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Epidemiologic study of the C-3 epimer of 25-hydroxyvitamin D_3 in a population-based sample

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Abstract

Background & aims—Vitamin D is associated with many health outcomes and the blood concentration of 25-hydroxyvitamin D [25(OH)D] is commonly measured in clinical practice. A C-3 epimer of this compound, 3-epi-25(OH)D_3, has recently been detected in blood samples. Few clinical assays currently detect this epimer and its physiological function is unknown, as are the demographic, behavioral, and physiologic factors that may be correlated with it. We sought to determine the correlation between these factors and 3-epi-25(OH)D_3.

Methods—We conducted a cross-sectional population-based study of 303 non-Hispanic white participants in the Survey of the Health of Wisconsin. Serum 25(OH)D_2, 25(OH)D_3 and 3-epi-25(OH)D_3 were measured by high-performance liquid chromatography tandem mass spectrometry. We measured vitamin D intake from foods and supplements via a food frequency questionnaire, sun exposure by spectrophotometry, waist circumference during a physical exam, and additional demographic and behavioral factors by questionnaire. We calculated the percent of 3-epi-25(OH)D_3 out of the total 25(OH)D_3.

Results—Summer P=0.009), higher alcohol intake (season (P=0.007), and higher vitamin D intake from supplements (P=0.0004), but not food (P=0.20), were significantly associated with a higher percent of 3-epi-25(OH)D_3 relative to the total 25(OH)D_3, although these associations appear to be partially driven by individuals with low 3-epi-25(OH)D_3. Moreover, the percent of 3-epi-25(OH)D_3 was significantly correlated with the total 25(OH)D_3 (r=0.37, P<0.0001).

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Statement of authorship

CDE conceived of and designed the study, performed the statistical analysis, and drafted the manuscript. RB carried out the data analysis. MZ and HS coded and analyzed the vitamin D intake data. TK coded and analyzed the sun exposure data. FJN conceived of and designed the SHOW study and contributed to the writing of the manuscript. All authors read and approved the final manuscript.

Conflict of interest statement

No author had any conflict of interest.

Conclusions—We report findings from an epidemiologic study of 3-epi-25(OH)D_3 and show that individuals with lower total 25(OH)D_3 tend to have a lower percent of 3-epi-25(OH)D_3 relative to the total. While this is the largest reported sample of adults with measured 3-epi-25(OH)D_3, the sample size of 303 is relatively small and replication of our findings is necessary.

Keywords

3-epi-25(OH)D_3; 25(OH)D_2; 25(OH)D_3; epimer; vitamin D; sun exposure

Introduction

Over the past ten to fifteen years, vitamin D has been associated with many health outcomes, including skeletal health, cancer, immune response, type 2 diabetes, metabolic syndrome, hypertension, cardiovascular disease, neuropsychological functioning, and all-cause mortality (1–7). The best indicator of vitamin D status is the blood concentration of 25-hydroxyvitamin D [25(OH)D], which is commonly measured in clinical practice.

The blood concentration of 25(OH)D includes both 25(OH)D_2 (obtained from the diet) and 25(OH)D_3 (obtained from the diet or through sun exposure). Recently, a C-3 epimer of 25(OH)D_3 [3-epi-25(OH)D_3] has been reported in infant, pediatric, and adult samples (8–10). A survey conducted in October 2011 by the International Vitamin D External Quality Assessment Scheme sought to determine which vitamin D assays were able to detect 3-epi-25(OH)D_3; only 5 out of 14 methods were able to detect it (11). The physiological function of 3-epi-25(OH)D_3 is largely unknown, making the importance of detecting it in clinical samples a topic for debate (11).

