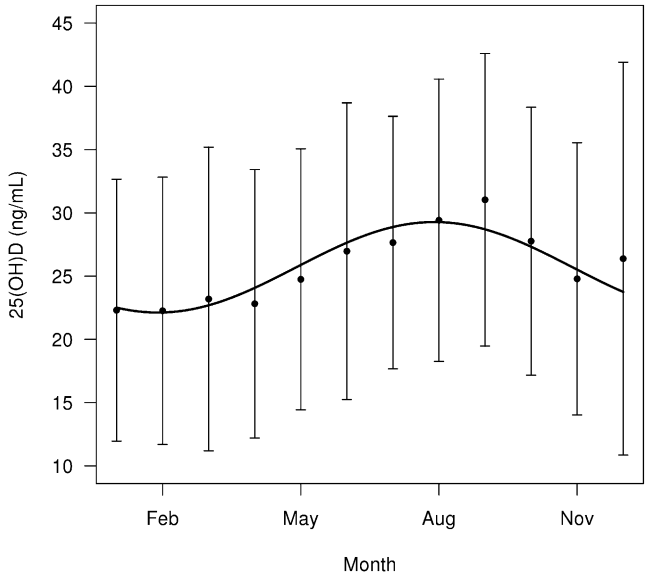

FIGURE 1. Mean (±SD) seasonal variation in 25(OH)D with the fitted sine curve superimposed. Error bars are ±1 SD. Aug, August; Feb, February; Nov, November; 25(OH)D, 25-hydroxyvitamin D.

Longitudinal analyses

For 368 participants, we repeated serum 25(OH)D measurements a median of 17 mo after the baseline study visit (range: 16–19 mo), which were uniformly distributed over the course of the calendar year. In this subset, the mean 25(OH)D concentration was slightly higher at follow-up than at baseline (25.0 compared with 23.5 ng/mL, respectively). However, the mean 25(OH)D concentration at follow-up was similar to the mean 25(OH)D concentration in the entire cohort (25.0 compared with 25.4 ng/mL, respectively). The correlation between baseline and follow-up 25(OH)D concentrations was 0.74, whereas the correlation between cosinor-predicted follow-up 25(OH)D and measured follow-up 25(OH)D was 0.82 (**Figure 2**). The empty cosinor model (without covariates) predicted the follow-up 25(OH)D concentration significantly better than simply carrying forward the baseline 25(OH)D concentration (difference in root mean squared error (RMSE): 1.3 ng/mL; $P < 0.001$; **Table 3**). For the cosinor model, 57% of predicted values laid within 4 ng/mL of the measured value compared with 43% of predicted values when baseline values were carried forward. The RMSE and bias were similar across categories of relevant demographic and clinical characteristics (*see* Table 1 under "Supplemental data" in the online issue). The cosinor model had a lower mean squared error than did the model which assumed a different mean 25(OH)D concentration for each of winter (December to February), spring (March to May), summer (June to August), and fall (September to November); however, this difference was not significant (difference in RMSE: 0.3 ng/mL; $P = 0.29$; Table 3). For the cosinor model without covariates, the estimated intercept for the cosinor curve was 25.7 (95% CI: 25.4, 26), the coefficient for r was −1.9 (95% CI: −2.3, −1.6), and the coefficient for s was −3 (95% CI: −3.4, −2.6). The addition of covariates to the cosinor model did not significantly alter the predictive performance (Table 3).

To estimate mean annual 25(OH)D exposure, we investigated the subset of 135 participants with 2 measurements that occurred at opposite sides of the sine curve (ie, 18 mo apart). For those participants, we compared the annual mean 25(OH)D estimated from the cosinor model to the computed mean of observed values, which is considered the gold standard. The cosinor model substantially reduced the bias and variance of the estimated annual mean 25(OH)D exposure compared with simply carrying forward the baseline 25(OH)D concentration (difference in RMSE: 0.8 ng/mL; $P = 0.005$; Figure 2, Table 3). For the cosinor model, 82% of estimated annual mean values fell within 4 ng/mL of the measured mean compared with 67% when carrying forward baseline values. Again, the addition of covariates to the cosinor

TABLE 2
Differences in the mean and amplitude of 25(OH)D modeled according to the cosinor model[1]

