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Determinants of serum 25 hydroxyvitamin D levels in a nationwide cohort of blacks and non-Hispanic whites

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Abstract
Objective To develop algorithms predicting serum 25 hydroxyvitamin D [s25(OH)D] for a large epidemiological study whose subjects come from large geographic areas, are racially diverse and have a wide range in age, skin types, and month of blood sample collection. This will allow a regression calibration approach to determine s25(OH)D levels replacing the more costly method of collection and analysis of blood samples.
Study design and setting Questionnaire data from a subsample of 236 non-Hispanic whites (whites) and 209 blacks from the widely dispersed Adventist Health Study-2 ($n = 96{,}000$) were used to develop prediction algorithms for races separately and combined. A single blood sample was collected from each subject, at different times throughout the year.
Results Models with independent variables age, sex, BMI, skin type, UV season, erythemal zone, total dietary vitamin D intake, and sun exposure factor explained 22 and 31% of the variance of s25(OH)D levels in white and black populations, respectively (42% when combined). UV season and erythemal zone determined from measured UV radiation produced models with higher R^2 than season and latitude.
Conclusion Combining races with a term for race and using variables with measured UV radiation capture the variance in s25(OH)D levels better than analyzing races separately.

Keywords Cancer · Serum 25-hydroxyvitamin D · Predictors · Adventist health study-2 · Blacks · Whites

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Introduction

The number of chronic diseases tentatively associated with low serum 25 hydroxyvitamin D [s25(OH)D] levels has increased markedly during the last decade [1]. Further studies are needed to verify these associations. The three sources of vitamin D are diet, supplement intake, and ultraviolet B (UVB; 290–315 nm) radiation [2, 3]. Factors such as race [4–10], age [11–15], body mass index (BMI) [4–6, 9, 16–19], sun-reactive skin type/color [2, 20–22], sunscreen use [23], geographic location/latitude [22], time of year of blood sample collection [6, 7, 9, 24–27], and genetic factors [28] modify resulting s25(OH)D levels.

Previously published multivariate models for determinants of s25(OH)D vary considerably [4–7, 9, 10, 13, 19, 21, 23–26, 29–35]. The assortment in type and precision of data collection suggest that many of the variables contributing to s25(OH)D levels are difficult to ascertain accurately [36, 37]. This is especially true of exposure to UVB radiation [2, 22, 38, 39]. The effect of UVB light on any individual depends on a complex mix of personal and environmental factors [2, 3, 36, 37] such as geographic location of the subject and month/season of year. These are usually represented by the surrogates latitude and season, respectively [22]. However, strength of UV radiation does not vary in parallel lines across the United

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States as implied by the use of latitude. Rather, its convoluted patterns are such that the noon UV intensity could vary by as much as a factor of two in different parts of the country at the same latitude on the same fine day [40]. Furthermore, comparison of maps of the average monthly noon UV intensity throughout the year across the contiguous US demonstrates that seasonal changes in UV intensity do not occur in 3 monthly segments represented by the traditional seasons. Putting months with similar UV intensity patterns together results in different groupings representing the seasons [40].

Our main goal is to develop a prediction equation for s25(OH)D levels for the AHS-2 cohort, a large geographically dispersed population of racially diverse subjects. The blood samples used to develop the prediction equation were collected throughout the year from a representative sample of the calibration study subgroup of the AHS-2. Finding variables that predict s25(OH)D levels and refining their measurement would allow a regression calibration approach substituting E(s25(OH)D | questionnaire data) for measured serum values. This would enable this large epidemiological study to examine the effects of s25(OH)D at lower costs than collecting 90,000 blood samples. We will also compare and contrast regression models when blacks and non-Hispanic whites (whites) are analyzed separately and together, and search for differences in effect of the important variables.

Materials and methods

Parent study

The AHS-2 has been described in detail elsewhere [41]. In brief, it is a prospective epidemiological study of 96,000 Seventh-day Adventists designed to examine the relationship of lifestyle to risks of prostate, breast, and colon cancers. Enrollment to AHS-2 occurred between 2002 and 2007. More than 25,000 of the enrollees are black, and study members live in every state and province of the United States and Canada. Every 2 years, a questionnaire to gather information about hospitalizations is mailed, the second of which included additional detailed questions about sun exposure.