In adults, 3-epi-25(OH)D_3 makes up 0% to 25.5% of the total 25(OH)D (9, 10, 12–15). The correlation between age and 3-epi-25(OH)D_3 has been examined (no correlation was found) (9, 10), but these studies did not have the data to examine the correlation with other variables known to influence total 25(OH)D. In fact, to our knowledge, no epidemiologic study of 3-epi-25(OH)D_3 has been conducted. Therefore, we sought to determine the correlation between both 3-epi-25(OH)D_3 and the proportion of 3-epi-25(OH)D_3 relative to the total 25(OH)D_3, and vitamin D intake from foods and supplements, sun exposure (measured by spectrophotometry), waist circumference, and additional demographic and behavioral factors in 303 participants from the cross-sectional, population-based Survey of the Health of Wisconsin (SHOW).

Materials and methods

Study population

Non-institutionalized/non-active duty adult residents (ages 21–74) from the general population of Wisconsin were recruited as part of SHOW, which is modeled after the National Health and Nutrition Examination Survey. Details of the study rationale and methods have been published (16). The current study is an ancillary study to SHOW, including 303 non-Hispanic white participants who were recruited between October 2009

and June 2011 and had complete data for all primary predictor and outcome variables. This study was conducted with the approval of the University of Wisconsin Institutional Review Board and all subjects provided signed informed consent before participation.

Measurement of serum 25(OH)D_2, 25(OH)D_3, and 3-epi-25(OH)D_3

Blood for serum samples was collected via venipuncture in bar-coded red top tubes, allowed to clot at room temperature for 40–90 minutes, centrifuged, and stored at −80°C. A 0.5 mL cryovial of serum was sent to the Clinical Toxicology Laboratory at the University of Wisconsin Hospital and Clinics. Serum 25(OH)D_2, 25(OH)D_3, and 3-epi-25(OH)D_3 were measured by high-performance liquid chromatography tandem mass spectrometry as previously described (10, 17). The assay was calibrated with a serum pool that has 25(OH)D_2, 25(OH)D_3, and 3-epi-25(OH)D_3 added to horse serum. The concentrations were determined by the molar extinction coefficient of their respective methanol solutions prepared for standards purchased from Sigma-Aldrich®. The actual concentrations of the serum calibrators were confirmed by analysis of the National Institute of Standards and Technology human serum reference materials that contain specified amounts of 25(OH)D_2, 25(OH)D_3, and 3-epi-25(OH)D_3 established by both National Institute of Standards and Technology and the Centers for Disease Control and Prevention. The inter-assay coefficient of variation for the assays was less than 7%. The percent of 3-epi-25(OH)D_3 out of the total 25(OH)D_3 was calculated [% 3-epi-25(OH)D_3].

Measurement of sun exposure via skin color

Skin color was measured at four sites using a DataColor CHECK™ portable reflectance spectrophotometer (Lawrenceville, NJ). Measurements of the upper inner arm (3 inches up from the medial elbow joint) and the inner forearm (3 inches up from the wrist) represented inherited (constitutive) skin color at sites not typically exposed to sun, whereas measurements of the upper outer arm (3 inches up from the lateral elbow joint) and the outer forearm (3 inches up from the wrist) represented inherited plus sun-induced (facultative) skin color. Measurements at each location were taken in triplicate and automatically averaged by the instrument. The data were reported using the Commission Internationale de l'Eclairage Lab system in which colors are described by their lightness value (L*; ranging from black to white), the amount of green or red (a*), and the amount of yellow or blue (b*). Skin pigmentation can be characterized by the Individual Typology Angle (ITA°) calculated as: ITA° = Arc Tangent $[(L^* - 50)/b^*] \times 180 \times \pi^{-1}$ (18). The inherited ITA° values were then categorized into the following skin color types: very light (ITA° >55), light (ITA° >41 – 55), intermediate (ITA° >28 – 41), tanned (ITA° >10 – 28), brown (ITA° >−30 – 10), and dark (ITA° ≤−30) (19). The independent effect of sun exposure was investigated by including both inherited and sun-induced ITA° values in the regression model (20).