	Unadjusted			Adjusted		
Clinical characteristic	Difference in mean 25(OH)D	Difference in amplitude	*P*-difference in amplitude	Difference in mean 25(OH)D	Difference in amplitude	*P*-difference in amplitude
	ng/mL			*ng/mL*		
Age (/10 y)	1.1 (0.8, 1.4)	−1.2 (−1.6, −0.8)	<0.001	0.9 (0.6, 1.2)	−1.1 (−1.4, −0.7)	<0.001
Age categories						
45–54 y	—	—	—	—	—	—
55–64 y	0.8 (0, 1.5)	−0.9 (−1.9, 0.2)	0.094	—	—	—
65–74 y	2.4 (1.6, 3.1)	−2.1 (−3.2, −1.1)	<0.001	—	—	—
75–84 y	2.6 (1.7, 3.5)	−3 (−4.3, −1.7)	<0.001	—	—	—
Ethnicity						
White	—	—	—	—	—	—
Chinese	−3.6 (−4.5, −2.7)	−1.3 (−2.6, −0.1)	0.034	−5.1 (−6.2, −4.1)	−1 (−2.4, 0.4)	0.162
Black	−11 (−11.6, −10.4)	−1.3 (−2.3, −0.4)	0.004	−11.2 (−11.8, −10.5)	−1 (−2, 0)	0.042
Hispanic	−5.3 (−6, −4.7)	−0.2 (−1.2, 0.8)	0.673	−6.4 (−7.1, −5.6)	0.1 (−1, 1.2)	0.868
Sex (M)	0.1 (−0.5, 0.6)	1.5 (0.7, 2.2)	<0.001	−0.2 (−0.7, 0.3)	1.6 (0.9, 2.4)	<0.001
Study sites						
Twin Cities, MN	—	—	—	—	—	—
Chicago, IL	−1.3 (−2.3, −0.4)	−0.9 (−2.2, 0.5)	0.211	−0.3 (−1.2, 0.7)	−0.4 (−1.8, 0.9)	0.526
New York, NY	−2.9 (−3.8, −1.9)	−1.4 (−2.8, −0.1)	0.04	0.8 (−0.1, 1.7)	−1.6 (−3, −0.3)	0.015
Baltimore, MD	−2.6 (−3.6, −1.6)	−0.3 (−1.8, 1.1)	0.649	−0.3 (−1.3, 0.7)	0.1 (−1.4, 1.5)	0.923
Forsyth County, NC	−1.5 (−2.5, −0.5)	−2.7 (−4.2, −1.3)	<0.001	0.7 (−0.2, 1.7)	−1.1 (−2.5, 0.4)	0.152
Los Angeles, CA	0.4 (−0.5, 1.3)	−0.9 (−2.2, 0.4)	0.166	3.1 (2.2, 4.1)	−1 (−2.3, 0.4)	0.154
BMI						
<25 kg/m^2	—	—	—	—	—	—
25 to <30 kg/m^2	−2.4 (−3, −1.7)	−1 (−2, −0.1)	0.032	−1.7 (−2.3, −1)	−0.8 (−1.7, 0.1)	0.08
30 to <40 kg/m^2	−6.1 (−6.8, −5.3)	−0.9 (−1.9, 0.1)	0.091	−4.3 (−5, −3.6)	−0.7 (−1.8, 0.3)	0.155
≥40 kg/m^2	−9.7 (−11.2, −8.2)	−1 (−3.1, 1.1)	0.356	−6.8 (−8.2, −5.3)	−1.2 (−3.2, 0.9)	0.257
eGFR <60 mL · min^{-1} · 1.73 m^{-2}	4 (3.1, 5)	−0.9 (−2.3, 0.4)	0.181	2 (1.1, 2.9)	0 (−1.4, 1.3)	0.942
Diabetes	−3 (−3.8, −2.1)	−2.2 (−3.4, −1)	<0.001	−1.4 (−2.2, −0.6)	−1.3 (−2.4, −0.2)	0.019
Physical activity						
Light	—	—	—	—	—	—
Moderate	1.8 (1.1, 2.5)	0.8 (−0.2, 1.7)	0.107	1.7 (1.1, 2.3)	0.5 (−0.4, 1.4)	0.311
High	0.5 (−0.3, 1.3)	2.3 (1.2, 3.4)	<0.001	2 (1.3, 2.8)	1.5 (0.4, 2.5)	0.006

[1] All values are estimated differences in mean or amplitude (as noted in columns); 95% CIs in parentheses. Adjusted differences were adjusted for age, ethnicity, sex, and study site. *P* values are from Wald tests of the null hypothesis that differences in amplitude are zero. Multivariate Wald test for ethnicity: $P = 0.008$ (adjusted), $P < 0.001$ (unadjusted). eGFR, estimated glomerular filtration rate; 25(OH)D, 25-hydroxyvitamin D.

