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The Contributions of Adjusted Ambient UVB at the Place of Residence and Other Determinants to Serum 25-Hydroxyvitamin D Concentrations

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Running Title: UVB radiation and 25-hydroxyvitamin D concentration in plasma

What is already known about this topic?

- Solar UVB radiation is the major source of vitamin D for humans and it is strongly associated with vitamin D status.

- UVB radiation at wavelengths that can induce vitamin D synthesis can be approximated by total UV or UVB radiation, sunshine hours or latitude; typically averaged over a large geographical region and long time period, yielding unreliable approximations.

What does this study add?

- Freely available data on measured ambient UVB exposure at wavelengths required for vitamin D synthesis (vitD-UVB) and adjusted for cloud cover and ozone layer is a powerful yet entirely underutilized epidemiological tool to study the relationships between UVB, vitamin D and health outcomes.

- There was a significant geographical variation in vitD-UVB, even within a small geographic area at a northern latitude.

- Measured ambient vitD-UVB dose at place of residence is a good predictor of vitamin D status in healthy adult population.

Abstract

Background. Solar ultraviolet radiation is the major source of vitamin D for humans.

Objectives. We use high-resolution geo-temporal measurements to describe ambient UVB radiation at wavelengths that induce vitamin D synthesis (vitD-UVB) in Scotland, and examine the relationship of this exposure to 25-hydroxyvitamin D (25OHD) measured in plasma of 1,964 healthy adults.

Methods. We estimated the average vitD-UVB dose for each day of the year, and for each postcode area in Scotland using the Tropospheric Emission Monitoring Internet Service database (TEMIS, 2005-2010). Cumulative and weighted vitD-UVB (CW-vitD-UVB) exposure at the place of residence, prior to the date of blood sampling was calculated for each participant. Plasma 25OHD was assayed in 1,964 healthy participants.

Results. Statistically significant seasonal (highest in June) and geographic (highest in south) variation in vitD-UVB was observed, even within a small geographic area in a northern latitude. Ambient vitD-UVB exposure at the place of residence was significantly associated with plasma 25OHD ($p<10E-10$). An average increase of 1 ng/mL in 25OHD was observed for every 1000 kJ/m^2 higher CW-vitD-UVB dose or for every 2.5 µg of daily supplement taken. Adequate 25OHD concentration (>16 ng/mL) was observed in majority of individuals when CW-vitD-UVB dose was above 6000 kJ/m^2, a level of ambient radiation achieved only in summer months in Scotland. When predicting vitamin D deficiency, dramatic improvement in AUC was observed after CW-vitD-UVB dose was added to the model in addition to a range of other covariates (from 0.55 to 0.70).

Conclusions. We conclude that ambient vitD-UVB is a measurable exposure that can be useful predictor of vitamin D status. Geo-temporally mapped measurements of vitD-UVB can be used as a proxy for vitamin D status or as an important covariate in epidemiological research, particularly if 25OHD is not available.

Introduction

Seasonality of vitamin D status follows seasonality of UVB radiation and attests to the key role of UVB as a source of vitamin D [1]. Because skin synthesis is directly related to the UVB exposure, there is a strong *a priori* expectation that ambient UVB dose predicts vitamin D status. However, there is a paucity of studies that exploit UVB measurement; this could be in part due to difficulties in obtaining accurate *individual* estimates, as ambient UVB dose does not directly map to latitude, sunshine, season or weather. Previous attempts have estimated UVB dose using crude proxies such as sunshine hours or latitude [2–4], typically averaged over a large geographical region and longer time period, yielding unreliable approximations. Apart from few rare exceptions [5], more accurate estimates of UVB relevant for vitamin D production have not been examined or utilised in epidemiological studies to date.

The amount of vitamin D synthetized in skin depends on two principal determinants: (i) available UVB at wavelengths relevant for vitamin D production and (ii) personal factors (**Figure 1**) [6]. While the ambient dose is easily measureable, personal characteristics and behaviours are not, because they are highly variable and difficult to capture (e.g. clothing, time spent outside, or tan) [7]. Therefore, an accurate estimate of ambient UVB dose at wavelengths relevant for vitamin D production is a critical *measurable* predictor of vitamin D status [2].

Once vitamin D is synthesised (or ingested), it undergoes hydroxylation in the liver to form 25-hydroxyvitamin D (25OHD). The concentration of 25OHD in the circulation is currently considered to be the best biomarker of vitamin D status [8]. However, it is not always feasible to measure circulating

25OHD due to assay costs and difficulty (practical or ethical) of obtaining blood samples, particularly if assessment at multiple time-points is desired [9].

In this paper, we set out to describe ambient exposure to UVB radiation at wavelengths that can induce vitamin D synthesis (vitD-UVB) in Scotland, and to examine the relationship between ambient vitD-UVB at the place of residence and vitamin D status. We also discuss the relevance and application of this novel approach for future studies.

Methods

Study Population

The study population consists of 1964 individuals (43% female) recruited as control subjects between February 2003 and March 2004 for a case-control study aimed at investigating factors associated with colorectal cancer (SOCCS) in Scotland. The study is described in detail elsewhere [10,11]. All participants were white Caucasians of Scottish ancestry (genetic markers were used to verify this). Participants completed a detailed socio-demographic and lifestyle questionnaire, a semi-quantitative food frequency and a supplements questionnaire. We administered the Scottish Collaborative Group Food Frequency Questionnaire (SCG-FFQ, Version 6.41), which has been validated in Scotland. Nutrient content was estimated for each food item using national nutritional database. Nutrients intakes were calculated from the consumption frequencies of specified portion size for each food item from the FFQ and were standardized for total energy intake.