Vitamin D intake

Dietary and supplemental vitamin D intake in the three weeks prior to the study visit were self reported using a food frequency questionnaire (FFQ) modeled after the Block FFQ (21). This FFQ was designed specifically to capture foods containing vitamin D based on the USDA National Nutrient Database for Standard Reference, a review of the literature, and food labels at local grocery stores and on company websites. These foods included milk,

eggs, cold cereal, shiitake mushrooms, fatty fish, and certain brands of orange juice, yogurt, margarine, and meal replacement drinks and bars. Intake from multivitamins, vitamin D supplements, and cod liver oil was also recorded. To assist in accurate completion of the FFQ, the participants were sent a letter prior to the study visit asking them to record brands of orange juice, yogurt, margarine, and meal replacement drinks and bars they consumed in the three weeks prior to the study visit and the amount of vitamin D in any supplements they were taking. The three-week window was selected for the FFQ and letter because the half-life of 25(OH)D is two to three weeks. Vitamin D values per serving were generally assigned based on the USDA National Nutrient Database for Standard Reference (http://www.ars.usda.gov/ba/bhnrc/ndl) (22). For the four fortified products included in the letter capturing brands, where there was wide variation in the amount of vitamin D by brand that was not distinguished by the USDA database, the vitamin D values on the nutrition label of each brand were used. Average daily vitamin D intake for the past three weeks was calculated by summing vitamin D intake from foods and supplements (IU/d).

Body size measures

The methods used to measure body composition have been described previously (16). Briefly, height (cm) was measured with a stadiometer, weight (kg) was measured with a calibrated digital scale, and BMI (weight/height^2 in kg/m^2) was calculated from these measures. Waist circumference (cm) was measured twice at the uppermost lateral border of the ilium and the average was taken.

Other potential correlates and confounders

Season of blood was categorized as summer/fall (June to November) or winter/spring (December to May) and served as a proxy measure of the available solar radiation in the environment. Self-reported gender, age, education, general health, average number of cigarettes smoked per day, and average number of alcoholic drinks per day, all in the three weeks prior to the study visit, were assessed by an in-person interview. Cigarettes smoked and alcoholic drinks per day were positively skewed so categories were formed to reduce the influence of extreme outliers.

Statistical analysis

All data were analyzed using SAS version 9.2 (SAS Institute, Cary, NC). Season, gender, age, inherited skin color, sun-induced skin color, vitamin D intake from food and from supplements, BMI or waist circumference (two separate models due to collinearity, one with each of these and all other variables), education, general health, cigarettes smoked per day, and alcoholic drinks per day were included as independent variables in the initial multiple regression model, using PROC REG. The dependent variables were: 25(OH)D_2, 25(OH)D_3, 3-epi-25(OH)D_3, and % 3-epi-25(OH)D_3. We took a backward elimination approach starting with the initial (full) model, removing the least significant variable, and then rerunning the analysis. We eliminated, one at a time, all variables that were not significantly associated with any of the four vitamin D metabolites and did not change the coefficients of any other covariate by more than 10%. Collinearity diagnostics were performed on the final models to ensure that correlation between independent variables would not lead to unstable parameter estimates with high standard errors. To determine if individuals with low 3-

epi-25(OH)D_3 were driving the associations with the epimer, we tested the final model with and without individuals in the lowest 10th percentile of 3-epi-25(OH)D_3.