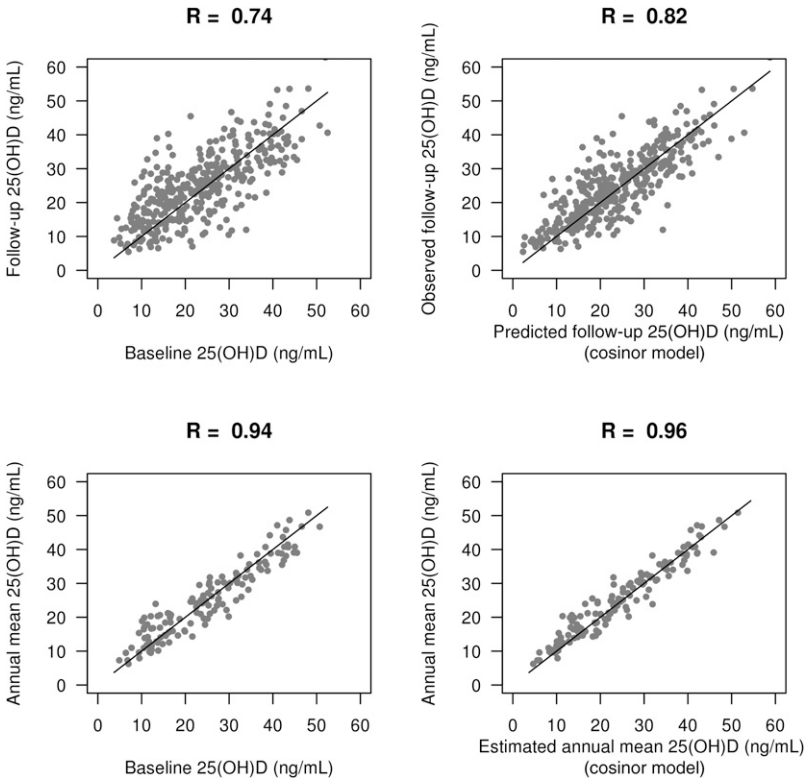

FIGURE 2. Scatter plots comparing the predictive performance of baseline 25(OH)D (left) to the null cosinor model (right). The top row shows correlations for predicting the follow-up 25(OH)D value (n = 368), and the bottom row shows correlations for predicting the empirically estimated annual 25(OH)D exposure (n = 135). The cosinor model was unadjusted. Annual mean plots were restricted to participants who were measured 6 mo apart. Solid lines are on the diagonal ($y = 1 \times x$). R, Pearson's correlation coefficient; 25(OH)D, 25-hydroxyvitamin D.

model did not significantly improve the predictive performance. An online tool that uses the cosinor model without covariates to calculate the estimated mean annual 25(OH)D concentration and estimated yearly peak and trough 25(OH)D concentrations from a single 25(OH)D measurement and measurement date is available at http://kri.washington.edu/calculator (25).

TABLE 3
Prediction error for follow-up 25(OH)D concentration (top set of rows) and for mean annual 25(OH)D (bottom set of rows)[1]

	MSE	RMSE	Bias	SD	*P* compared with baseline model	*P* compared with cosinor model without covariates
Model for follow-up 25(OH)D (n = 368)						
Baseline 25(OH)D carried forward	58.8	7.7	1.5	7.5	—	—
Dummy variables for season, no covariates	45.7	6.8	0.30	6.8	0.004	0.29
Cosinor, no covariates	39.5	6.3	0.9	6.2	<0.001	
Cosinor, age, sex	39.7	6.3	0.8	6.3	—	0.987
Cosinor, plus ethnicity	41.9	6.5	0.9	6.4	—	0.782
Cosinor, plus study site	42.9	6.6	1.0	6.5	—	0.572
Cosinor, plus physical activity	43.2	6.6	1.0	6.5	—	0.573
Model for annual mean 25(OH)D (n = 135)						
Baseline 25(OH)D carried forward	15.5	3.9	0	3.9	—	—
Dummy variables for season, no covariates	13.3	3.6	0.1	3.7	0.213	0.135
Cosinor, no covariates	10	3.2	0.7	3.1	0.005	—
Cosinor, age, sex	9.3	3	0.7	3	—	0.688
Cosinor, plus ethnicity	9.8	3.1	0.7	3.1	—	0.915
Cosinor, plus study site	10.1	3.2	0.6	3.1	—	0.929
Cosinor, plus physical activity	10.2	3.2	0.6	3.1	—	0.893

[1] Bias, average difference between measured and predicted values; MSE, mean squared error (average of squared differences between measured values and predicted values); RMSE, root mean squared error (square root of the mean squared error); SD, SD of predicted values; 25(OH)D, 25-hydroxyvitamin D.