Residential postcode was mapped within 30' of geographical latitude and longitude to UVB radiation database grid (55 km south-to-north and 33 km east-to-west at the latitudes of Scotland). The final sample size (n=1964) followed exclusion of participants (n=284) with missing data on any of: residential location; level of physical activity; BMI; plasma 25OHD level; or extreme outliers of plasma vitamin D (>100 ng/ml). Approval for the study was obtained from the Multicentre Research Ethics Committee for Scotland and Local Research Ethics committees and all participants gave written informed consent.

Plasma vitamin D measurement and deficiency categories

Blood samples were taken throughout the year (**Supplementary Table 1**). All plasma samples were batched and assayed at the same laboratory. Total 25-OHD (25-OHD$_2$ and 25-OHD$_3$) was measured by liquid chromatography-tandem mass spectrometry which is considered the gold-standard method [12]. The lower limit of detection for this method is 4 ng/mL; values below the limit were replaced with a value randomly sampled from the 0-4 ng/mL range from a tail of normal distribution, to improve normality. To characterise the degree of deficiency, categorical 25OHD cut-off points were set to 10, 16 and 20 ng/mL in accordance with Ross et al. (2011)[13], although risk categories cut-offs are still under debate [14,15].

Genetic Score

We have extracted the genotypes for three SNPs (rs12785878, rs10741657 and rs2282679) that have been associated with 25OHD concentration in genome-wide association study[16]. Genetic score has been calculated as the number of risk alleles for rs12785878 and rs2282679, variants that have been associated with circulating 25OHD in this population. Genotyping protocol has been described in detail elsewhere[17]. Approximately one third of the cohort had no risk alleles (n=527), 63% (n=1021) had 1 or 2 risk alleles, while 4% had 3 or 4 risk alleles (n=65).

UVB Data Resource

We extracted ambient UVB dose relevant for vitamin D production at residential locations for every participant using the Tropospheric Emission Monitoring Internet Service (TEMIS) database (www.temis.nl/uvradiation). Daily estimates are based on satellite UV measurements from sunrise to sunset, with a time step of 10 minutes. The readings are adjusted for the terrain elevation, the total ozone column and cloud cover (**Figure 1**) [18].

The exposure dose is the energy associated with the radiation divided by the surface area of the receptor, expressed in kilo-Joules per square meter (kJ/m^2). A biological function is applied to the intensity of UV radiation, to isolate the narrow band of wavelengths relevant for vitamin D production. Synthesis occurs within the UVB wavelengths, but while action spectrum is between 260 and 315 nm, the peak conversion occurs at a very narrow range: between 295 and 297 nm [19]. The final estimate is the daily dose of UV radiation at the earth's surface that can induce vitamin D synthesis if absorbed by the human skin (**vitD-UVB**).

Because observed vitD-UVB measurement was not available prior to 2005 when this cohort was being recruited, average daily vitD-UVB dose was calculated for each day of the year (DD/MM) using data recorded between 2005 and 2010 (DD/MM/YYYY).

Seasonal and Geographic Analysis of VitD-UVB in Scotland

Total monthly vitD-UVB exposure averaged over five (or six) years (July 2005 to October 2010) was calculated and is reported for eight exemplar areas selected to represent diverse geographical locations while accounting for population density: Glasgow, Edinburgh, Oban, Aberdeen, Inverness, Stornoway, Kirkwall and Lerwick. Multivariate linear regression was carried out to examine the association between total monthly vitD-UVB dose and month, location and year.

Mean monthly vitD-UVB dose was calculated for 79 grid cells that cover the entire land area of Scotland. The map of Scotland was created using Postal Boundaries Open 2012, a spatial dataset detailing the extent of the 9,232 geographic postal sectors (e.g. HP21 8) covering Great Britain (last modified: October 2012). Postal sectors are grouped together to form 2,736 Postal Districts (e.g. HP21) which in turn group together to form 120 Postal Areas (e.g. HP). The data is released under the same terms as the OS OpenData license [20].

Individual cumulative and weighted vitD-UVB (CW-vitD-UVB) exposure dose

Mean daily vitD-UVB dose for each day of the year (DD/MM) for each region was calculated using data from 2005 to 2010 (DD/MM/YYYY) for that region. A cumulative and weighted vitD-UVB dose prior to date of blood sampling was calculated for each individual based on the average estimated dose. "Cumulative" means that daily vitD-UVB doses from a number of preceding days are added up to contribute to the final exposure estimate, but using a method where daily contributions to the cumulative exposure estimate are weighted so that vitD-UVB exposures immediately prior to blood sampling contribute more than exposures from a more distant past (**Supplementary Figure 1**). This weighting is akin to the "half-life" of vitD-UVB effect and reflects the half-life of vitamin D in the body (whole-body vitamin D: 2 months; 25OHD in the circulation: 15 days [21]). Our analysis suggests a 35-day half-life of vitD-UVB effect and 135-day period are optimal parameters (see **Supplementary Methods**). The model presumes that the amount of vitD-UVB *currently* contributing to plasma concentration is negligible 135 days prior to sampling. The estimates are unique for every individual, because they are determined by the place of residence and date of blood sampling. For example, if blood sample was taken on 27/01, data on daily vitD-UVB doses between 14/09 and 26/01 will be extracted, weighted and added up. This is illustrated in **Supplementary Figure 1** and **Equation 1,** where x = days ago (starting day before and up to 135 days prior to sampling), y = rate of disappearance of effect of UVB in days (half-life set at 35 days), and e^(-ln2*(x/y) is the weighing formula applied.

$$Cumulative\ and\ Weighted\ \textbf{UVB}\ (x) = \sum_{x=1:135} \left(\textbf{vitDUVB}(x)\right) * e^{-\left(\frac{ln2}{y}\right)x}$$

(Equation 1)

Statistical Analysis

We tested whether cumulative and weighted vitD-UVB (CW-vitD-UVB) dose was associated with 25OHD, in regression analysis after adjustment for a range of factors that have been shown to affect 25OHD concentration (age, sex, body mass index, number of risk alleles, socio-economic status, level

of physical activity, dietary vitamin D and vitamin D supplements use). The proportion of variance explained by each predictor in this population was estimated, to assess relative contributions to 25OHD levels in this population. A drawback with population variance measures is that the major determinants of population variation may not be the main *causes* of the condition; therefore it is not possible to rank the absolute importance of factors according to their contribution to population variance.

