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Original Contribution

Predictors of 25-Hydroxyvitamin D Concentration Measured at Multiple Time Points in a Multiethnic Population

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The evidence for a relationship between serum vitamin D levels and nonskeletal health outcomes is inconsistent. The validity of single or predicted measurements of 25-hydroxyvitamin D (25(OH)D) concentration is unknown, as levels of this biomarker are highly seasonally variable. We compared models of 25(OH)D measured at baseline, at multiple time points throughout the year, and averaged over the year among 309 persons in Toronto, Ontario, Canada (43°N latitude) during 2009–2013. Information and blood samples were collected every 2 months. Baseline and average 25(OH)D concentrations were correlated ($r = 0.88$). Major factors associated with 25(OH)D level were similar across models and included race/ethnicity (concentrations in non-European groups were lower than those in Europeans), vitamin D supplement use of ≥1,000 IU/day (18.9 nmol/L (95% confidence interval (CI): 16.1, 21.8) vs. no supplement use in a full data set with all factors), and the presence of the group-specific component/vitamin D binding protein gene (*GC*/*DBP*) rs4588 functional polymorphism (AA vs. CC: −16.7 nmol/L (95% CI: −26.2, −7.1); CA vs. CC: −10.7 nmol/L (95% CI: −14.9, −6.5)). Most factors had similar associations in Europeans and non-Europeans. Genetic factors may play a greater role in average 25(OH)D concentrations. Prediction models for 25(OH)D are challenging and population-specific, but use of genetic factors along with a few common population-relevant, quantifiable nongenetic factors with strong associations may be the most feasible approach to vitamin D assessment over time.

25-hydroxyvitamin D; linear models; risk factors; seasons; vitamin D

Abbreviations: CI, confidence interval; 25(OH)D, 25-hydroxyvitamin D; SNP, single nucleotide polymorphism; UVB, ultraviolet B.

Vitamin D has an established role in bone metabolism and calcium absorption (1). Despite considerable research evidence suggesting that vitamin D could play a much broader role in health and disease, with potential links to infectious diseases, multiple sclerosis, cancer, diabetes, hypertension, and total mortality, among other outcomes, none of the relationships are consistent or convincing (1). Large randomized controlled trials are in progress, but there are issues regarding relevant intervention doses and study populations (2). Much of the current evidence comes from large cohort studies using the primary indicator of vitamin D status, serum 25-hydroxyvitamin D (25(OH)D) concentration. However, these studies generally use single measurements with seasonal adjustment or use measurements in a subset to create 25(OH)D prediction models which are then applied to the full cohort. Outside equatorial latitudes, 25(OH)D levels can be highly seasonal (3, 4), but the degree of variation may differ, particularly by racial/ethnic group (5). The variation in 25(OH)D captured by prediction models has generally been low (20%–40%), and the specific predictors available are variable (6–16).

The primary sources of vitamin D are skin exposure to ultraviolet B (UVB) radiation, a small number of natural or fortified food sources, and vitamin supplements (1, 17). The amount of UVB radiation received from the sun can vary considerably over the seasons in many geographic areas and is influenced by weather, pollution, and behavioral factors such sun protection or avoidance (1). In addition to the primary sources, a variety of factors can influence 25(OH)D concentrations, including age, body mass index, skin pigmentation, and genetics (1, 18–20).

We conducted a study in a multiethnic population of young and middle-aged adults in which 25(OH)D was measured at multiple time points during the year and in which information on a variety of potential predictors was collected. The goal of the study was to identify and compare predictors of single-point baseline concentrations, multiple concentrations over the course

of the year, and average concentrations in order to determine the utility of single-point measurements in the context of seasonal variation. We also examined the relationship of these predictors to 25(OH)D separately in persons of European and non-European heritage.

METHODS

Study population and data collection

We recruited volunteer men and women aged 18–59 years in Toronto, Ontario, Canada, from a variety of sources, including advertisements at several hospitals, universities, and fitness/community centers and advertisement in a free newspaper. The census metropolitan area of Toronto includes more than 6 million people, and the population is diverse. The latitude is 43°40′N. We sought to recruit a study population in which about half of the participants were of non-European heritage. Persons who reported kidney or liver disease were excluded because of the potential major influence of these conditions on vitamin D metabolism. Those interested in participating in the study contacted the coordinator by telephone or e-mail, and eligibility was confirmed before the baseline study visit was scheduled. Recruitment occurred in 2 waves. In an initial pilot phase, 53 persons were recruited from July 2009 to January 2010. During the second phase, 257 new participants were recruited between June 2011 and August 2013. Data from both phases were combined. One person was subsequently excluded because no study diary had been completed. The final study population included 309 individuals (22 in the pilot phase only, 31 in both phases of the study, and 256 in the full phase only). There was no evidence for differences between 25(OH)D levels during the same season (May–October and November–April) in different years (data not shown).

Prior to the baseline study visit and all subsequent visits, a daily diary was sent to each participant to complete over the course of the week prior to the visit. The diary involved detailed reporting of each activity the participant engaged in for at least 15 minutes during the day, including whether the activity was undertaken outdoors (yes/no) and the level of physical activity (3 levels). Consumption of specific vitamin D-containing foods (milk, margarine, juice, yogurt, cereal, cheese, eggs, mushrooms, fish, other) and supplements and information on sunscreen use, cigarette smoking, and alcohol intake was also collected. At the study visit, the diary was returned, and additional information was collected in a brief interview. This information included travel and sunbed/sunlamp use since the past visit (or over the past 2 months, at baseline). Information on race/ethnicity was collected only at baseline. Height, weight, waist circumference, and hip circumference were measured at each visit. Skin melanin content was measured at each visit with reflectance spectroscopy using a DSM II ColorMeter (Cortex Technology A.p.S., Hadsund, Denmark). We measured melanin content on the upper inner arm, the forehead, and the forearm. One blood sample was collected at each visit for measurement of 25(OH)D in serum, which was frozen in aliquots in liquid nitrogen the same day. One additional tube of blood was collected at baseline for DNA extraction.