Results

Characteristics of the SHOW participants are shown in Table 1. Average vitamin D intake from supplements ranged from 0 (in 145 participants) to 25,000 IU/d (the second highest intake was 7,200 IU/d). To minimize the influence of the one extreme outlier, the vitamin D supplement value of 25,000 IU/d was winsorized (transformed) to the next highest value of 7,200 and used in all subsequent analyses. The concentration of 25(OH)D_2 was <5 ng/mL for all but two participants where the values were 10 and 21 ng/mL. The non-epimeric 25(OH)D_3 ranged from 4.6 to 84.7 ng/mL and the 3-epi-25(OH)D_3 ranged from 0 (in 26 samples) to 4.9 ng/mL (the lowest detectable value was 0.18 ng/mL). The correlation between 25(OH)D_3 and 3-epi-25(OH)D_3 was 0.70 (P <0.0001; r = 0.66). The % 3-epi-25(OH)D_3 ranged from 0 (in 26 samples) to 13.3 and did not vary by 5-y age groups (data not shown). There was a significant correlation between % 3-epi-25(OH)D_3 and total 25(OH)D_3 (r = 0.37, P <0.0001). The mean (±S D) % 3-epi(25)D $_3$ by quartile of total 25(OH)D_3 is displayed in Table 2.

In the final multivariable model, summer season, female gender, more sun exposure (sun-induced skin color adjusted for inherited skin color), higher vitamin D intake from supplements, and lower waist circumference were significantly associated with higher 25(OH)D_3 (Table 3). The fully adjusted model explained 37% of the variation in 25(OH)D_3. Summer season, more sun exposure, higher vitamin D intake from supplements, lower waist circumference, and higher alcohol intake were significantly associated with higher 3-epi-25(OH)D_3, with the fully adjusted model explaining 26% of the variation. When individuals in the lowest 10th percentile of 3-epi-25(OH)D_3 were excluded from the analysis, sun exposure was only marginally significant (P = 0.10), but the other variables remained significant and the parameter estimates of the effect of all four variables remained the same (data not shown). Only summer season, higher vitamin D intake from supplements, and higher alcohol intake were significantly associated with a higher percent of 3-epi-25(OH)D_3 relative to the total 25(OH)D_3. The fully adjusted model explained 13% of the variation in % 3-epi-25(OH)D_3. When individuals in the lowest 10th percentile of 3-epi-25(OH)D_3 were excluded from the analysis, the parameter estimates of the effect of season and vitamin D intake from supplements were reduced by nearly 50% with larger P-values (0.08 and 0.03, respectively), but the effect of alcohol intake remained the same and was significant (P= 0.001; data not shown). Interestingly, none of the variables included in the initial model were significantly associated with 25(OH)D_2 and the percent of variation that was accounted for by all of the variables listed in Table 3 was only 0.07%. Even though total 25(OH)D_3 was correlated with % 3-epi-25(OH)D_3 (see previous paragraph), it was not included in the model due to multicollinearity with the predictor variables. Although age and vitamin D intake from food were not significantly associated with any of the four outcome variables, they were retained in the model because they were primary predictors of interest. Waist circumference was more strongly associated with all three outcomes than BMI (data not shown).

Discussion

In this study of 303 non-Hispanic white participants from SHOW, we demonstrate empirically that season, sun exposure (measured by spectrophotometry), vitamin D intake from supplements, alcohol intake, and waist circumference influence concentrations of 3-epi-25(OH)D_3. Moreover, season, vitamin D intake from supplements, and alcohol intake are also associated with the percent of 3-epi-25(OH)D_3 relative to the total 25(OH)D_3, although the associations with season and vitamin D intake from supplements appear to be partially driven by individuals with low 3-epi-25(OH)D_3. Similar to Lensmeyer et al. (2012), we did not observe an association between age and either the concentration or percent of 3-epi-25(OH)D_3. We did observe a significant correlation between % 3-epi-25(OH)D_3 and total 25(OH)D_3, where the proportion of the epimer out of the total 25(OH)D_3 increased with increasing total 25(OH)D_3 (Table 2). This is the first epidemiologic study to examine the association between 3-epi-25(OH)D_3 and vitamin D intake, sun exposure, waist circumference, and additional demographic and behavioral factors, a gap in the literature noted by Strathmann et al. (9) and Lensmeyer et al. (10).