TABLE 4
Classification of 25(OH)D concentrations comparing single measured values with estimated annual means[1]

Measured baseline 25(OH)D (n = 6476)	Estimated annual mean 25(OH)D ≥20 ng/mL	Estimated annual mean 25(OH)D <20 ng/mL	Percentage reclassified
All seasons			
≥20 ng/mL	4079	179	4.2
<20 ng/mL	279	1936	12.6
Total	—	—	7.1
Summer			
≥20 ng/mL	889	119	11.8
<20 ng/mL	0	256	0
Total	—	—	9.4
Winter			
≥20 ng/mL	879	0	0
<20 ng/mL	124	519	19.3
Total	—	—	8.1

[1] 25(OH)D, 25-hydroxyvitamin D.

Classification of vitamin D deficiency

Compared with use of the baseline 25(OH)D concentration alone, the use of the mean annual 25(OH)D concentration estimated from the cosinor model reclassified 7.1% of participants with regard to risk of 25(OH)D deficiency, which was defined as <20 ng/mL as per Institute of Medicine recommendations (**Table 4**) (1). In the summer, the cosinor model reclassified 11.8% of participants who had a single 25(OH)D concentration ≥20 ng/mL as vitamin D deficient, whereas in the winter, the cosinor model reclassified 19.3% of participants who had a 25(OH)D concentration <20 ng/mL as sufficient.

Associations with BMD

We examined how the estimation of the annual mean 25(OH)D concentration affected associations of a low 25(OH)D concentration with BMD, which is known to be reduced with vitamin D deficiency (2). Both 25(OH)D and lumbar BMD at L3 were successfully measured in a subset of 1898 MESA participants. After adjustment for age, sex, ethnicity, and BMI, an unadjusted baseline 25(OH)D concentration <20 ng/mL was associated with a 2.5-g/cm^3 lower BMD (95% CI: 6.0, −1 g/cm^3 lower; P = 0.164). In addition, after adjustment for the season of 25(OH)D measurement as dummy variables, an unadjusted baseline 25(OH)D concentration was <20 ng/mL was associated with a 2.4-g/cm^3 lower BMD (95% CI: 6.0, −1.2 g/cm^3 lower; P = 0.194). In a parallel adjusted model, an estimated annual mean 25(OH)D concentration <20 ng/mL by using the cosinor model was associated with a 3.9-g/cm^3 lower BMD (95% CI: 7.6, 0.2 g/cm^3 lower; P = 0.037).

DISCUSSION

In a large, multiethnic, community-based population, the seasonal variation in serum 25(OH)D concentrations was large relative to mean values and was well approximated by a cosinor model. Longitudinal measurements showed that the unadjusted cosinor model improved the prediction of the subsequent 25(OH)D concentration and generated mean annual 25(OH)D concentrations that were highly correlated with observed mean 25(OH)D concentrations. The mean annual 25(OH)D concentration estimated from the cosinor model reclassified 7.1% of participants with regard to 25(OH)D deficiency, which was defined as a concentration <20 ng/mL. The association of a low mean annual 25(OH)D concentration estimated from the cosinor model with BMD was stronger than that of the untransformed low 25(OH)D concentration with BMD, which suggesting that an improved 25(OH)D classification reduced bias. Together, these observations suggested that use of the cosinor model to estimate the mean annual 25(OH)D concentration is a valid approach to improve 25(OH)D classification.

This study built on previous studies that showed a substantial seasonal variation in the 25(OH)D concentration (8, 9, 11, 14, 15) and used cosinor models to describe this variation (8, 9, 11, 15). Like these studies, we began by using cross-sectional data with single measurements per person to evaluate the population mean 25(OH)D concentration. We confirmed that the cosinor model fit the seasonal variation well in a diverse, multi-ethnic population. Notably, the cross-sectional approach assumes that the mean seasonal variation in a population approximates the mean of seasonal variations in its constituents. This assumption may be reasonable in a large population, but cross-sectional studies cannot determine the distribution of within-person seasonal variation or the degree to which modeling fits individual patterns of variation.

Therefore, to our knowledge, the novel component of our study was the use of longitudinal data to validate our approach. Our cosinor model predicted follow-up 25(OH)D concentrations with significantly less bias and more precision than simply carrying forward baseline 25(OH)D values. In addition, the mean annual 25(OH)D concentration estimated from the cosinor model correlated strongly (r = 0.96) with the mean of the 2 measured 25(OH)D concentrations. These observations suggested that the cosinor model is a valid method to estimate 25(OH)D concentration over the year in individuals. Similar results were reported in a longitudinal study from New Zealand (15). Because we studied a diverse North American population, and the prediction with the cosinor model was similar across strata defined by important demographic and clinical characteristics, the tool we developed appears to be broadly applicable to adults in North America.