The ability of the 25OHD-UVB proxy to predict plasma vitamin D status in three categories (<10ng/ml, <16 ng/ml and <20ng/ml) was tested using random forests prediction (RF). While logistic regression models predict the category of an observation based on linear combinations of the predictor variables, RF uses classification trees. A classification tree is fitted for each predictor variable, with each variable given a cut-off value corresponding to being vitamin D deficient or not. RF prediction fits many classification trees to randomly selected subsets of the data, and then combines the predictions from all the trees. The success of the prediction for each observation is calculated by combining the results of all the classification trees. The main advantage of RF compared to logistic regression is it has very high classification accuracy. Repeated classification trees are made from separate bootstrap samples of the training data using the Classification and Regression Tree (CART) algorithm. Each tree provides a prediction rate for the number of individuals correctly classified below or above the cut-off point. Receiver operator curves (ROC curves) were constructed to measure the performance of the test and the area under the curve (AUC) was reported. The AUC is the percentage of randomly drawn pairs correctly classified. The training dataset, on which the predictions were made, consisted of 982 observations, while the validation set, which was used for the ROC curves, had remaining 982 measurements. The analysis was carried out in the randomForest package in R [22].

Results

Ambient VITD-UVB Exposure: regional and seasonal differences

Substantial regional differences in UVB radiation dose at wavelengths that can induce vitamin D synthesis (VitD-UVB) were observed across Scotland and throughout the year. As expected, vitD-UVB dose was inversely related to latitude (**Figure 2** and **Supplementary Table 2**). Location within Scotland was significantly associated with VitD-UVB radiation, despite the restricted geographic area (54.8-62.3° North and 0.25-8.25° West): for example, Edinburgh (55.9° N) received on average 560 kJ/m^2 vitD-UVB more per month than Lerwick (60.2° N) (**Supplementary Table 3)**.

VitD-UVB dose had a marked seasonal pattern and comparisons by month of the year revealed very large differences (**Figure 2** and **Supplementary Table 2**). Both the highest daily and highest cumulative monthly VitD-UVB dose were consistently observed in the month of June and the lowest in December (2005-2010): monthly vitD-UVB dose in June was approximately 100 times higher than in December. In the multivariate regression model, month remained very strongly associated with vitD-UVB dose (p<0.001); for example, vitD-UVB in June was on average 5,621 kJ/m^2 higher than in January.

Association between VITD-UVB exposure and 25OHD in the SOCCS cohort

In total 1964 healthy participants (843 female) from Scotland aged between 22 and 82 were included in this study. The mean 25OHD was 14.14 ng/mL (SD=9.01). Peak concentrations and highest proportion of sufficient samples were observed in August (**Supplementary Figure 2A-B**). In total 510 (45% female) participants reported taking supplements, median dose was 5 μg (SD=3.58). For other characteristics of this population see Zgaga et al. [15].

Mean cumulative and weighted vitD-UVB in this cohort was 3894 kJ/m2 (SD=2745; median 3923 kJ/m^2, IQR: 1217-6510). While ambient daily vitD-UVB radiation dose in Scotland is the highest in June, CW-vitD-UVB estimate peaks approximately 1-2 months later **(Supplementary Figure 2C-D).**

We observed a very strong association between plasma 25OHD and the cumulative and weighted vitD-UVB exposure estimate, in both the unadjusted and adjusted analysis (p<2x10^{-16}, **Table 1 and Figure 3)**. CW-vitD-UVB was associated with an average 25OHD concentration increase of 1 ng/mL for every 1000 kJ/m^2. After adjustment for age, sex, BMI, socioeconomic class, level of physical activity, dietary vitamin D and supplements, the proportion of variance explained (PVE) by the CW-vitD-UVB exposure estimate was 10.94% in the whole cohort, 13.22% among those who do not take vitamin D supplements (n=1,455) and 6.09% among those who do take supplements (n=510). Differences in 25OHD concentration according to the level of CW-vitD-UVB exposure were more pronounced in supplement non-takers (**Supplementary Figure 3A-B**). Adequate vitamin D status (>16 ng/mL, "sufficient or at low risk of deficiency") was mostly achieved when CW-vitD-UVB was above 6000 kJ/m^2 (a dose is typically achieved only in summer months in Scotland) in individuals below the age of 50 (**Supplementary Figure 3C-D**).

Not surprisingly, after stratification by the season of blood sample, in winter months we did not observe any association between UVB estimate and 25OHD. In contrast, the association between supplement use and plasma 25OHD was the strongest in winter and spring, when skin synthesis is low (p=2.35x10^{-6} and p=6.49x10^{-6}) and explained 5.63% and 4.0% of the variance in 25OHD (only 0.59% in the summer). Vitamin D supplementation was associated with an average of 0.4 ng/mL greater plasma level for every 1 μg of daily supplement. For every additional risk allele 25OHD concentration was on average 1 ng/mL lower (p=0.0004). Notably, vitamin D from dietary sources excluding supplements, explained only a small proportion of the variance in plasma 25OHD (0.4%).