Participants (defined as those completing a baseline visit) were asked to come in for a visit during each of six 2-month periods throughout the year: January–February, March–April, May–June, July–August, September–October, and November–December. During the pilot phase of the study, a maximum of 4 visits occurred. We provided reimbursement of $25 for each study visit. Some individuals recruited during the pilot phase also participated during the full study, resulting in a maximum of 10 visits. The distribution of completed visits is shown in Table 1. Most participants were measured during at least five 2-month periods during the full study ($n = 219$; 76%). The total number of visits completed, including both phases of the study, was 1,588. Because some information was missing at some visits ($n = 26$ visits; 1.6%), the final number of visits included in the analysis was 1,562. The study protocol was approved by the Mount Sinai Hospital Research Ethics Board (Toronto, Ontario, Canada), and all participants provided signed consent.

25(OH)D assays

At the end of the study, 1 frozen aliquot from each visit was shipped to Quest Diagnostics (Madison, New Jersey), where 25(OH)D assays were performed using liquid chromatography–tandem mass spectrometry. Quest was unable to analyze all samples for each individual in the same batch. However, we included 31 blinded duplicate samples (different batches), and the coefficient of variation for these samples was 7%. Both $25(OH)D_3$ and $25(OH)D_2$ levels were measured, and values were combined to obtain total 25(OH)D concentration. In the pilot phase of the study, we had measured 25(OH)D in 1 aliquot from each visit using liquid chromatography–tandem mass spectrometry at a local laboratory experienced in the use of this assay. Among the 155 samples for which we had 25(OH)D concentrations assessed using the same approach in 2 laboratories, the Pearson correlation between the assay results was very high (Pearson's $r = 0.97$, $P < 0.0001$).

Selection of SNPs and genotyping

We identified a list of candidate genes and single nucleotide polymorphisms (SNPs) linked to these genes, primarily from the literature (Appendix Table 1), including some associated with circulating 25(OH)D levels in 2 large genome-wide association studies carried out in primarily Caucasian populations (19, 20). Specific SNPs within genes were identified from the literature, supplemented by additional tagSNPs within relevant genes. A total of 19 SNPs were successfully (100%) genotyped at the Clinical Genomics Centre at Mount Sinai Hospital (Toronto, Ontario, Canada) using the Sequenom iPLEX MassARRAY (Sequenom, San Diego, California). Duplicate samples were included ($n = 47$), and the genotype agreement of these samples was 100%. Some SNPs turned out to be in complete linkage disequilibrium. Therefore, the number of loci studied was actually 16, linked to 9 genes: group-specific component/vitamin D binding protein (*GC/DBP*); 7-dehydrocholesterol reductase/nicotinamide adenine dinucleotide synthetase 1 (*DHCR7/NADSYN1*); cytochrome P-450 family 2, subfamily R, member 1 (*CYP2R1*); cytochrome P-450 family 24, subfamily A, member 1

Table 1. Baseline Characteristics of Participants in a Study of Predictors of 25-Hydroxyvitamin D Concentration and Distribution of Numbers of Visits in the Pilot (2009–2010) and Full (2011–2013) Phases of the Study, Toronto, Ontario, Canada, 2009–2013

Characteristic	No. of Persons	%	Mean (SD)	Range
Age, years[a]	309		37 (12)	19–59
Sex[a]				
Male	92	29.8		
Female	217	70.2		
Race/ethnicity[a]				
European	173	56.0		
South Asian	31	10.0		
African	13	4.2		
Other Asian	51	16.5		
Other	41	13.3		
Body mass index[a,b]	309		25 (5)	16–49
Waist circumference, cm			83 (13)	59–129
25(OH)D level, nmol/L[a]				
Baseline	309		63.3 (25.9)	11.9–156.6
Average[c]	219		64.7 (23.7)	14.8–146.5
No. of visits completed[d]				
Pilot study ($n = 53$)				
1	2			
2	13			
3	25			
4	13			
Full study ($n = 287$[e])				
1	26			
2	11			
3	10			
4	21			
5	43			
6	176			

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; SD, standard deviation.

[a] Data recorded at the participant's first visit, regardless of whether it occurred in the pilot phase of the study or the full study (53 in the pilot phase and 256 in the full study).

[b] Weight (kg)/height (m)^2.

[c] Persons who completed 5 or 6 visits in the full study contributed to analyses of average 25(OH)D level ($n = 219$).

[d] The total number of study visits was 1,588; the total number of visits with complete information was 1,562.

[e] Including 31 persons who participated in both the pilot phase and the full study.

(*CYP24A1*); cytochrome P-450 family 27, subfamily B, member 1 (*CYP27B1*); solute carrier family 24, member 5 (*SLC24A5*); phosphodiesterase 3B (*PDE3B*); oculocutaneous albinism II melanosomal transmembrane protein (*OCA2*); and melanocortin 1 receptor (*MC1R*).

Statistical analysis

We examined the distribution of 25(OH)D values at baseline, and among persons with levels measured in at least 5 of the six 2-month periods throughout 1 year, we examined the distribution of the ranges of 25(OH)D values and the correlation between average and baseline values. We then conducted linear regression modeling of baseline 25(OH)D concentrations using several steps. Each factor was assessed individually and also adjusted for age, sex, and race/ethnicity. We then performed forward stepwise modeling, beginning with factors that had the lowest *P* values from the previous age-, sex-, and race/ethnicity-adjusted analyses. We considered multiple factors related to UVB exposure, diet, body size, lifestyle (e.g., cigarette smoking, alcohol drinking, and physical activity), and genetics. Because we wanted to obtain the best model without skin reflectance measures, which are not always available, we added these as a last step. Variables were added one at a time, and a single variable was removed at any step if the *P* value was above 0.10, with the exception of age and sex, which were always included in the model. We repeated the process using data (25(OH)D and covariates) from all available visits of all study participants, using generalized estimating equations to account for the multiple measurements within individuals. In a final step, the process was repeated using average 25(OH)D level as the outcome in the subset of persons with measurements taken in 5 or 6 2-month periods over the course of a year, and all covariates were also averaged.