Compared with a single 25(OH)D measurement, the mean annual 25(OH)D concentration estimated by using the cosinor model reclassified a substantial proportion of participants with regard to risk of 25(OH)D deficiency, defined as <20 ng/mL as recommended by the Institute of Medicine (1). Moreover, the association of estimated mean annual 25(OH)D concentration <20 ng/mL with BMD was stronger than that of a single 25(OH)D measurement with BMD, with both a point estimate further from null and a lower P value. One potential explanation for this observation is that reduced bias in measurement of 25(OH)D facilitates more accurate evaluation of associations with relevant health outcomes.

In cross-sectional analyses, we showed that several clinical characteristics affected the amplitude of seasonal 25(OH)D variation, the overall mean 25(OH)D concentration (height of the sine curve), or both. For example, the amplitude of seasonal variation was greater in younger participants, men, and more physically active participants and varied slightly by study site.

Race-ethnicity and BMI were strongly associated with mean 25(OH)D concentration but not amplitude. These findings are consistent with our prior results in the Cardiovascular Health Study (11) and point to age, sex, physical activity and location as potential modifiers of seasonal variation in 25(OH)D. However, in longitudinal analyses, these associations were not sufficiently strong to impact the prediction performance of the cosinor model. Therefore, we believe the simple and conservative approach of applying a cosinor model without covariates is generally most appropriate.

In epidemiologic studies of 25(OH)D, investigators often account for seasonal variation by adjusting for season of 25(OH)D measurement as dummy variables in regression models. We observed that the model for 25(OH)D with dummy variables for season had a smaller bias than that with the cosinor model but a larger variation in predicting follow-up 25(OH)D measurements and the mean annual 25(OH)D. The model with dummy variable was more complex than the cosinor model (estimating 4 compared with 3 variables); therefore, this appears to be an example of the bias-variance tradeoff (26). The concept of the bias-variance tradeoff states that as the complexity of a model increases, a reduction in bias will be observed while observing a corresponding increase in the variance of an out-of-sample prediction. The cosinor model appears to be in the sweet spot where the mean squared error (which includes both bias squared and variance) is minimized. In addition, a recent simulation study suggested that adjustment for seasons as dummy variables in estimating associations with an outcome variable is an ineffective approach to eliminate bias (13).

The use of a cosinor model to estimate the mean annual 25(OH)D concentration or future 25(OH)D concentrations has many potential applications. In clinical care, patients and providers may find estimated mean annual 25(OH)D concentrations or predicted peak and trough concentrations of 25(OH)D over the calendar year to be a useful aid to guide decisions regarding vitamin D supplementation. We have enabled this by installing a free online tool at kri.washington.edu/calculator (25). In research, the use of an estimated mean annual 25(OH)D concentration may reduce bias when testing associations with outcomes that are examined in a cross-section but do not vary by season (as with BMD in this study) and when testing associations with longitudinal outcomes (13). In particular, associations of 25(OH)D with health outcomes have often been described as nonlinear, and the assessment of the mean annual 25(OH)D concentration may help identify appropriate 25(OH)D thresholds (4, 21, 27–31).

Although our study was performed in a diverse, community-based population, it was restricted to communities in North America and, thus, was limited in the range of latitude. Because latitude is the most-important predictor of sunlight intensity, the cosinor model that we have estimated in this study may not be applicable in other populations. In addition, because of limited resources, we had a small sample size of longitudinal measurements. It is possible that we did not detect covariate effects on the accuracy because of a lack of power. However, we have validated the general approach of estimating the cosinor model from cross-sectional data, and we encourage other researchers to consider doing so in other populations around the world.

In conclusion, we developed and validated an approach to estimate the mean annual 25(OH)D concentration from a single 25(OH)D measurement by using a cosinor model. This model represents an acceptably accurate and precise method for classifying vitamin D status and should be considered for use in research and clinical care.

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The authors' responsibilities were as follows—MCS: designed the study, analyzed data, wrote the manuscript, and was responsible for the final content of the manuscript; AS: designed the research; GPL and CR-C: designed the research and analyzed data; ANH: conducted the research and provided essential reagents; NS-J and MB: conducted the research; JHI and PLL: wrote the manuscript; DSS: conducted the research and wrote the manuscript; and BK and IHdB: designed the research and wrote the manuscript. None of the authors had a conflict of interest.

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