Stratification by age confirmed particularly strong relationship between 25OHD and CW-vitD-UVB in those under 50: average 25OHD levels were over 15 ng/mL higher in those with highest levels of CW-vitD-UVB exposure compared to those who had CW-vitD-UVB below 1000 kJ/m^2. UVB exposure estimate explained 19% of variance, while supplement use was not associated with 25OHD concentration in this group.

Predictive ability of cumulative weighted vitD-UVB estimate for vitamin D deficiency

Cumulative weighted vitD-UVB exposure estimate was found to make a large contribution to predicting vitamin D deficiency (**Figure 4**). The final model (Model 6) included cumulative weighted vitD-UVB exposure estimate and: age, sex, BMI, number of risk alleles, socioeconomic class, level of physical activity, dietary vitamin D and supplements use. When evaluated in the validation dataset, improved AUC was observed for models that included cumulative weighted vitD-UVB exposure estimate, particularly for predicting severe deficiency: for cut-off at 10 ng/mL AUC was 0.70 (improved from 0.55), for deficiency cut-off at 16 ng/mL AUC was 0.69 (improved from 0.60), and for 20ng/ml AUC was 0.70 (improved from 0.64). The variables with the largest determined importance in the final model are vitD-UVB, supplement use and age (**Supplementary Figure 4)**.

Discussion

We set out to examine the relationship between the dose of UVB relevant for vitamin D production at the place of residence and vitamin D status, and determine if this exposure can be used to predict vitamin D status. The UVB radiation relevant for vitamin D production is determined daily for Europe and is readily available through the Tropospheric Emission Monitoring Internet Service (TEMIS). In addition to much greater geo-temporal resolution, these data offer a further improvement over UVB estimates used previously: *measured* daily dose of solar radiation is considered rather than an approximation of it; wavelengths relevant for vitamin D production *only* are extracted, and adjustments are made for terrain elevation, local ozone column and cloudiness [18]. Earlier estimates have been based on surveys of hours of sunshine [7], full UV spectrum expressed as the erythemal dose, measured at a single geographic location [2] or a small number of locations [3], or fixed ozone and cloud cover [23].

As a result of the gradual accumulation of vitamin D during summer months and gradual diminution of reserves in the months when solar radiation is low, a "lag of seasons" is observed for 25OHD concentration. We found that in Scotland peak vitD-UVB occurs in June, while peak 25OHD occurs 1-

2 months later, as has been observed previously [24]. We developed a simple method to calculate exposure *for each individual*: a cumulative and weighted ambient UVB exposure that accounts for gradual accumulation and diminution and allows estimation of vitamin D status at a particular *point* in time (here date of blood sample).

We showed a very strong association between measured 25OHD concentration and cumulative and weighted ambient UVB exposure at the place of residence, at wavelengths relevant for vitamin D production (vitD-UVB). Although predictive ability of the final model with all relevant covariates was not complete, a dramatic improvement in the AUC curve was observed after addition of vitD-UVB estimate. This suggests that ambient UVB is major determinant of vitamin D deficiency, even in a high-latitude region [25,26]. The association is particularly strong in summer and autumn (when UVB dose is high) and in individuals under 50, which is consistent with previous research [27].

We observed significant regional variations in vitD-UVB exposure within Scotland, controlling for temporal and seasonal differences. This highlights that variation exists even in a relatively small northerly country without great differences in latitude as a result of local conditions, accentuating that precise local measurements which we consider should be favoured over crude macro-estimates of UVB exposures. The regional variation in vitD-UVB within the small area has not been previously reported. During the summer months there was a high daily variability in vitD-UVB between the different postcodes within Scotland, which decreased dramatically as level of UVB fell during the rest of the year. This suggests that geographical differences are likely to be even greater in lower latitude regions where UVB exposures are overall higher.

Many studies previously have shown a positive relationship between supplementation and circulating vitamin D levels [28]. In this study, the association with 25OHD levels was the strongest in winter and spring (when UVB dose is very low or following months of diminution), and this finding emphasises the role of vitamin D supplementation in winter months. Interestingly, supplementation was not associated with vitamin D status in younger participants (<50 y). Dietary intake had minimal effect on in vitamin D status, with was expected because it is now widely accepted that food sources

of vitamin D are scarce [15,29]. The relationship between genetic factors and 25OHD was similar to what was reported previously[16].

It is important to emphasise that the proportion of variance explained is not a measure of the effect[30]. PVE is strongly determined by the variation of the exposure in the population: in a population living an outdoor-oriented lifestyle and residing in a region where sunshine UVB is abundant, majority of *variation* in 25OHD may be due to genetic factors or skin tone, and not UVB (because it is uniformly abundant everywhere). However, UVB radiation would still be the primary *cause* of vitamin D production. We found that UVB explained 11% of variance in 25OHD in this population overall, but it explained almost no variation in 25OHD in winter. It is reasonable to assume that proportion of variance explained by UVB could be greater in regions where the annual oscillations in UVB radiation dose are greater.

Applications and Uses of Method

With the exception of few studies, accurate individual measures of ambient UVB have been largely underexploited in research. We suggest that the routinely collected data on UVB exposure can be used to approximate vitamin D status when 25OHD levels are not measured or blood sample is not available. Existing past UVB dose recordings allow investigation of the relationship between UVB estimate as a proxy of vitamin D and outcomes *retrospectively* in existing cohorts at virtually no cost. Alternatively, UVB exposure data can be used to adjust 25OHD measurement for date of sampling, or it can be used in conjunction with 25OHD to predict *average yearly* 25OHD of each individual [31]. Furthermore, randomized control trials (RCT) into the effects of vitamin D intakes on health outcomes can suffer from confounding by high exposure to naturally occurring VitD-UVB. Skin synthetized vitamin D can dominate 25OHD concentration and mask any impact of vitamin D supplementation on circulating level and/or study outcomes. We suggest that ambient UVB should be added as a covariate in the analysis, as ignoring "personal" vitamin D supplements use among participants in vitamin D RCTs has been shown to affect the findings previously [32]. Finally, UVB

exposure can be used as an instrument for Instrumental Variable Analysis and provide some insights into causality [2,33].