Study population

Subjects included in this report are a sample of members from the AHS-2 calibration study who were enrolled by June 2006, had provided detailed sun exposure information for the 2 months prior to their clinic attendance, and reported their race as either black ($n = 209$) or white ($n = 236$). These subjects had their blood samples analyzed for s25(OH)D. Details of the calibration study methods [41, 42] have been described elsewhere. Briefly, calibration subjects ($n = 1{,}011$) were randomly selected from among the 96,000 enrollees to the AHS-2. They were required to attend a clinic where weight and height were measured, and fasting blood samples collected. These clinics were held from November 2003 to May 2007 (excluding February, June, and July because of weather or vacation time). Calibration subjects also provided six 24-h telephone dietary recalls and completed a food frequency questionnaire (FFQ) within 1–3 months of blood sample collection. The study was approved by the institutional review board of Loma Linda University.

Determination and assessment of factors contributing to S25(OH)D Levels

BMI

BMI was determined from measured height (without shoes) and weight (with light clothing) of participants at time of blood collection.

Dietary, supplemental, and total vitamin D intake

Vitamin D intake was assessed from the AHS-2 FFQ which requested information about the previous 1 year and was validated against two blacks (each of three) of recalls taken 5–6 months apart to cover opposite seasons. The FFQ has corrected validity correlations of 0.60 and 0.64 against 24-h telephone recalls in black and white subjects, respectively [42]. Dietary vitamin D included D_2 and D_3. The naturally occurring and fortified vitamin D content of foods was obtained using the Nutrition Data System for Research (NDS-R) software version 5.03 database (The Nutrition Coordinating Center, Minneapolis, MN). Subjects were asked to name all supplements they were consuming, together with brand names and quantities. Values of vitamin D from supplements were verified from the manufacturers' websites. No differentiation was made for D_2 or D_3 as this could not always be determined.

Dietary vitamin D was adjusted for energy intake using the residual method [43]. Supplemental intake was not energy adjusted. Total vitamin D intake was the sum of the population mean dietary intake, the energy-adjusted residual and supplemental intake.

Smoking history and alcohol consumption

Neither was included in our models as these habits are not part of the AHS-2 subjects' lifestyle.

Skin pigmentation

Subjects were categorized according to Fitzpatrick sun-reactive skin types I through VI [44] based on their answer to the question "What happens to your skin if it is exposed many times to bright sunlight in the summer without protection?" Types I and II (no tan or tan very lightly) were collapsed for both blacks and whites to skin type I/II since there were only 11 white and 2 black subjects reporting skin type I. Fitzpatrick skin type III (tan moderately), IV (tan darkly), and V (already brown) and VI (already black) were coded skin type III, IV, and V/VI, respectively. These collapsed categories were scored 1, 2, 3, and 4, respectively, for the independent regression variable, skin type.

Percentage of body exposed

The detailed sun exposure questionnaire asked which parts of the body were typically exposed when in the sunshine on Sundays, Saturdays, and weekdays during the 2 months prior to clinic attendance. Adapting burn exposure charts [45], percentages of 4, 2, 6, 13, and 13 were assigned to face and neck, hands, most of the arms, most of the legs and upper torso, respectively. Our percentages are lower than Wachtel's [45] because ours represent "most", not "all" of that body part, and compensate for UVB radiation affecting only the side of the body facing the sun at any one time.

Duration of sun exposure

Subjects were asked how long they were in the sun on a typical weekday, Saturday, and Sunday during the 2 months prior to clinic attendance. Categories were 0, $\leq$29 min, 30–59 min, 1–2 h, and 2–3 h except for the hours 11 a.m. to 3 p.m., when the categories were 0, up to 14 min, 15–29 min, 30–59 min, 1–2.9 h, 3–4 h. Midpoints of each of these time categories were used to calculate total time per week which was converted to a daily average. Duration was also calculated weighting the hours from 11 a.m. to 3 p.m. by 2 to allow for higher intensity of UVB at that time of day [46, 47].

Sun exposure factor

The amount of vitamin D produced in the skin is the result of the percentage of skin surface exposed to the sun and duration of that exposure. Sun exposure factor, the product of these two variables, was calculated for (a) total time exposed, (b) 11 a.m. to 3 p.m., when the strength of UV radiation is strongest, and (c) when midday hours were weighted by 2 [46].

Latitude

Latitude is a surrogate for UVB exposure due to geographic location. Latitude categories 1–3 were defined in the baseline questionnaire by designating the state of subject's residence as north, mid or south when more than 50% of the state fell between the latitudes of >40°N, 35–40°N, and <35°N, respectively.