We then reran the analysis for each final model separately among self-reported Europeans and non-Europeans. Note that during the modeling process we found that either sunbed/sunlamp use or physical activity was retained in the model, but not both. Nine of the 10 individuals reporting sunbed/sunlamp use also reported a high level of physical activity. When these 10 persons were excluded, physical activity was no longer retained in the model, and therefore we retained sunbed/sunlamp use but not physical activity. All analyses were performed using Stata 13 (StataCorp LP, College Station, Texas). All *P* values were 2-sided.

RESULTS

In the final sample, 56% of the population was of European descent and 44% was non-European (Table 1). Most non-Europeans were Asian, including 10% South Asian (India, Pakistan, or Bangladesh) and 17% from other parts of Asia (mostly China). Another 4% were of African heritage, and the remaining 13% of participants were members of all other groups combined, including those of mixed heritage. Most study participants (70%) were female, and measures of body size varied widely. The average baseline 25(OH)D concentration was 63.3 nmol/L, similar to the average of the mean values for those measured across at least five 2-month periods and similar to the average value for the Canadian population (64 nmol/L) observed in the Canadian Health Measures Survey, cycle 2 (August 2009–November 2011) (21).

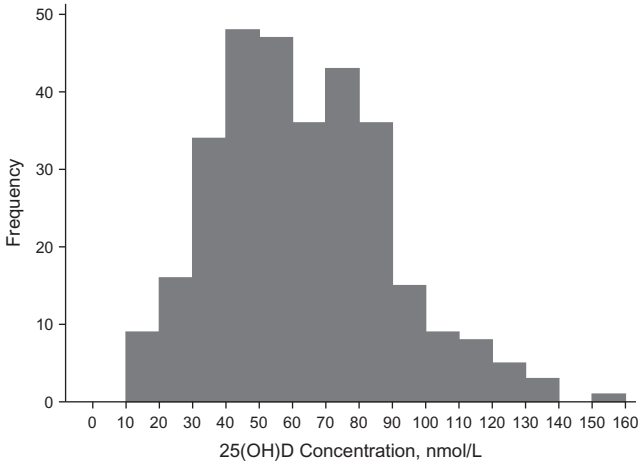

Figure 1. Distribution of 25-hydroxyvitamin D (25(OH)D) levels at the baseline visit in a study population of adults aged 18–59 years ($n = 309$), Toronto, Ontario, Canada, 2009–2013.

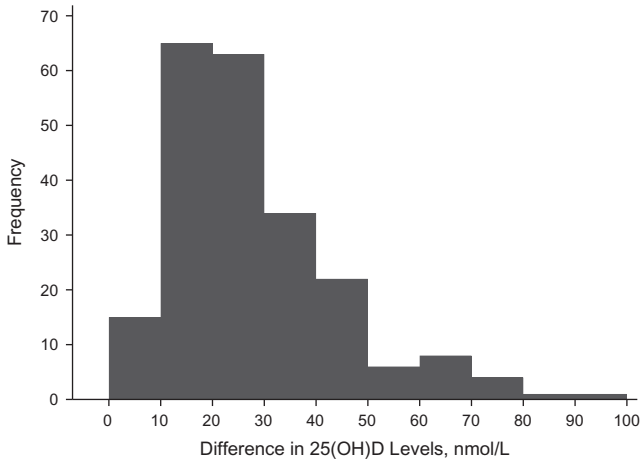

Figure 2. Distribution of the difference between the highest and lowest 25-hydroxyvitamin D (25(OH)D) concentrations in adults aged 18–59 years with 5 or 6 study visits across the course of a year ($n = 219$), Toronto, Ontario, Canada, 2011–2013.

Baseline 25(OH)D concentrations were approximately normally distributed, with a few individuals having high concentrations (Figure 1). The distribution of differences between the highest and lowest values in persons with measurements taken in 5 or 6 2-month periods was slightly skewed, with some individuals exhibiting large differences (Figure 2). However, there was high correlation (Pearson's $r = 0.88$, $P < 0.0001$) between baseline and average concentrations (Figure 3).

Results from the final models without skin reflectance measures of melanin are shown in Table 2. The total adjusted R^2 value for both baseline and average models was 0.46 (R^2 cannot be calculated using generalized estimating equations). The variables with the largest β coefficients and R^2 values were similar across models using baseline 25(OH)D concentration, 25(OH)D concentrations from all visits, or average 25(OH)D concentration in persons who had measurements taken in 5 or 6 2-month periods as the dependent variable. Racial/ethnic groups varied considerably in their 25(OH)D concentrations, even after we accounted for a variety of other factors, with all non-European groups having considerably lower concentrations than Europeans. The power to determine differences among non-European groups was limited. As expected, there was seasonal variation, with higher concentrations in May–October and the lowest concentrations in January–April. Use of vitamin D-containing supplements had a major impact on 25(OH)D concentration, particularly for intakes of 1,000 IU/day or more. The well-known *GC/DBP* rs4588 variant was associated with 25(OH)D in all models, and persons who were homozygous for the variant had considerably lower concentrations. Two loci, *DHCR7/NADSYN1* rs12785878 and *CYP24A1* rs6013897, were associated with average concentrations only. Sunbed/sunlamp use contributed to all of the models, though it was not very common in this population. Indicators of recent sun exposure, such as amount of time spent outside and travel to a southern location (<35°N latitude), contributed to the baseline and all-visits models but not the average model. Persons who reported usually wearing a hat in the winter (for each day, the study diary asked, "Did you wear a hat?") had slightly lower average concentrations. Daily milk drinkers had slightly higher 25(OH)D levels at baseline and higher average concentrations, particularly if they drank milk during the winter. Alcohol consumption was associated only with baseline concentrations. The only skin reflectance melanin measure associated with 25(OH)D was the sun-exposed forearm measurement, which was positively associated (Table 3). The addition of this melanin variable did not have a major impact on the other variables in the model.