Strengths. The principal major strength of this study is the use of an all-encompassing UVB estimate from daily satellite measurements, restricted to wavelengths relevant for vitamin D production, and adjusted for regional terrain and atmospheric conditions (cloudiness and ozone)[34,35]. To our knowledge, this study used the highest geographical resolution of the exposure to date with respect to the place of residence, and was the first study that correlated accurate individualised ambient vitD-UVB exposure and 25OHD levels. Study comprised large number of participants that are homogeneous regarding skin type (all participants were white Scottish.

Limitations. Information on personal characteristics and behaviours were largely unknown, for example "sun holidays" or sunscreen use, or time spent outside. However, sunlight exposure questionnaires were shown to provide poor estimates of vitamin D status [7,9]. The additional benefit of adjusting the vitD-UVB estimate for these factors is currently unknown. However, this means that the vitD-UVB exposure used here is not confounded by personal behaviours. Because of the narrow range of both UVB dose (high latitude) and supplements (conservative intake recommendations [36,37]) at low doses, we were not able to examine these exposures at higher levels. As observed vitD-UVB data is only available since July 2005, daily UVB dose had to be estimated for each day of the year (DD/MM) from available data. As a consequence, the random measurement error in our UVB estimate could bias our results towards the null (ie. attenuate effect size)[38] and more accurate measurements are likely to yield even stronger associations and better predictors in the future.

Conclusions. There is a significant seasonal and geographical variation in ambient vitD-UVB exposures in Scotland, despite this being a relatively small country without large differences in latitude. The cumulative and weighted ambient vitD-UVB exposure estimate was a good predictor of vitamin D status. The TEMIS database of ambient VitD-UVB exposures can be useful for

epidemiological research, aimed at examining relationships between UVB/vitamin D and health outcomes.

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Table 1. Multivariate Linear regression models describing the personal characteristics relevant for the synthesis of vitamin D. Plasma 25OHD levels were associated with cumulative weighted vitD-UVB exposure estimate, supplement use and dietary Vitamin D, adjusting for age, sex, BMI, socioeconomic class and level of physical activity. Percentage of variance explained was calculated by partitioning the sum of squares between predictors for covariates of interest.

Model	n	Plasma 25OHD ng/ml, Median (IQR)	Cumulative Weighted vitD-UVB			Supplements			Dietary Vitamin D			Genetic Risk Score		
			PVE	Beta Coefficient (in 000s)	P Value	PVE	Beta Coefficient	P Value	PVE	Beta Coefficient	P Value	PVE	Beta Coefficient	P Value
All cohort	1963	12.9 (7.8 – 19.0)	11.16	1	< 2E-16	1.42	0.368	3.19E-09	0.27	0.153	0.011	0.65	-0.864	3.90E-04
Supplement taking														
Yes	510	15.0 (10.4 – 21)	6.89	1	4.06E-08	0.32	0.142	0.198	0.033	-0.041	0.746	2.25	-1.561	0.001
No	1543	12.0 (7.0 – 18.0)	13.18	1	< 2E-16	n/a	n/a	n/a	0.49	0.252	0.001	0.33	-0.619	2.81E-02
BMI <25	726	13.0 (7.0 – 19.7)	9.04	1	1.29E-12	2.1	0.45	1.46E-04	0.27	0.165	0.169	1.56	-1.37	0.001
BMI ≥25	1237	12.5 (8.0 – 18.2)	12.56	1	< 2E-16	1.19	0.336	1.12E-04	0.23	0.139	0.128	0.28	-0.561	0.06
Age														

|	N	Median (IQR)												
<50	246	14.8 (7.3 − 21.3)	22.01	2	3.18E-12	0.026	0.078	0.79	0.051	-0.11	0.69	0.007	0.1	0.89
50-59	579	13.5 (8.1 − 20.9)	8.82	1	1.96E-11	1.89	0.461	0.001	0.33	0.195	0.184	1.55	-1.511	0.004
60-69	579	13.0 (8.0 − 18.0)	9.51	1	5.09E-12	1.31	0.309	0.005	0.48	0.161	0.15	1.04	-0.915	0.018
>70	559	11.4 (7.0 − 17.0)	11.29	1	1.57E-14	2.05	0.37	8.41E-04	0.66	0.259	0.044	0.46	-0.675	0.118
Season														
Winter*	387	10.0 (5.0 − 14.0)	0.002	-1	0.844	4.69	0.618	1.39E-04	0.03	0.07	0.698	0.83	-0.931	0.094
Spring	369	10.0 (6.0 − 16.0)	4.24	2	3.25E-04	4.19	0.519	2.98E-04	0.11	-0.097	0.487	0.04	0.177	0.725
Summer	621	16.0 (11.1 − 23.0)	4.38	1	2.84E-06	0.47	0.25	0.0885	0.86	0.292	0.041	0.41	-0.69	0.131
Autumn	586	13.0 (8.0 − 19.0)	3.13	1	7.32E-04	0.99	0.28	0.012	0.48	0.187	0.126	1.98	-1.479	7.29E-03
Male	1121	13 (8.0 − 19)	12.39	1	< 2E-16	1.02	0.295	0.0007	0.072	79	0.38	0.42	0.692	0.032
Female	842	12.9 (7.0 − 19)	9.77	1	< 2E-16	1.95	0.472	4.58E-05	0.95	0.306	0.012	0.92	-1.03	0.006
Physical Activity														
1	1055	12.0 (7.0 − 17.05)	10.3	1	< 2E-16	1	0.001	1.41E-03	0.11	0.093	0.344	0.13	-0.395	0.246
2	533	13.3 (8.0 − 19.3)	12.4	1	2.66E-08	2.1	0.457	8.07E-04	1.34	0.409	9.24E-03	2.4	-1.685	0.004
3	253	15.0 (9.7 − 21.0)	10.24	1	6.14E-03	1.98	0.434	0.034	0.69	0.204	0.318	0.55	-0.895	0.262
4	122	15.0 (9.1 − 21.1)	16.45	1	2.70E-04	0.84	0.249	0.279	0.29	-0.121	0.584	0.28	-0.562	0.556
Genetic risk score														
0	527	13.7 (7.7 -21.0)	12	1	< 2E-16	4.38	0.687	1.13E-07	0.36	0.189	0.211	n/a	n/a	n/a
1 or 2	1021	12.7 (8.0- 18.3)	10.3	1	< 2E-16	0.54	0.208	0.011	0.29	0.145	0.089	n/a	n/a	n/a
3 or 4	65	10.1 (70. - 17.2)	16.9	1	0.014	0.01	-0.076	0.912	0.007	-0.025	0.938	n/a	n/a	n/a