Erythemal zone

Erythemal zones are based on UV index maps showing the convoluted patterns of UV radiation intensity across the United States. Using the maps available from the National Aeronautics and Space Administration (NASA) (August 1996–August 2003) [40], each subject was assigned an erythemal zone based on the average of those years of the average monthly strength of noon erythemal radiation during the two months prior to the date of blood sample collection at the location of their residence. Although erythemal radiance (which causes reddening or erythema of the skin) includes UVA and C, as well as UVB radiation, it is a more accurate indicator than latitude of relative UVB strength due to geographic location. The erythemal zones were coded 1–5, beginning at <60 mW/m^2, and increasing by 60 W/m^2 to 240–300 mW/m^2. Erythemal zones are somewhat confounded by season, as they depend on the two months preceding the clinic visit.

Season

Season categories were designated using traditional breakpoints: Season 1, winter—December to February; Season 2, spring—March to May; Season 3, summer—June to August (although no samples were collected in June or July); Season 4, fall—September to November.

UV season

From the same maps used to determine Erythemal zone, UV seasons were formed by grouping together the months which had similar patterns of noon erythemal radiation strength [40] forming UV winter—November to February; UVspring—March; UV summer—April to August; and UV fall—September to October. UV winter and UV spring were collapsed to form UV season 1 since only 12 blood samples were collected in March, UV season 2 and 3 represent UV summer and UV fall, respectively. This modification for season was made to test whether it would be a more precise measure of UV exposure due to month of blood collection.

Sunscreen use and altitude

So few subjects in this cohort used sunscreen regularly or lived at altitudes greater than 3,000 feet that we did not include these variables in our analyses.

Biochemical methods

Plasma and cells were separated by centrifuge at the clinic sites. Blood collected at field clinics from calibration subjects was sent on frozen gel packs overnight to reach the processing lab at Loma Linda University, CA within 30 h of sample collection, then stored in liquid nitrogen. S25(OH)D was measured using a two-step radioimmunoassay procedure (Diasorin, Stillwater, MN). The selected samples were couriered on dry ice from the Loma Linda laboratory to the Reproductive Endocrine Research Laboratory, Department of Obstetrics and Gynecology, USC Keck School of Medicine, Los Angeles, and stored again in liquid nitrogen until time of assay which was carried out in three batches. Typical intra and interassay coefficients of variation at this laboratory are 10 and 16%, respectively.

Statistical analyses

All analyses were conducted using S-Plus software, version 7.0 (TIBCO software, Inc, Palo Alto, CA). Chi-square difference of means and two sample *t*-tests were used to determine the difference between the white and black populations for categorical and continuous variables, respectively. Partial Spearman correlations adjusted for age and sex were determined between all predictive variables. Blacks and whites were analyzed together and separately. Subjects with missing values for variables being tested were omitted from that analysis. Linear regressions were at first only age and sex adjusted, then multivariate models were used to examine the relationships between s25(OH)D levels and independent variables. Selected second-order terms were used to check evidence of non-linearity. Of these, skin type^2 and UV season^2 were statistically significant, and therefore included in the model as reported in Table 3. Sex and age-adjusted models were also used to examine the relationships between sun exposure variables, and skin types, and season.

Regressions for blacks were not log-transformed to allow for easier comparison of effect between races, although distribution of their s25(OH)D levels was skewed slightly to the right. The same variables were significantly associated with s25(OH)D levels using either the transformed or non-transformed data.

All multivariate models included variables measuring age, sex, BMI, dietary vitamin D, supplemental vitamin D, month of blood sample collection, geographic location of the subject, skin type, and skin surface and duration of sun exposure. The model combining the races also included a term for race, and the product terms with race for those variables which were significantly different between the races. Low numbers of males in both white and black populations limited power when testing interactions with gender.

Results

Relevant baseline characteristics of the study population are described in Table 1. Of the 209 blacks and 236 whites, more than two-thirds were female. It was an older population, the mean age for whites being somewhat higher than for blacks. Mean s25(OH)D levels were 20.0 (SD 10.2) and 30.8 (SD 10.3) ng/mL, with 15.8 and 52.1% attaining sufficiency ($\geq$30 ng/mL) [1] in blacks and whites, respectively. BMI was higher in blacks compared to whites. There were no significant differences between races for nutritional vitamin D intake or sun exposure variables.