Table 4 shows results separately for Europeans and non-Europeans using the all-visits data incorporating melanin measured on the forearm. The results were generally similar, including those for many of the UVB-related variables, vitamin D supplement

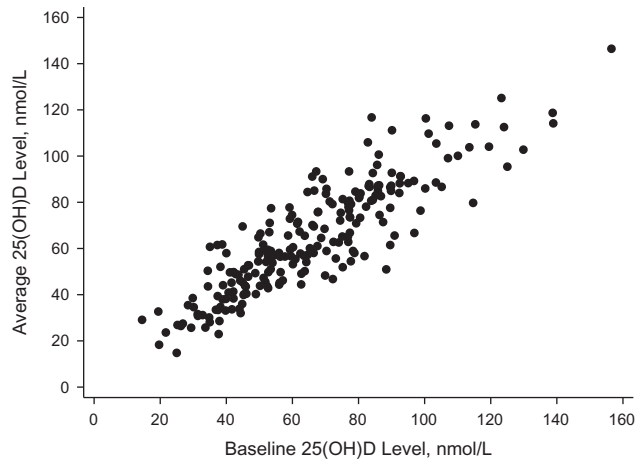

Figure 3. Baseline versus average 25-hydroxyvitamin D (25(OH)D) concentrations in adults aged 18–59 years with 5 or 6 study visits across the course of a year ($n = 219$), Toronto, Ontario, Canada, 2011–2013. Pearson correlation: $r = 0.88$.

Table 2. Predictors of 25-Hydroxyvitamin D Concentration[a] (Excluding Skin Reflectance) Derived Using Data Collected at Baseline, Data Collected at Multiple Time Points (All Study Visits), and Averaged Data, Toronto, Ontario, Canada, 2009–2013[b]

	Model									
	Baseline				All Study Visits (n = 1,562)		Average[c]			
	No. of Persons	β	95% CI	R^2	β	95% CI	No. of Persons	β	95% CI	R^2
Age, years	309	0.0	−0.2, 0.2	<0.01	0.3	0.1, 0.4	219	0.2	−0.1, 0.4	<0.01
P value		0.70			0.01			0.24		
Sex				0.01						<0.01
Male	92	0	Referent		0	Referent	68	0	Referent	
Female	217	7.3	2.4,12.3		5.6	0.9, 10.3	151	3.5	−2.5, 9.4	
P value		0.004			0.02			0.25		
Race/ethnicity				0.10						0.03
European	173	0	Referent		0	Referent	129	0	Referent	
South Asian	31	−20.4	−28.1, −12.7		−19.3	−26.4, −12.1	16	−13.8	−23.9, −3.7	
African	13	−25.8	−36.9, −14.6		−24.2	−34.8, −13.6	8	−15.5	−28.9, −2.0	
Other Asian	51	−16.0	−22.3, −9.6		−18.5	−24.3, −12.6	36	−11.6	−19.2, −4.0	
Other	41	−11.0	−17.6, −4.1		−12.8	−19.0, −6.6	30	−10.1	−17.7, −2.4	
P value		<0.001			<0.001			0.003		
Month				0.06						
January–February	38	0	Referent		0	Referent				
March–April	36	−5.0	−14.3, 4.2		0.8	−1.1, 2.6				
May–June	27	13.9	4.1, 23.7		9.0	7.1, 10.9				
July–August	69	12.5	4.5, 20.5		15.7	13.7, 17.6				
September–October	72	14.5	6.8, 22.3		11.2	9.4, 13.1				
November–December	67	8.0	0.2, 15.9		3.3	1.4, 5.1				
P value		<0.001			<0.001					
GC/*DBP* rs4588 genotype				0.04						0.04
CC	153	0	Referent		0	Referent	114	0	Referent	
CA	140	−10.5	−15.1, −5.8		−10.6	−14.8, −6.4	98	−8.2	−13.2, −3.2	
AA	16	−15.9	−26.1, −5.7		−16.2	−25.8, −6.6	7	−20.1	−35.1, −6.9	
P value		<0.001			<0.001			<0.001		
DHCR7/*NADSYN1* rs12785878 genotype										0.02
TT							79	0	Referent	
GT							95	−5.7	−11.4, 0	
GG							45	−12.5	−20.2, −4.9	
P value								0.006		
CYP24A1 rs6013897 genotype										0.01
TT							147	0	Referent	
AT							63	0.6	−4.8, 6.0	
AA							9	15.1	2.9, 27.2	
P value								0.05		
Use of vitamin D-containing supplements, IU/day				0.12						0.16
0 (none)	182	0	Referent				89	0	Referent	
<1,000	76	11.0	5.6, 16.3		7.8	5.8, 9.7	88	9.9	4.5, 15.4	
1,000	16	22.0	11.6, 32.0		13.8	10.2, 17.4		—[d]	—	
>1,000[d]	35	27.0	19.4, 34.2		18.8	15.9, 21.7	42	27.0	20.2, 33.9	
P value		<0.001			<0.001			<0.001		

Table continues

Table 2. Continued

	Model									
	Baseline				All Study Visits (n = 1,562)		Average[c]			
	No. of Persons	β	95% CI	R^2	β	95% CI	No. of Persons	β	95% CI	R^2
Use of a sunbed/sunlamp[e]				0.01						0.01
Never use	303	0	Referent		0	Referent	209	0	Referent	
Ever use	6	23.2	7.2, 39.2		9.2	2.9, 15.6	10	13.0	1.2, 24.8	
P value			0.01			0.004			0.03	
Travel south (<35°N latitude), days[e]				0.01						
<6	289	0	Referent		0	Referent				
≥6	20	10.4	1.4, 19.4		8.7	6.2, 11.1				
P value			0.02			<0.001				
Waist circumference, cm					−0.3	−0.4, −0.1	219	−0.2	−0.4, 0.0	0.01
P value						<0.001			0.06	
Milk intake, glasses/day[f]				0.01						0.01
<1	196	0	Referent				150	0	Referent	
≥1	113	5.2	0.7, 9.8				69	6.3	1.1, 11.6	
P value			0.03						0.02	
Total time spent outside, hours/day				0.01						
<1.5	144	0	Referent							
≥1.5	165	5.6	0.9, 10.3							
P value			0.02							
Time spent outside between 10 AM and 2 PM, hours/day										
<1					0	Referent				
≥1					2.7	0.8, 4.6				
P value						0.01				
Alcohol intake, glasses/day				0.02						
<1	254	0	Referent							
≥1	55	9.4	3.5, 15.2							
P value			0.002							
Regular hat use[f]										0.01
No							88	0	Referent	
Yes							131	−5.4	−10.4, −0.3	
P value									0.04	

Abbreviations: CI, confidence interval; *CYP24A1*, cytochrome P-450 family 24, subfamily A, member 1; *DHCR7/NADSYN1*, 7-dehydrocholesterol reductase/nicotinamide adenine dinucleotide synthetase 1; *GC/DBP*, group-specific component/vitamin D binding protein; 25(OH)D, 25-hydroxyvitamin D.