Footnote:

* Seasons defined as December to February, March to May, June to August and September to November respectively.

PVE: proportion of variance explained. Proportion of variance explained by the exposure will depend on: (a) strength of the association between exposure and the outcome, but also on the (b) variation of the exposure in the population. Therefore, while causal relationships remain unchanged, varying exposure level in a population will strongly impact on the proportion of variance explained by that exposure. Therefore, it does not make sense to evaluate the contribution of exposures in absolute terms, but it can be a useful descriptive measure for relative comparisons in a population. IQR: interquartile range; PA: Level of physical activity (hours of running, cycling, and other sports activities; four categories).

Genetic risk score was calculated as the number of risk alleles (associated with decreasing 25OHD) for two SNPs: rs12785878 and rs2282679
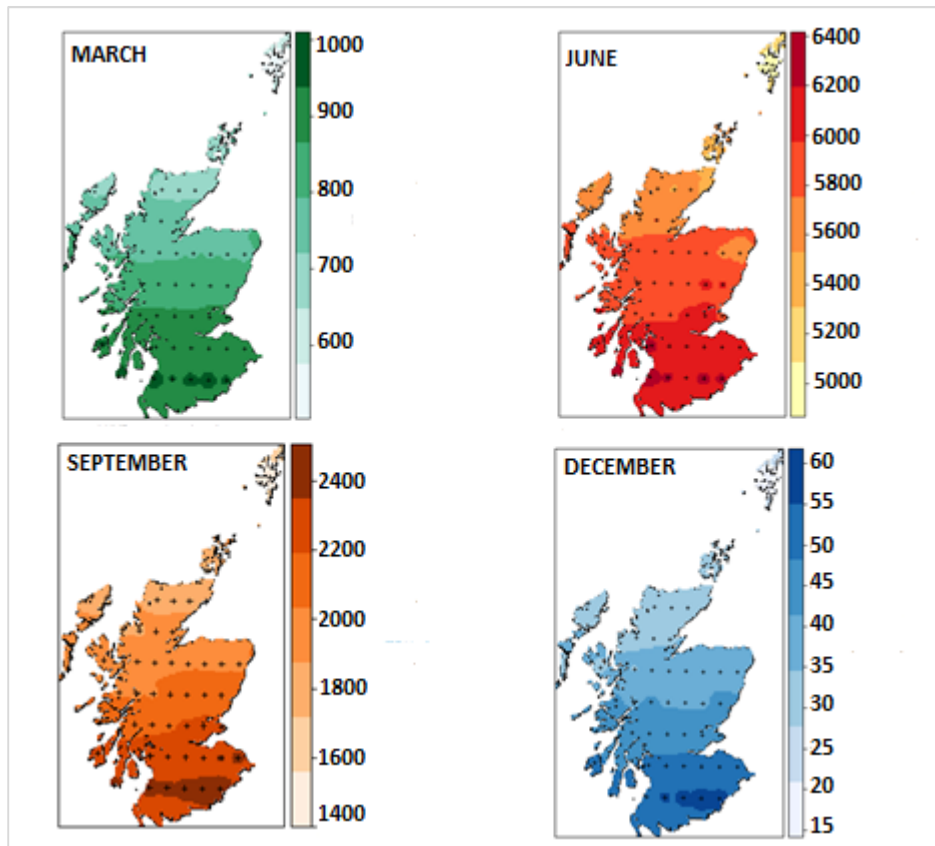
Figures