Spearman's age and sex-adjusted (where appropriate) pair-wise correlations between all independent variables in the multivariate model revealed no significant correlations for blacks. For whites, sun exposure factor was found to be positively correlated with skin type (0.14, $p = 0.05$), UV season (0.22, $p = 0.002$) and erythemal zone (0.22, $p = 0.002$) and negatively correlated with age (-0.25, $p = 0.0005$). In age and sex-adjusted linear regression, positive associations were found between min spent in the sunshine ($p = 0.03$) and both the percentage of body exposed to the sunshine and UV season ($p = 0.004$).

In age and sex-adjusted linear regression, blacks and whites shared several variables significantly associated with s25(OH)D levels, including BMI, season, UV season, vitamin D from supplements and total vitamin D intake (Table 2). Age, skin type, and sun exposure factor were significant predictors in whites only. Erythemal zone was significantly associated with s25(OH)D levels for blacks but not whites. Sex, latitude, percentage of body exposed to sunshine, time spent in the sunshine, and vitamin D from food were not significant predictors for either racial group. Neither midday hours alone, midday hours weighted by 2, or sun exposure factor using midday hours alone or midday hours weighted by 2 were significant in any regression. We did find positive associations between sun exposure factor with skin type ($p = 0.03$) and percentage of body exposed with UV season ($p = 0.004$).

For multivariate analyses, all the variables that were significant in the age and sex-adjusted models for either racial group, plus sex, were included. Significant variables in the age and sex-adjusted models remained significant for whites. For blacks, BMI became non-significant. For the

Table 1 Means (SD) and proportions of selected baseline characteristics of study population by racial group

Characteristic	Non-Hispanic whites $n = 236$	Blacks $n = 209$	p[a]
Males, %	36.9	26.3	0.016
Age, years	63.0 (13.8)	58.3 (12.5)	0.0002
Range, years	34–96	31–88	
Serum 25 hydroxyvitamin D [1], ng/mL	30.8 (10.3)	20.0 (10.2)	<0.0001
Severe deficiency (<25), %	0.9	12.0	
Deficiency (≥25–≤50), %	14.4	45.5	
Insufficiency (≥50–≤75), %	32.6	26.8	
Sufficiency (≥75), %	52.1	15.8	
BMI, Kg/m^2	26.9 (5.2)	30.2 (6.7)	<0.0001
Unknown[b], %	4.2	3.8	
Fitzpatrick skin types			
I No tan/freckles, %	8.9	1.0	<0.0001
II Tan lightly, %	32.2	14.8	
III Tan moderately, %	42.4	15.3	
IV Tan darkly, %	16.5	21.1	
V/VI skin brown or black, %	0	43.5	
Unknown[b], %	0	4.3	
Dietary Vitamin D intake[c], mcg	3.4 (2.4)	3.3 (2.4)	0.60
Unknown[b], %	5.9	14	
Supplemental Vitamin D intake, mcg	6.3 (7.9)	5.0 (7.4)	0.39
Unknown[b], %	4.2	9.6	
Total Vitamin D intake[d], mcg	9.9 (6.2)	8.8 (7.9)	0.22
Unknown[b], %	5.9	20.6	
Season of the year[e] (for blood collection)			<0.0001
Winter (months 12–2), %	13.3	4.8	
Spring (months 3–5), %	14.4	35.4	
Summer (months 6–8), %	18.6	13.9	
Fall (months 9–11), %	53.8	45.9	
UV season[f] (blood collection)			0.0004
UV season1 (months 11–3), %	30.9	20.0	
UV season 2 (months 4–8), %	29.2	46.9	
UV season 3 (months 9–10), %	39.8	33.0	
Latitude of residence			<0.0001
>40°N, %	42.4	25.4	
35–40°N, %	49.6	43.5	
<35°N, %	8.0	31.1	
Erythemal zone[g] of residence			0.20
<60 mW/m^2, %	8.5	9.1	
60–119 mW/m^2, %	11.0	12.4	
120–179 mW/m^2,%	24.2	29.7	
180–240 mW/m^2, %	30.9	33.0	
240–300 mW/m^2, %	25.4	15.8	
Duration of daily sun exposure (min)	98.7 (93.1)	91.2 (91.0)	0.50
Unknown[b], %	12.7	14.8	
Percentage of body exposed to sunshine[h]	9.3 (6.5)	8.7 (6.9)	0.31
Unknown[b], %	9.7	1.9	