[a] β coefficients represent the estimated change in 25(OH)D concentration (nmol/L) associated with the factor after adjusting for all other factors in the model.

[b] The total R^2 value for both the baseline and average models was 0.46.

[c] Average values were calculated only among persons with 5 or 6 study visits over the course of a year.

[d] Supplement use in the model of average 25(OH)D concentration was for the winter months only (November–April), and in this model there were only 3 categories: none, <1,000 IU/day, and ≥1,000 IU/ day.

[e] In the past 2 months at baseline; since the last study visit for other visits.

[f] During the winter months only for the model of average 25(OH)D concentration.

use, and the *GC/DBP* rs4588 variant. Although in some cases a specific variable seemed to have a stronger association in one group or the other, the direction of effect was the same and the 95% confidence intervals overlapped.

Because predicting average concentrations is probably more relevant for long-term health outcomes and because direct measurements of melanin are unlikely to be available in many large studies, we also compared the models of average concentrations

Table 3. Predictors of 25-Hydroxyvitamin D Concentration[a] (Including Skin Reflectance) Derived Using Data Collected at Baseline, Data Collected at Multiple Time Points (All Study Visits), and Averaged Data, Toronto, Ontario, Canada, 2009–2013[b]

	Model									
	Baseline				All Study Visits (n = 1,562)		Average[c]			
	No. of Persons	β	95% CI	R^2	β	95% CI	No. of Persons	β	95% CI	R^2
Age, years	309	0.0	−0.2, 0.2	<0.01	0.2	0.0, 0.4	219	0.1	−0.2, 0.3	<0.01
P value			0.87			0.02			0.57	
Sex				0.02						0.01
Male	92	0	Referent		0	Referent	68	0	Referent	
Female	217	9.1	3.9, 14.4		6.7	2.0, 11.5	151	6.2	0.8, 12.3	
P value			0.001			0.006			0.05	
Race/ethnicity				0.10						0.06
European	173	0	Referent		0	Referent	129	0	Referent	
South Asian	31	−25.0	−34.1, −15.9		−22.3	−29.7, −15.0	16	−22.9	−34.6, −11.3	
African	13	−32.6	−45.9, −19.3		−28.7	−39.7, −17.8	8	−28.4	−44.1, −12.6	
Other Asian	51	−15.7	−22.1, −9.4		−18.3	−24.1, −12.5	36	−10.7	−18.3, −3.3	
Other	41	−12.1	−18.9, −5.2		−13.5	−19.7, −7.3	30	−12.5	−20.2, −4.9	
P value			<0.001			<0.001			<0.001	
Month				0.04						
January–February	38	0	Referent		0	Referent				
March–April	36	−4.7	−14.3, 4.2		1.0	−1.1, 2.6				
May–June	27	12.9	4.1, 23.7		8.5	7.1, 10.9				
July–August	69	11.4	4.5, 20.5		14.7	13.7, 17.6				
September–October	72	13.3	6.8, 22.3		10.5	9.4, 13.1				
November–December	67	7.6	0.2, 15.9		3.0	1.4, 5.1				
P value			<0.001			<0.001				
GC/*DBP* rs4588 genotype				0.04						0.04
CC	153	0	Referent		0	Referent	114	0	Referent	
CA	140	−10.5	−15.2, −5.9		−10.7	−14.9, −6.5	98	−8.2	−13.1, −3.3	
AA	16	−16.4	−26.6, −6.2		−16.7	−26.2, −7.1	7	−23.6	−37.5, −9.6	
P value			<0.001			<0.001			<0.001	
DHCR7/*NADSYN1* rs12785878 genotype										0.03
TT							79	0	Referent	
GT							95	−5.9	−11.5, −0.3	
GG							45	−13.6	−21.2, −6.1	
P value									0.002	
CYP24A1 rs6013897 genotype										0.01
TT							147	0	Referent	
AT							63	0.8	−4.5, 6.1	
AA							9	13.0	1.0, 25.0	
P value									0.11	
Use of vitamin D-containing supplements, IU/day				0.11						0.17
0 (none)	182	0	Referent		0	Referent	89	0	Referent	
<1,000	76	10.9	5.6, 16.2		8.0	6.0, 9.9	88	10.3	4.9, 15.6	
1,000	16	22.2	12.0, 32.4		13.9	10.4, 17.5		—[d]	—	
>1,000[d]	35	26.3	18.9, 33.7		18.9	16.1, 21.8	42	28.1	21.4, 34.9	
P value			<0.001			<0.001			<0.001	

Table continues

Table 3. Continued

	Model									
	Baseline				All Study Visits (n = 1,562)		Average[c]			
	No. of Persons	β	95% CI	R^2	β	95% CI	No. of Persons	β	95% CI	R^2
Use of a sunbed/sunlamp[e]				0.01						0.01
Never use	303	0	Referent		0	Referent	209	0	Referent	
Ever use	6	22.1	6.0, 38.1		8.6	2.3, 14.9	10	12.4	0.8, 24.0	
P value			0.007			0.008			0.04	
Travel south (<35°N latitude), days[e]				0.01						
<6	289	0	Referent		0	Referent				
≥6	20	10.3	1.4, 19.3		8.2	5.7, 10.7				
P value			0.02			<0.001				
Waist circumference, cm					−0.3	−0.4, −0.1	219	−0.2	−0.4, 0.0	0.01
P value						<0.001			0.05	
Milk intake, glasses/day[f]				0.01						0.02
<1	196	0	Referent				150	0	Referent	
≥1	113	5.4	0.8, 9.9				69	6.9	1.7, 12.0	
P value			0.02						0.01	
Total time spent outside, hours/day				0.01						
<1.5	144	0	Referent							
≥1.5	165	5.0	0.2, 9.7							
P value			0.04							
Time spent outside between 10 AM and 2 PM, hours/day										
<1					0	Referent				
≥1					2.5	0.7, 4.4				
P value						0.008				
Alcohol intake, glasses/day				0.02						
<1	254	0	Referent							
≥1	55	9.2	3.3, 15.0							
P value			0.002							
Regular hat use[f]										0.01
No use							88	0	Referent	
Any use							131	−5.7	−10.6, −0.7	
P value									0.03	
Melanin[g]	309	0.3	0.0, 0.6	<0.01	0.2	0.1, 0.3	219	0.6	0.2, 0.9	0.02
P value			0.07			0.001			0.003	