Figure 1. Determinants of Vitamin D Synthesis in Skin.
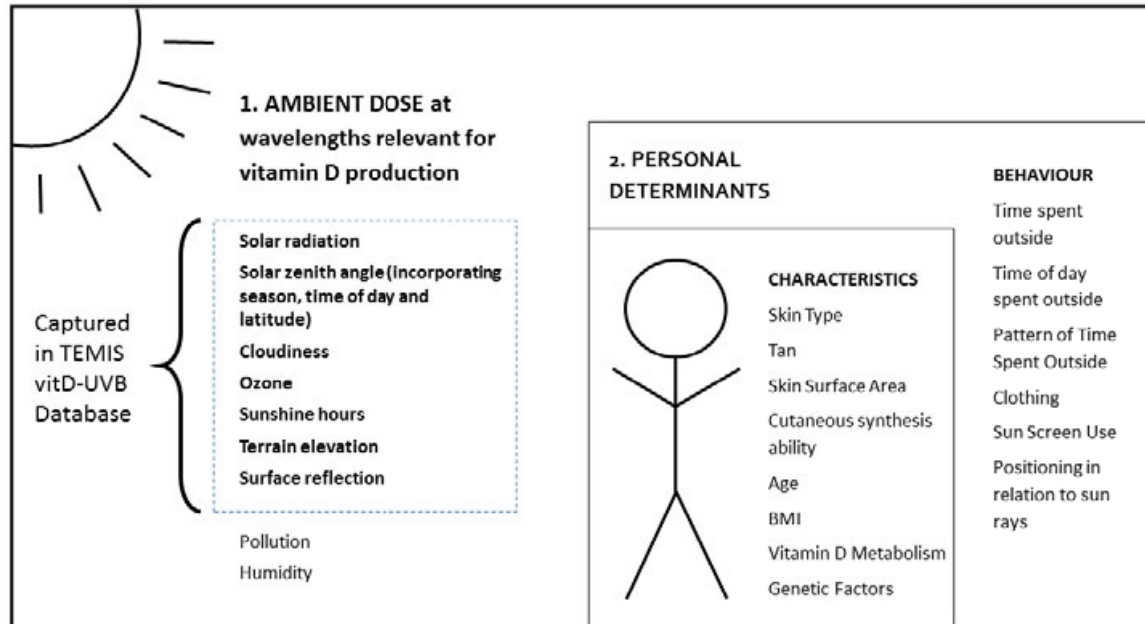
Footnote: The amount of vitamin D synthetized in skin will depend on two principal parameters: (i) ambient dose of UVB at wavelengths relevant for vitamin D production and (ii) personal factors. Important difference between the two is that ambient dose is easily measureable, while personal factors are not. Personal factors include a wide range of personal characteristics (such as skin tone and surface area, cutaneous synthesis ability, known to decrease with age) and behaviours (duration and timing of time spent outside, clothing, sun screen use). These factors are often difficult and/or costly to capture accurately, due to their constant change (clothing, tanning, time/time of day spent outside, angle of sun rays reaching skin) or are not measurable (vitamin D synthesis ability, deposition in fat, metabolism). The Tropospheric Emission Monitoring Internet Service (TEMIS) provides dose of UVB relevant for vitamin D production: daily solar radiation is measured by satellites, dose is weighted with a biological function to isolate the narrow band of wavelengths relevant for vitamin D production and adjusted this dose for all main local factors: ozone, cloudiness and terrain. The main ambient factor not accounted for in the estimate is pollution, however air pollution in Scotland is low.

TEMIS: Tropospheric Emission Monitoring Internet Service.

Figure 2. Average monthly ambient dose of UVB radiation that can induce Vitamin D synthesis (vitD-UVB; kJ/m²) in Scotland for March, June, September and December.

Footnote: *Colour gradient indicates regional variation in vitD-UVB dose in a given month using average monthly vitD-UVB (July 2005 - October 2010). Note a very large differences in the scale between four selected months.*