Table 1 continued

Characteristic	Non-Hispanic whites $n = 236$	Blacks $n = 209$	p[a]
Sun exposure factor[i]	1,005.7 (1,268.4)	978.8 (1,471.6)	0.9
Unknown[b], %	12.7	14.8	

[a] Chi-square difference of means and two sample t-test comparing blacks to whites, for categorical and continuous variables, respectively

[b] Percentage of cohort with unknown value for the variable

[c] Calorie adjusted by residual method. Conversion factor to IU = ×40

[d] Sum of population mean dietary vitamin D intake, the energy-adjusted residual and supplemental vitamin D intake. Conversion factor to IU = ×40

[e] Traditional seasons: winter (months 12–2), spring (months 3–5), summer (months 6–8), fall (months 9–11)

[f] Months of year grouped according to similarity of erythemal zone patterns

[g] Average of average monthly erythemal radiance for subject's location for 2 months prior to blood collection

[h] Percentages of 4, 2, 6, 13, and 13 were assigned to face and neck, hands, most of the arms, most of the legs, upper torso, respectively. Adapted from burn exposure charts [46]

[i] Product of duration of daily sun exposure and percentage of body exposed to sunshine

white cohort, this multivariate model explained 22% of the variance of s25(OH)D levels, and for the black cohort, 31% (Table 3). Significant differences in effects on s25(OH)D between races were found for age ($p = 0.037$) and sun exposure factor ($p = 0.007$). When the racial groups were combined and product terms between these two variables and race, as well as a term for race were included in the model, 42% of the variance in s25(OH)D levels was explained. Using season and latitude, the R^2 for multivariate white, black, and combined races models were 0.20, 0.21, and 0.37, respectively. Thus, variables for UV exposure based on recorded UV radiation rather than season and latitude improved the R^2 of these models by 11, 44, and 13% in whites, blacks, and combined races models, respectively.

The relative effect of 1 SD change for the significant non-quadratic variables, age, BMI, skin type, total vitamin D intake, and sun exposure factor on s25(OH)D levels in whites was 0.7, 0.6, 0.5, 0.8, and 1.0 ng/mL, respectively; and for blacks, effects of 1 SD change of sun exposure factor and total vitamin D intake produced changes of 0.1 and 1.1 ng/mL. Changing to the next higher erythemal zone of residence predicted an increase of 0.9 ng/mL in blacks. Skin type in blacks had a curvilinear association with s25(OH)D, this being similar to whites for lighter skin tones, but when skin type was brown/black, there was dramatically less effect. The effect of season is also complex and is shown in Fig. 1. In our data, for both blacks and whites the highest s25(OH)D levels were found in fall or early winter, respectively.

The three models shown in Table 3 can provide predicted levels of s25(OH)D when applied to the relevant populations. These predicted levels (ng/mL) are such that the 10th and 90th percentiles are 25.0 and 37.1 (white subjects alone); 13.5 and 27.3 (black subjects alone); and 16.6 and 35.3 (combined populations). For each race, separately, these percentile ranges correspond to vitamin D dietary intake differences of 60 mcg or 2,400 IU (whites), 34 mcg or 1,360 IU (blacks), according to these same regressions. These ranges of predicted values are sufficient to potentially detect differences in risk of some endpoint when such equations are used in regression calibration.

Discussion

We developed algorithms that explained 31, 22, and 42% of the variance in measured s25(OH)D levels in our black, white, and racially combined populations, respectively. The higher R^2 of the combined model indicates that the race variable captures factors not accounted for, nor yet understood. Only age and sun exposure factor were found to have significantly different effects between races. Among blacks, sun exposure had a lesser impact than diet due to diminished cutaneous production of s25(OH)D. For both races, season had a higher impact on changes in s25(OH)D levels than any other factor, even after we included variables for sun exposure.

The difference we found in mean s25(OH)D levels between races is no doubt due in part to the differences in skin tone. Not only is the lighter toned skin of whites capable of greater production of s25(OH)D, cutaneously produced vitamin D_3 has been reported to result in more sustained levels of s25(OH)D than does oral dosing of vitamin D_2 [48, 49].