It has been demonstrated that 3-epi-25(OH)D_3 is metabolized to 3-epi-1α,25-dihydroxyvitamin D_3 [3-epi-1α,25$(OH)_2D_3$], the biologically active vitamin D metabolite, by a cytochrome P450 protein, CYP27B1 (23). A nice systematic review of existing literature on the physiological function of 3-epi-1α,25$(OH)_2D_3$ was recently published (11). Bailey et al. concluded that 3-epi-1α,25$(OH)_2D_3$ provides some, but not all of the physiological properties of the non-epimeric form. More research is needed to definitively determine the function of this molecule in order to decide whether it is necessary for clinical laboratories to separate and quantify the epimer.

Under the assumption that the epimer is an inactive or less active metabolite of 25(OH)D_3, Strathmann et al. estimated the frequency that a deficient [25(OH)D <20 ng/mL] patient could be misclassified as sufficient, due to the inclusion of the epimer by one of the five types of clinical assays that currently detects the epimer, to be 3% in children and adults (1–94 y) (9). In our sample, only 1% of adults (21–74 y) would have been classified as sufficient if the epimer was included in the 25(OH)D concentration, but deficient if it was excluded. This low percent of misclassification may be because the percent of the epimer is low (mean of 2.7%; Table 2) in individuals in the lowest quartile of 25(OH)D_3 who are more at risk for vitamin D deficiency. This low percent of the epimer means that very few individuals will cross the cut-off for deficient status when the concentration of the epimer is excluded (or not detected by an assay). The percent of the epimer increases approximately linearly to a mean of 5.1% in individuals in the highest quartile of 25(OH)D_3 for whom misclassification after excluding the epimer concentration is not likely due to their high 25(OH)D_3 concentration.

The existing literature on the epimer has focused on the D_3 metabolite, not the D_2 form. Moreover, existing epidemiologic studies combine the concentrations of D_2 and D_3, examining total 25(OH)D. Although the D_2 metabolite was not a focus of our current research, we did examine it in the multivariable models performed for the D_3 metabolites. Interestingly, none of the variables we included in the initial model were significantly

associated with the concentration of 25(OH)D_2 and the percent of variation that was accounted for by all of the variables listed in Table 3 was extremely small (0.07%). Although the mean concentration of 25(OH)D_2 in our population was quite low (0.7 ng/mL), it was still surprising that vitamin D intake from foods and supplements, the sources of the D_2 metabolite, were not associated with 25(OH)D_2 concentration.

This study only included non-Hispanic white individuals. Future research should determine if the findings from this study are generalizable to other racial and ethnic groups. Moreover, while this is the largest reported sample of adults with measured 3-epi-25(OH)D_3, the sample size of 303 was still relatively small. As data become available from larger epidemiologic studies that have begun to measure 3-epi-25(OH)D_3, such as the National Health and Nutrition Examination Survey, replication of these associations will be possible. A recent report by Shah and colleagues presented an LC-MS/MS method to separate and quantitate not only 3-epi-25(OH)D_3, but also two isobars, 1α-hydroxyvitamin D_3 [1α(OH)D_3] and 7α-hydroxy-4-cholesten-3-one (24). Our laboratory method can separate and quantitate both 3-epi-25(OH)D_3 and 1α(OH)D_3, however, while we believe it could also separate and quantitate 7α-hydroxy-4-cholesten-3-one, this has not been examined so interference by this isobar cannot be ruled out. Finally, while we were able to explain 37% of the variation in 25(OH)D_3, we were only able to explain 26% of the variation in 3-epi-25(OH)D_3 and 13% of the variation in % 3-epi-25(OH)D_3. Clearly additional factors influence the concentration and percent of 3-epi-25(OH)D_3.