Abbreviations: CI, confidence interval; *CYP24A1*, cytochrome P-450 family 24, subfamily A, member 1; *DHCR7*/*NADSYN1*, 7-dehydrocholesterol reductase/nicotinamide adenine dinucleotide synthetase 1; *GC*/*DBP*, group-specific component/vitamin D binding protein; 25(OH)D, 25-hydroxyvitamin D.

[a] β coefficients represent the estimated change in 25(OH)D concentration (nmol/L) associated with the factor after adjusting for all other factors in the model.

[b] The total R^2 values for the baseline and averaged models were 0.46 and 0.48, respectively.

[c] Average values were calculated only among persons with 5 or 6 study visits over the course of a year.

[d] Supplement use in the model of average 25(OH)D concentration was for the winter months only (November–April), and in this model there were only 3 categories: none, <1,000 IU/day, and ≥1,000 IU/ day.

[e] In the past 2 months at baseline; since the last study visit for other visits.

[f] During the winter months only for the model of average 25(OH)D concentration.

[g] Melanin was measured on the forearm using reflectance spectroscopy (in the "average" model, it was the average measurement from May to October).

Table 4. Predictors of 25-Hydroxyvitamin D Concentration[a] (Including Skin Reflectance) Derived Using Data Measured at All Study Visits (Final Model), by Race/Ethnicity (European vs. Non-European), Toronto, Ontario, Canada, 2009–2013

	Race/Ethnicity					
	European (n = 892 Visits)			Non-European (n = 670 Visits)		
	Baseline No. of Persons	β	95% CI	Baseline No. of Persons	β	95% CI
Age, years	173	0.1	−0.2, 0.4	136	0.4	0.1, 0.6
P value		0.37			0.002	
Sex						
Male	53	0	Referent	39	0	Referent
Female	120	9.3	2.3, 16.3	97	3.7	−2.1, 9.5
P value		0.009			0.21	
Race/ethnicity						
South Asian				31	0	Referent
African				13	−6.8	−16.7, 3.2
Other Asian				51	2.8	−4.3, 9.9
Other				41	7.6	0.4, 14.8
P value					0.02	
Month						
January–February	19	0	Referent	19	0	Referent
March–April	18	0.6	−2.1, 3.2	18	2.2	−0.3, 4.6
May–June	15	9.0	6.2, 11.7	12	8.2	5.7, 10.7
July–August	41	17.0	14.2, 19.8	28	11.8	9.1, 14.4
September–October	43	13.1	10.5, 15.8	29	7.5	5.1, 9.9
November–December	37	3.9	1.3, 6.6	30	2.2	−0.1, 4.6
P value		<0.001			<0.001	
GC/*DBP* rs4588 genotype						
CC	83	0	Referent	70	0	Referent
CA	83	−11.7	−17.9, −5.5	57	−9.9	−15.1, −4.8
AA	7	−19.9	−35.9, −4.0	9	−14.4	−24.7, −4.1
P value		<0.001			<0.001	
Use of vitamin D-containing supplements, IU/day						
0 (none)	98	0	Referent	84	0	Referent
<1,000	44	6.8	3.9, 9.7	32	9.0	6.6, 11.4
1,000	6	11.2	6.3, 16.1	10	17.7	12.9, 22.4
>1,000	25	17.1	13.0, 21.1	10	20.9	17.2, 24.7
P value		<0.001			<0.001	
Use of a sunbed/sunlamp[b]						
Never use	168	0	Referent	135	0	Referent
Ever use	5	6.6	−1.6, 14.8	1	10.7	1.2, 20.3
P value		0.11			0.03	
Travel south (<35°N latitude), days[b]						
<6	160	0	Referent	129	0	Referent
≥6	13	9.4	6.0, 12.8	7	6.2	2.8, 9.5
P value		<0.001			<0.001	
Waist circumference, cm	173	−0.3	−0.5, −0.1	136	−0.2	−0.4, 0.0
P value		0.004			0.02	

Table continues

Table 4. Continued

	Race/Ethnicity					
	European (n = 892 Visits)			Non-European (n = 670 Visits)		
	Baseline No. of Persons	β	95% CI	Baseline No. of Persons	β	95% CI
Time spent outside between 10 AM and 2 PM, hours/day						
<1	25	0	Referent	19	0	Referent
≥1	148	2.6	0.0, 5.2	117	2.9	0.3, 5.4
P value		0.01			0.03	
Melanin[c]	173	0.2	0.1, 0.4	136	0.1	0.0, 0.3
P value		0.004			0.08	

Abbreviations: CI, confidence interval; *GC*/*DBP*, group-specific component/vitamin D binding protein.
[a] β coefficients represent the estimated change in 25-hydroxyvitamin D concentration (nmol/L) associated with the factor after adjusting for all other factors in the model.
[b] In the past 2 months at baseline; since the last study visit for other visits.
[c] Melanin was measured on the forearm.

in Europeans and non-Europeans excluding the melanin measurement (Table 5). In this comparison, some cell sizes were small and the models somewhat less stable. Again many of the effect estimates were similar. However, there were some differences, including the lack of any evidence of association between drinking milk during the winter and average 25(OH)D level in non-Europeans. Additionally, the increased average 25(OH)D level in *CYP24A1* variant homozygotes occurred only in Europeans. Given the reduced sample size in the subgroup analysis, the results should be interpreted with caution.