Decreased production of cutaneous 7 dehydroxy cholesterol with age has been reported in Caucasians with skin type III, the most common skin type in the United States [14]. This may contribute to the negative association of s25(OH)D levels with age which, in our white cohort, remained after adjustment for sun exposure factor, the only other variable found to decrease with age in sex-adjusted

Table 2 Beta coefficients, p-values, and R^2 for predictor variables for serum 25 hydroxyvitamin D levels (ng/mL) in age and sex-adjusted linear regressions among blacks and non-Hispanic whites separately and combined

Predictor variables	Non-Hispanic whites ($n = 236$)		Blacks ($n = 209$)		Combined Non-Hispanic whites and blacks ($n = 445$)	
	Beta (p)	R^2	Beta (p)	R^2	Beta (p)	R^2
Age[a] (years)	−0.16 (0.001)	0.044	0.10 (0.07)	0.016	0.02 (0.61)	0.006
Sex[b]	−1.19 (0.40)	0.044	−0.47 (0.77)	0.016	−1.82 (0.12)	0.006
Race[c]	–	–	–	–	−10.95 (<0.0001)	0.221
Body mass index, Kg/m^2	−0.37 (0.006)	0.077	−0.24 (0.02)	0.039	−0.52 (<0.0001)	0.081
Skin type[d] alone	2.79 (0.003)	0.081	–	–	–	–
Skin type	N/A		7.17 (0.08)	0.030	12.03 (<0.0001)	0.088
Skin type^2			−1.26 (0.10)		−2.77 (<0.0001)	
Season[e]	−10.66 (0.009)	0.072	−2.73 (0.001)	0.09	−14.77 (<0.0001)	0.074
Season^2	1.97 (0.01)		–		2.99 (<0.0001)	
UV season[f]	−16.51 (0.006)	0.082	−27.46 (<0.0001)	0.117	−30.51 (<0.0001)	0.105
UV season^2	3.77 (0.01)		6.35 (<0.0001)		7.12 (<0.0001)	
Latitude[g]	0.27 (0.8)	0.045	0.23 (0.81)	0.016	−1.88 (0.015)	0.020
Erythemal zone[h]	0.19 (0.70)	0.045	1.83 (0.002)	0.059	1.27 (0.006)	0.023
Vitamin D from food[i], mcg	0.40 (0.17)	0.052	0.50 (0.13)	0.023	0.59 (0.017)	0.018
Vitamin D from supplements, mcg	0.23 (0.008)	0.074	0.35 (0.0005)	0.075	0.32 (0.003)	0.056
Total vitamin D intake[j], mcg	0.25 (0.004)	0.080	0.33 (0.001)	0.072	0.31 (0.0001)	0.044
Time spent in sunshine, min per day	0.01 (0.14)	0.053	0.01 (0.2)	0.034	0.01 (0.047)	0.021
Percentage of body exposed to sunshine	0.16 (0.14)	0.048	0.003 (0.97)	0.016	0.15 (0.09)	0.017
Sun exposure factor[k]	0.002 (0.002)	0.087	0.0007 (0.19)	0.035	0.001 (0.004)	0.032

[a] Adjusted for sex only

[b] Adjusted for age only; coded 1 for male and 2 for female

[c] Coded 1 for whites, 2 for blacks

[d] Adapted from Fitzpatrick skin type; coded 1–4, for Types I + II, III, IV, and V + IV, respectively

[e] Traditional seasons: winter (months 12–2), spring (months 3–5), summer (months 6–8), fall (months 9–11); coded 1–4, respectively

[f] Months of year grouped according to similarity of erythemal zone patterns; coded 1–3 for months 11–3, 4–8, and 9–10, respectively

[g] Coded 1–3 for latitudes >40°N, 35–40°N, and <35°N, respectively

[h] Average of average monthly erythemal radiance for subject's location for 2 months prior to blood collection; coded 1–5 beginning at <60 mW/m^2, and increasing by 60 W/m^2 to 240–300 mW/m^2

[i] Calorie adjusted by residual method. Conversion factor to IU = ×40

[j] Sum of population mean dietary vitamin D intake, the energy-adjusted residual and supplemental vitamin D intake. Conversion factor to IU = ×40

[k] Product of duration of daily sun exposure and percentage of body exposed to sunshine

analyses. As in NHANES III reports [9], we found no association between s25(OH)D and age among blacks, this difference with white subjects being nearly statistically significant ($p = 0.06$).

For whites, we found a negative association between BMI and s25(OH)D levels which is consistent with the literature [8, 9, 50]. For blacks, the association in the age and sex-adjusted model disappeared in the multivariate model. In the literature, when only blacks are included in the model, the results vary [4, 9, 27, 51].