In conclusion, we have shown that summer season, more sun exposure, higher vitamin D intake from supplements, higher alcohol intake, and lower waist circumference are associated with higher 3-epi-25(OH)D_3 and that summer season, higher vitamin D intake from supplements, and higher alcohol intake are also associated with a higher percent of 3-epi-25(OH)D_3 relative to the total 25(OH)D_3, although the associations with season and vitamin D intake from supplements appear to be partially driven by individuals with low 3-epi-25(OH)D_3. Further research is necessary to establish the source and physiologic function of 3-epi-25(OH)D_3. It is unknown whether inclusion of 3-epi-25(OH)D_3 in the total 25(OH)D concentration is appropriate for research studies or clinical practice, however the percent of misclassification from doing so is likely to be low due to the low fraction of the epimer in individuals with low total 25(OH)D_3.

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Abbreviations

% 3-epi-25(OH)D_3	percent of C-3 epimer of 25(OH)D_3 out of the total 25-hydroxyvitamin D_3
25(OH)D	25-hydroxyvitamin D
25(OH)D_2	25-hydroxyvitamin D_2
25(OH)D_3	25-hydroxyvitamin D_3
3-epi-25(OH)D_3	C-3 epimer of 25(OH)D_3
FFQ	food frequency questionnaire
ITA°	Individual Typology Angle
SHOW	the Survey of the Health of Wisconsin

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Table 1

Characteristics of the SHOW participants, $n = 303$[a].

Characteristic	Percent or mean (SD)	Range
Season		
Summer (June-November)	60.1%	
Winter (December-May)	39.9%	
Gender		
Male	48.5%	
Female	51.5%	
Age, y	48.1 (14.0)	21 – 74
Inherited skin color, ITA°	50.3 (9.8)	12.0 – 68.9
Inherited skin type		
Very light	37.0%	
Light	45.9%	
Intermediate	14.2%	
Tanned	3.0%	
Sun-induced skin color, ITA°	22.7 (16.6)	−69.7 – 57.4
Percent increase in skin color due to sun exposure[b]	56.7 (32.1)	8.5 – 224.5
Vitamin D intake from food, IU/d	195.1 (145.7)	0 – 1037
Vitamin D intake from supplements, IU/d	541.1 (1000.9)	0 – 7200
Total vitamin D intake, IU/d	734.0 (1000.9)	0 – 7225
Body mass index, kg/m^2	29.2 (6.2)	17.1 – 55.4
Waist circumference, cm	98.3 (15.8)	67.5 – 142.8
Education completed		
Less than high school	4.0%	
High school or GED	20.2%	
Some post-secondary	39.4%	
Bachelor’s degree	24.2%	
Master’s, professional or doctoral degree	12.3%	
General health		
Excellent	9.7%	
Very good	48.7%	
Good	33.7%	
Fair	6.3%	
Poor	1.7%	
Average # cigarettes smoked per day		
None	83.4%	
1–10	5.7%	
≥10	11.0%	
Average # alcoholic drinks per day		
None	34.9%	
1	43.2%	

Characteristic	Percent or mean (SD)	Range
2	12.3%	
≥3	9.6%	
25(OH)D_2, ng/mL	0.7 (1.4)	0 – 21.0
25(OH)D_3, ng/mL	30.3 (10.6)	4.6 – 84.7
3-epi-25(OH)D_3, ng/mL	1.3 (0.9)	0 – 4.9
% 3-epi-25(OH)D_3	3.9 (2.2)	0 – 13.3

[a] % 3-epi-25(OH)D3, percent of C-3 epimer of 25(OH)D3 out of the total 25-hydroxyvitamin D3; 25(OH)D3, 25-hydroxyvitamin D3; 3-epi-25(OH)D3, C-3 epimer of 25(OH)D3; ITA°, Individual Typology Angle; SHOW, the Survey of the Health of Wisconsin.

[b] Percent increase in skin color due to sun exposure relative to inherited skin color was calculated as 100 × (inherited skin color ITA° - sun-induced skin color ITA°)/inherited skin color ITA°.