Figure 3. Relationships between season, cumulative and weighted vitD-UVB (CW-vitD-UVB) and 25OHD are shown. (**A**) CW-vitD-UVB dose and season; (**B**) 25OHD concentration and season; (**C**) CW-vitD-UVB and 25OHD; (**D**) categories of CW-vitD-UVB exposure and 25OHD (**E**) Scatterplot of cumulative vitD-UVB vs. CW-vitD-UVB illustrates the impact of accounting for accumulation and diminution through weighing: for the same cumulative exposure, at times when UVB dose is increasing CW-vitD-UVB is going to be smaller than at times when UVB dose is increasing; (F) 25OHD concentration and number of risk alleles.
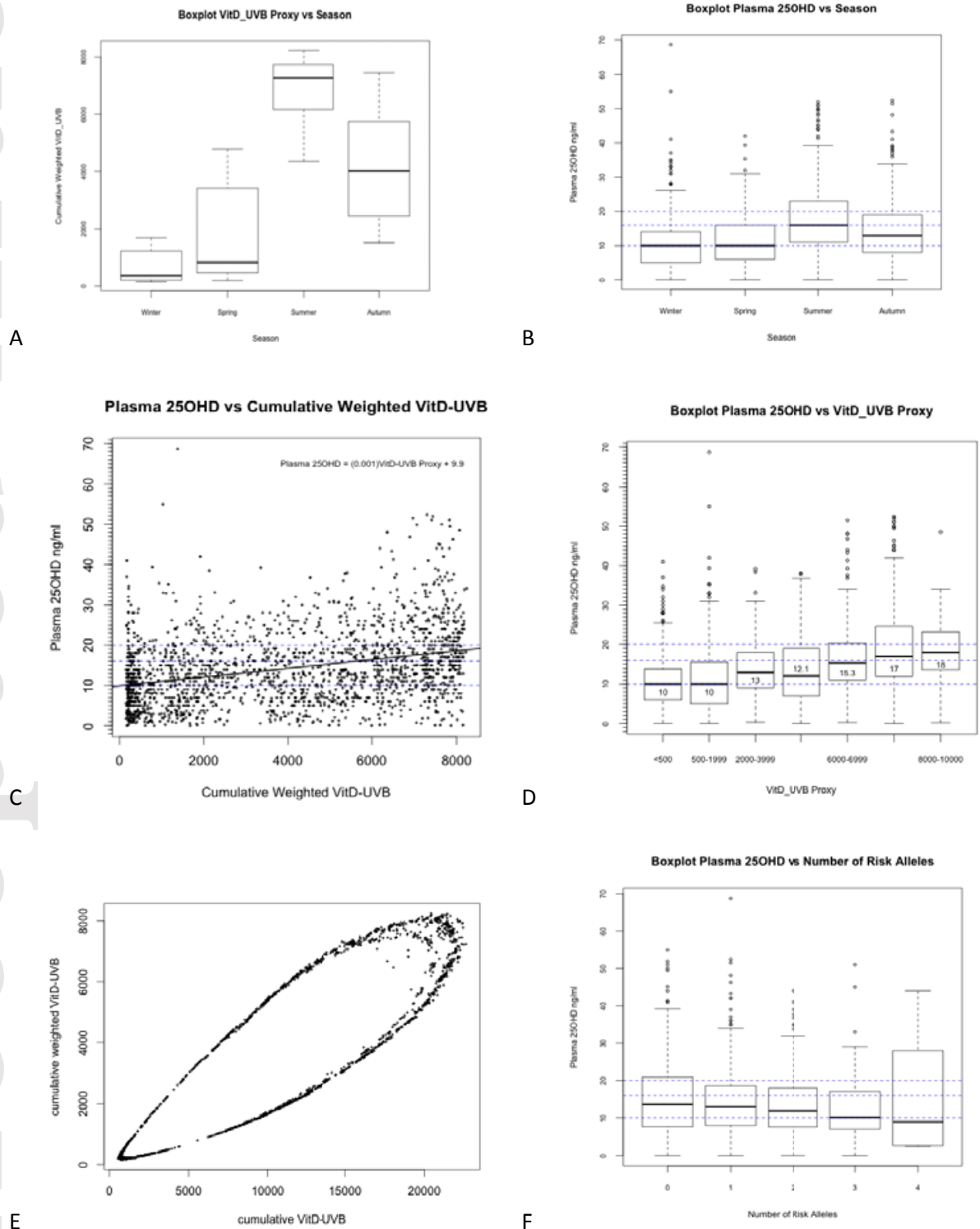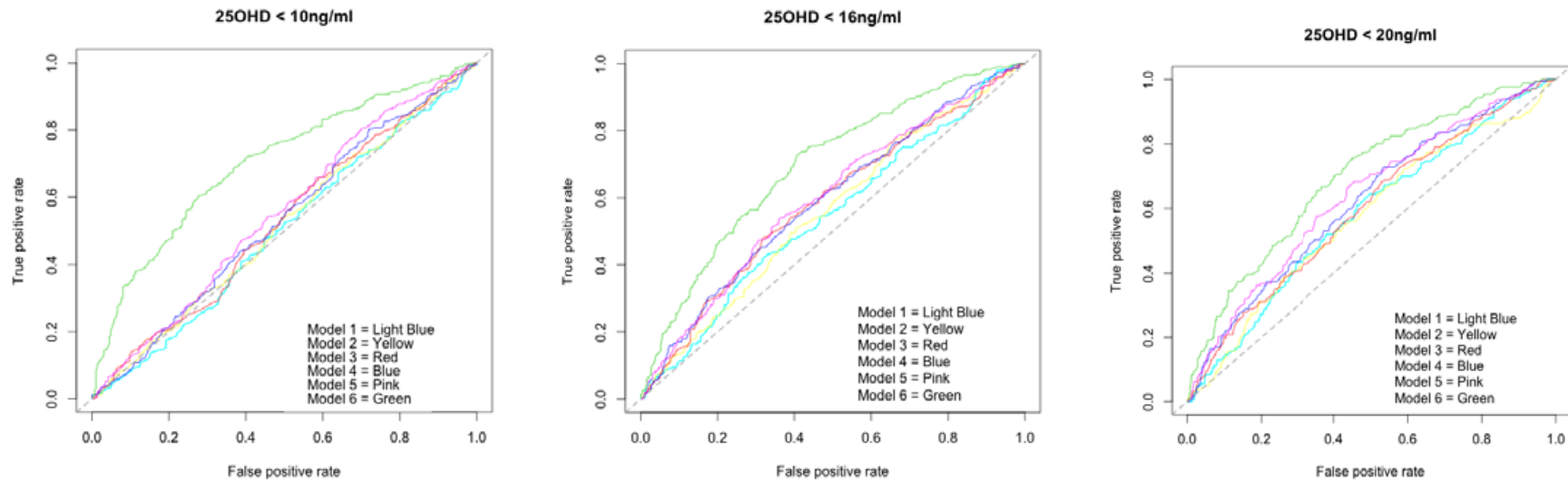
Figure 4. Receiver Operator Curves (ROC) for the prediction of Plasma Vitamin D with cumulative weighted vitD-UVB exposure estimate using randomForest method. *Six models were fitted to predict plasma 25OHD with <10ng/ml and 25ng/ml and the predictors and Area Under the Curve results are listed below.*

| n=1613 | | AUC | | |
Model	Predictors	<10ng/ml	<16ng/ml	<20ng/ml
1	Sex, age, BMI, Ses	0.496	0.547	0.579
2	Model 1 + number of risk alleles	0.514	0.558	0.573
3	Model 2 + dietary vitamin D	0.524	0.581	0.594
4	Model 3 + Physical activity	0.528	0.59	0.619
5	Model 4 + Supplementary vitamin d	0.551	0.598	0.636
6	Model 5 + vitD-UV	0.703	0.694	0.697

* Footnote:

SES = Socioeconomic Class. *667 (33.96%) participants were severely deficient (25OHD below 10 ng/mL), 583 (29.68%) were at high and 311 (15.84%) at low risk of deficiency (10-16 ng/mL and 16-20 ng/mL, respectively) and only 403 (20.52%) were vitamin D sufficient (25OHD >20 ng/mL).*