At first glance, the positive linear association that we found in whites between skin types I to IV and s25(OH)D levels and the curvilinear relationship in blacks appear to conflict with the understanding that the fairer the skin, the higher the conversion of pre-vitamin D to cholecalciferol per unit of UVB radiance [2, 22]. But photosensitive skin type is determined by the skin's potential for tanning [44, 52] while skin color is determined by the amount of melanin in the skin [2]. Skin types I and II correlate well with skin color, but skin types III and IV can have fair as well as darker tones prior to tanning. We found, along with others [52, 53], that those with greater ability to tan spend more time in the sunshine ($p = 0.03$) explaining the higher levels of s25(OH)D with increasing skin type. That some

Table 3 Beta coefficients and *p*-values for variables in multivariate linear regression models predicting serum 25 hydroxyvitamin D levels (ng/mL) in non-Hispanic whites and blacks separately and combined

Variables in final model	Non-Hispanic whites ($n = 236$)	Blacks ($n = 209$)	Blacks plus Non-Hispanic whites ($n = 445$)
	$R^2 = 0.221$ ($p < 0.0001$)	$R^2 = 0.308$ ($p < 0.0001$)	$R^2 = 0.415$ ($p < 0.0001$)
	Beta (*p*)	Beta (*p*)	Beta (*p*)
Age, years	−0.13 (0.02)	0.04 (0.55)	−0.27 (0.02)
Age × Race	–	–	0.15 (0.06)
Sex[a]	−0.22 (0.90)	−0.75 (0.6)	−0.69 (0.53)
Race[b]	–	–	−15.92(0.003)
BMI, Kg/m^2	−0.29 (0.04)	−0.18 (0.1)	−0.21 (0.02)
Skin type[c] alone	1.97 (0.05)	–	–
Skin type	–	6.98 (0.07)	6.67 (0.01)
Skin type^2		−1.51 (0.04)	−1.37 (0.01)
UV season[d]	−17.05 (0.008)	−29.21 (<0.0001)	−21.32 (<0.0001)
UV season^2	3.38 (0.03)	6.08 (0.0004)	4.30 (0.0001)
Erythemal zone[e]	1.34 (0.09)	1.82 (0.017)	1.72 (0.001)
Total Vitamin D intake, mcg[f]	0.20 (0.02)	0.35 (0.0006)	0.23 (0.0003)
Sun exposure factor[g]	0.0016 (0.007)	0.0001 (0.9)	0.0031 (0.008)
Sun exposure factor × race			−0.0015 (0.04)

[a] Coded 1 for male and 2 for female

[b] Coded 1 for whites, 2 for blacks; included only in model where blacks and non-Hispanic white data sets were combined

[c] Adapted from Fitzpatrick skin type; coded 1–4, for Types I + II, III, IV, and V + IV, respectively

[d] Months of year grouped according to similarity of erythemal zone patterns; coded 1–3 for months 11–3, 4–8 and 9–10), respectively

[e] Average of average monthly erythemal radiance for subject's location for 2 months prior to blood collection; coded 1–5 beginning at <60 mW/m^2, and increasing by 60 W/m^2 to 240–300 mW/m^2

[f] Sum of population mean dietary vitamin D intake, the energy-adjusted residual, and supplemental vitamin D intake. Conversion factor to IU = ×40

[g] Product of duration of daily sun exposure and percentage of body exposed to sunshine

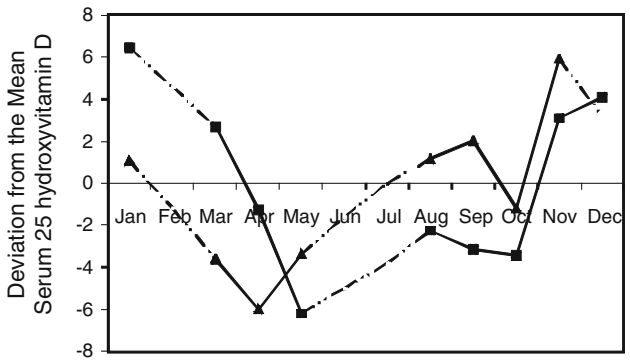

Fig. 1 Monthly change in serum 25 hydroxyvitamin D levels using deviations from multivariate-adjusted annual means. *Black square* non-Hispanic whites, *Black up pointing triangle* blacks, - -..- -.. No samples Collected

effect of skin type on s25(OH)D persists after adjustment for sun exposure may be due to residual confounding.