DISCUSSION

Vitamin D has been linked to many different health outcomes, but the evidence for these associations is inconsistent, and the role of vitamin D remains uncertain (1). Studies of vitamin D are challenging because of the variability in the indicator biomarker, 25(OH)D, and the variety of factors that contribute to it. We explored differences in predictors of individual, multiple, and average concentrations of 25(OH)D in a mixed population of young and middle-aged adults living at 43°N latitude. We observed a strong correlation between baseline and average concentrations. In addition, the major predictors were generally similar across different measures of 25(OH)D and in Europeans and non-Europeans. Race/ethnicity and vitamin D supplement intake of at least 1,000 IU/day had a major influence on 25(OH)D concentrations in this population, where supplement use is common. The functional *GC*/*DBP* genetic variant rs4588 also had a strong impact in all models, but genetic factors appeared to play a greater role in average concentrations of 25(OH)D. Indicators of UVB exposure, dietary variables, age, sex, and body fatness contributed to 25(OH)D concentrations to a lesser extent.

There have been many efforts to develop models for 25(OH)D that are applicable to a general adult population (6–16). These have been based on single-point measurements of 25(OH)D and therefore are equivalent to our baseline model. The variance explained based on R^2 ranges from 21% to 42%, with the exception of 1 model in the Australian population with a large number of factors and interactions, which explained 54% (13). Our baseline model explained 46% of variation. These models were derived from populations that varied in age and sex distribution, race/ethnicity, latitude, and the information available.

UVB radiation is a major source of 25(OH)D, but individual UVB exposure from sunlight is difficult to measure and individual monitoring is often not feasible. Ambient ultraviolet radiation can be assessed, including relatively simple measures such as season and latitude, but individual behaviors such as time spent outside, time spent in the shade when outside, and clothing coverage will influence the actual dose of UVB received. Detailed information is difficult and time-consuming to capture. The difficulty in quantifying sun exposure probably explains some of the failure to account for more of the variation in 25(OH)D within geographic locations.

The other major source of vitamin D is diet and supplements, but the impact of diet is usually limited. Food sources of vitamin D, including those that are fortified, generally contain modest amounts, and those with higher amounts, such as some types of fish, are rarely consumed in many populations. Diet can have a greater impact in specific populations, such as those living in the far north, in which intake of vitamin D-containing fish is high and ambient UVB exposure is low (22, 23). In most models, the estimated effect of vitamin D supplement use on 25(OH)D level is modest, but the variable is usually categorized as any supplement use versus none or is capped at an intake of ≥400 IU. With recent publicity around vitamin D and the availability of higher-dose supplements, intake of supplemental vitamin D has increased in some populations. In cycle 2 (2009–2011) of the Canadian Health Measures Survey, use of vitamin D supplements was reported by 34% of the Canadian population and was more common in females (41%) than in males (28%) (21). In our study population, which was 70% female, 41% of participants reported using vitamin D-containing

Table 5. Predictors of Averaged 25-Hydroxyvitamin D Concentration[a] (Excluding Skin Reflectance) Among Persons With Study Visits in at Least Five 2-Month Periods Over the Course of a Year (Final Model), by Race/Ethnicity (European vs. Non-European), Toronto, Ontario, Canada, 2009–2013[b]

	Race/Ethnicity							
	European				Non-European			
	No. of Persons	β	95% CI	R^2	No. of Persons	β	95% CI	R^2
Age, years	129	0.1	−0.3, 0.4	0.00	90	0.3	0.0, 0.7	0.02
P value		0.61				0.08		
Sex				<0.01				<0.01
Male	41	0	Referent		27	0	Referent	
Female	88	5.7	−2.4, 13.7		63	−2.7	−11.5, 6.2	
P value		0.17				0.55		
Race/ethnicity								<0.01
South Asian						0	Referent	
African						−2.7	−16.9, 11.4	
Other Asian						−0.8	−10.6, 9.1	
Other						4.0	−6.1, 14.0	
P value						0.60		
GC/*DBP* rs4588 genotype				0.02				0.08
CC	63	0	Referent		51	0	Referent	
CA	64	−7.0	−13.9, −0.2		34	−9.0	−16.0, −1.9	
AA	2	−19.9	−47.2, 7.4		5	−22.8	−38.3, −7.2	
P value		0.07				0.004		
DHCR7/*NADSYN1* rs12785878 genotype				0.02				<0.01
TT	69	0	Referent		10	0	Referent	
GT	50	−4.2	−11.4, 2.9		45	−4.0	−15.0, 7.0	
GG	10	−16.0	−29.0, −3.0		35	−7.6	−19.3, 4.1	
P value		0.05				0.40		
CYP24A1 rs6013897 genotype				0.06				0.00
TT	88	0	Referent		15	0	Referent	
AT	36	0.7	−6.9, 8.3		39	−0.3	−8.1, 7.5	
AA	5	32.4	14.6, 50.2		36	−8.0	−24.5, 8.4	
P value		0.002				0.62		
Use of vitamin D-containing supplements, IU/day[c]				0.17				0.23
0 (none)	53	0	Referent		36	0	Referent	
<1,000	49	8.8	1.0, 16.6		39	10.8	3.3, 18.4	
≥1,000	27	27.9	18.7, 37.0		15	27.0	17.1, 37.0	
P value		<0.001				<0.001		
Use of a sunbed/sunlamp				<0.01				0.01
Never use	122	0	Referent		87	0	Referent	
Ever use	7	12.0	−3.7, 27.6		3	14.0	−4.8, 32.8	
P value		0.13				0.14		
Waist circumference, cm	129	−0.1	−0.4, 0.2	<0.01	90	−0.3	−0.7, 0.1	0.02
P value		0.38				0.09		
Milk intake, glasses/day[c]				0.04				<0.01
<1	82	0	Referent		68	0	Referent	
≥1	47	10.4	3.4, 17.5		22	−0.9	−9.2, 7.7	
P value		0.004				0.83		

Table continues

Table 5. Continued

	Race/Ethnicity							
	European				Non-European			
	No. of Persons	β	95% CI	R^2	No. of Persons	β	95% CI	R^2
Regular hat use[c]				0.01				0.02
No	46	0	Referent		42	0	Referent	
Yes	83	−6.2	−13.2, 0.9		48	−6.3	−13.3, 0.8	
P value		0.09				0.08		

Abbreviations: CI, confidence interval; *CYP24A1*, cytochrome P-450 family 24, subfamily A, member 1; *DHCR7/NADSYN1*, 7-dehydrocholesterol reductase/nicotinamide adenine dinucleotide synthetase 1; *GC/DBP*, group-specific component/vitamin D binding protein

[a] β coefficients represent the estimated change in 25-hydroxyvitamin D concentration (nmol/L) associated with the factor after adjusting for all other factors in the model.