Table 2

Mean (±SD) 3-epi-25(OH)D_3 and % 3-epi-25(OH)D_3 by quartile of total 25(OH)D_3, n = 303[a].

	Lowest quartile total 25(OH)D_3: ≤24.4 ng/mL (n = 75)	**2nd quartile total 25(OH)D_3: >24.4 – 31.4 ng/mL (n = 77)**	**3rd quartile total 25(OH)D_3: >31.4 – 37.7 ng/mL (n = 76)**	**Highest quartile total 25(OH)D_3: >37.7 ng/mL (n = 75)**
3-epi-25(OH)D_3, ng/mL	0.5 (0.4)	1.0 (0.5)	1.4 (0.8)	2.4 (1.0)
% 3-epi-25(OH)D_3	2.7 (2.1)	3.5 (1.7)	4.2 (2.3)	5.1 (1.9)

[a]% 3-epi-25(OH)D3, percent of C-3 epimer of 25(OH)D3 out of the total 25-hydroxyvitamin D3; 25(OH)D3, 25-hydroxyvitamin D3; 3-epi-25(OH)D3, C-3 epimer of 25(OH)D3.

Table 3

Multivariable model for concentration of 25(OH)D_3, 3-epi-25(OH)D_3, and % 3-epi-25(OH)D_3, $n = 301$[a].

	25(OH)D_3, ng/mL		3-epi-25(OH)D_3, ng/mL		% 3-epi-25(OH)D_3	
Characteristic	**â (95% CI)**	***P*-value**	**â (95% CI)**	***P*-value**	**â (95% CI)**	***P*-value**
Winter season (versus summer)	−4.4 (−6.4, −2.3)	<0.0001	−0.4 (−0.6, −0.2)	0.0003	−0.7 (−1.2, −0.2)	0.009
Female (versus male)	3.0 (0.7, 5.3)	0.01	0.03 (−0.2, 0.3)	0.80	−0.2 (−0.8, 0.3)	0.43
Age, 10 y increase	0.4 (−0.4, 1.1)	0.28	−0.03 (−0.1, 0.5)	0.48	−0.1 (−0.3, 0.1)	0.20
Sun-induced skin color ITA°, 10° increase[b]	−0.9 (−1.7, −0.2)	0.02	−0.1 (−0.2, −0.01)	0.04	−0.1 (−0.3, 0.04)	0.13
Vitamin D intake from foods, 1000 IU/d increase	4.8 (−1.8, 11.4)	0.15	0.3 (−0.8, 0.5)	0.63	−1.0 (−2.6, 0.5)	0.20
Vitamin D intake from supplements, 1000 IU/d increase	4.6 (3.6, 5.6)	<0.0001	0.3 (0.2, 0.4)	<0.0001	0.4 (0.2, 0.7)	0.0004
Waist circumference, 10 cm increase	−1.4 (−2.1, −0.7)	<0.0001	−0.1 (−0.2, −0.02)	0.01	−0.1 (−0.2, 0.1)	0.41
Average # alcoholic drinks per day[c]	0.9 (−0.2, 2.0)	0.12	0.2 (0.05, 0.3)	0.004	0.4 (0.1, 0.6)	0.007
Fully adjusted model R^2	0.37		0.26		0.13	

[a] % 3-epi-25(OH)D3, percent of C-3 epimer of 25(OH)D3 out of the total 25-hydroxyvitamin D3; 25(OH)D3, 25-hydroxyvitamin D3; 3-epi-25(OH)D3, C-3 epimer of 25(OH)D3; ITA°, Individual Typology Angle.

[b] Adjusted for inherited skin color ITA° to capture sun exposure; a negative correlation means that lower ITA° (i.e., darker skin color due to sun exposure) results in a higher value of the outcome.

[c] Parameter estimate is for each increase in the average number of alcoholic drinks per day up to 3 drinks relative to none; individuals consuming, on average, more than 3 drinks per day were included in the 3 drinks category.