Many studies have reported that s25(OH)D levels are lowest in winter and highest in summer when UV strength is at its peak [4, 5, 13, 17, 18]. At least one study reported highest levels in the fall [9]. Our study found levels in whites were lowest and began to rise when UVB intensity began its annual considerable increase in April [40], continuing past the summer peak of UVB strength until January (Fig. 1). The continued rise through fall may imply a cumulative effect, s25(OH)D continuing to be stored while UVB strength is high, thereafter being released from storage for a time. Additionally, a positive association between amount of body exposed and UV season ($p = 0.004$) in whites but not blacks indicates an increasing body exposure among whites during fall, which may explain the continuing rise in their s25(OH)D levels during this time. A similar trend in s25(OH)D levels in blacks occurred 1 and 2 months ahead of the whites (Fig. 1). The earlier start in decreasing s25(OH)D levels in blacks may be explained, at least in part, by the inability of their darker skin to produce vitamin D at the lower intensities of UVB radiation in the fall and by their lack of increased body exposure during that time.

The variables, UV season and erythemal zone which we constructed from maps of UV radiation weighted for erythemal reaction and which included UVA, B, and C were intended to improve the accuracy of season and latitude as surrogates for UVB exposure. While they did produce moderate improvements in R^2, further improvement is likely with the use of recently published maps of UV radiation which have been refined and weighted for previtamin D_3 production [54] rather than erythemal reaction in human skin.

Whether our model will allow effective regression calibration remains to be seen in actual trials with disease endpoints. Giovannucci et al. [5] used this approach to predict colon cancer risk with an R^2 for predicting s25(OH)D of 0.28 in a model that included race. Compared to the (unattainable) average of a large number of serum values, using our predictive equations would reduce power by about 50%. However, using a single serum measure without adjustment for its with-in person random error will also result in a 30–40% reduction in power, but in addition, will bias effect estimates toward the null by about 50% [55, 56].

There are several limitations to this study including the relative inaccuracy of measuring certain exposure variables. There are the well-known effects of errors in dietary questionnaires [57]. We did not separate cholecalciferol and ergocalciferol [58, 59], or account for other vitamin D metabolites found in animal products [60]. The nutrient database values for vitamin D in foods and supplements are inaccurate, a problem currently being addressed by the Nutrient Data Laboratory [61].

As with many questionnaire items, questions for duration of vitamin D-producing sun exposure and amount of body exposed requested 'usual', not 'actual', exposure times. We could not correct for the variation in strength of UVB that occurs throughout the day. We found neither advantage in separating exposure by time of day, nor weighting midday hours by 2 [46]. We were also unable to adjust for the point at which cutaneous production of s25(OH)D levels plateau for each individual. Total sun time may have exceeded this point for many, especially those with lighter skin and longer daily sun exposure times.

The dynamics of lag time between UV exposure/vitamin D intake and s25(OH)D levels has not yet been fully elucidated. No calculations were made to differentiate between cutaneous production of vitamin D which results in a more sustained supply of s25(OH)D compared to oral vitamin D [49]. Reports for the half-life of s25(OH)D vary from 2 weeks to 2 months [58, 62–64].

The R^2s we obtained in our study compare favorably with other studies on US populations [4–7, 13, 18, 19, 26], but are still relatively low. Other factors such as the common genetic variants of vitamin D binding protein which result in as much as a threefold difference in s25(OH)D levels [28], and others yet unknown, may contribute to this.

Conclusion

A higher R^2 for predicting s25(OH)D levels is obtained in the model where the races are combined, compared to separate models for blacks and whites. Studying the racial groups separately allowed us to determine that age and sun exposure factor affect s25(OH)D levels in blacks and whites differently. The higher R^2 of the combined population results from the race variable and its significant product terms. This persists even after allowing for skin type and sun exposure. Other metabolic/genetic differences between the races may account for this. Seasonal changes are strong in both races. In whites, sun exposure was less in the summer than in the fall and this was probably reflected in serum levels. In blacks, dietary vitamin D has a proportionately greater influence than in whites. Skin type has a complex association with s25(OH)D. Mildly darker tones are associated with higher levels in both races, but blacks with the darkest skin tones have much lower levels. Replacing the usual surrogates of UV exposure, season, and latitude, with measured UV intensities determined from the erythemal index for the geographic location and season of each subject improves the R^2. A predictive model developed by one study may not be transferable to other study groups. The common features of each study, nevertheless, provide useful insights into the factors that affect s25(OH)D levels.

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