[b] The total R^2 values for Europeans and non-Europeans were 0.41 and 0.39, respectively.

[c] During the winter months only (November–April).

supplements at baseline, with 17% taking ≥1,000 IU/day. The frequency of higher-dose supplement use probably explains the impact of supplement use on variation in 25(OH)D levels in our population.

The amount of UVB radiation required to achieve the same increase in 25(OH)D concentration increases with higher melanin content in the skin (24). Thus, race/ethnicity is expected to be a determinant of 25(OH)D levels in diverse populations (9–12, 14). However, the association cannot be due entirely to skin color, as a strong association persisted after melanin content was included in the model, consistent with 1 other study that accounted for skin type (9). Race/ethnicity is probably related to many factors that can influence 25(OH)D levels, such as UVB-related behaviors, diet, and genetics, including factors not fully captured in the model.

Several other factors can influence 25(OH)D concentration to a lesser extent. Measures of body fatness are associated with lower 25(OH)D levels and are generally included in prediction models. The relationship of age and sex to 25(OH)D concentration is variable (6–16). In our study, there was a tendency toward higher 25(OH)D concentrations with increasing age and also higher concentrations in women compared with men, consistent with Canadian Health Measures Survey cycle 2 (21). Some studies have found a positive relationship with physical activity (6, 10–12, 15, 16), but in our study an initial relationship with physical activity appeared to be accounted for by use of sunbeds/sunlamps. A positive relationship between alcohol intake and 25(OH)D that we observed only at baseline seemed likely to be a chance association, but other studies have observed a similar relationship (12, 15). Alcohol may also be a proxy for other lifestyle factors.

Genome-wide association studies have identified consistent and biologically plausible genetic markers associated with 25(OH)D concentrations in European populations (19, 20), and these variants may be relevant in other populations (25). Predictive models have only recently begun to include genetic markers (13, 15), although the individual estimated effects of these markers have been modest. We observed a fairly strong and consistent association of the *GC/DBP* rs4588 variant with 25(OH)D in both Europeans and non-Europeans. Vitamin D allele scores have been developed for Mendelian randomization studies (26). Mendelian randomization is appealing, as genotyping is feasible in many studies and genetic variation may better capture variation in 25(OH)D levels over the longer term. However, some caution is needed, as there is the potential for pleiotropy, where a relationship between a vitamin D-related gene locus and a health outcome is not mediated through vitamin D (26).

A potential contributor to the relatively low R^2 values observed and to variation between models is inconsistency in 25(OH)D assays. Lack of consistency and standardization is an ongoing issue both between assays (27–29) and between laboratories (30). The assay method we used, liquid chromatography–tandem mass spectrometry, is considered a gold standard by some (27), and it was reassuring that we had both a high correlation between results measured in 2 different laboratories and a low interbatch coefficient of variation, particularly given that all samples from an individual could not be analyzed in the same batch.

To our knowledge, this is the first study to have evaluated determinants of 25(OH)D concentration based on multiple measurements taken in individuals over the course of a year. This allowed us to compare models of single-point, multiple-point, and average measurements. There have also been few studies involving a population of diverse race/ethnicity. Another strength is that statistical power was good for our generalized estimating equations models incorporating all visits. However, the power was reduced when the analyses were conducted separately in Europeans and non-Europeans, and it was also limited in models of 25(OH)D averaged over the year. Although we collected extensive information, some data, such as individual UVB monitoring data, were not available. The study was conducted at 1 location, and results may differ for more geographically diverse populations or at extreme locales.

It is unlikely that it will be possible to develop a universally applicable 25(OH)D prediction model or score, primarily because the relative importance of different factors and, in some cases, the role of different factors will vary across populations and studies. In addition, detailed information on factors such as UVB exposure is difficult to collect. It is possible that

the use of genetic information combined with a few common, measurable variables with larger impact on 25(OH)D in a specific population will be a feasible approach. An example in our population is vitamin D supplement intake of ≥1,000 IU/day. When developing future models, researchers could consider including a genetic score for 25(OH)D derived from a larger selection of SNPs in a genome-wide study. It is probably not worth expending considerable effort to collect information on contributors to 25(OH)D concentration that are likely to be minor.

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Appendix Table 1. Single Nucleotide Polymorphisms Evaluated as Predictors of 25-Hydroxyvitamin D Concentration

Gene	SNP
CYP2R1	rs10741657
	rs11023374
	rs12794714
CYP27B1	rs10877012
GC/DBP	rs1155563
	rs17467825/rs2282679/rs3755967
	rs4588
DHCR7/NADSYN1	rs12785878/rs7944926
	rs3794060
	rs1790349
SLC24A5	rs1426654
CYP24A1	rs6013897
	rs2762941
PDE3B	rs7116978
OCA2	rs7495174
MC1R	rs8045560

Abbreviations: *CYP2R1*, cytochrome P-450 family 2, subfamily R, member 1; *CYP24A1*, cytochrome P-450 family 24, subfamily A, member 1; *CYP27B1*, cytochrome P-450 family 27, subfamily B, member 1; *DHCR7/NADSYN1*, 7-dehydrocholesterol reductase/nicotinamide adenine dinucleotide synthetase 1; *GC/DBP*, group-specific component/vitamin D binding protein; *MC1R*, melanocortin 1 receptor; *OCA2*, oculocutaneous albinism II melanosomal transmembrane protein; *PDE3B*, phosphodiesterase 3B; *SLC24A5*, solute carrier family 24, member 5; SNP, single nucleotide polymorphism.