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Sunshine is an Important Determinant of Vitamin D Status Even Among High-dose Supplement Users: Secondary Analysis of a Randomized Controlled Trial in Crohn's Disease Patients

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ABSTRACT

Sunshine is considered to be the most important source of vitamin D. Due to an increased risk of skin cancer, sun avoidance is advised, but this directly contributes to the high prevalence of vitamin D deficiency. The simple solution is to advise vitamin D supplementation. The aim of this study was to examine the absolute and relative contribution of sunshine and supplementation to vitamin status. This study was a secondary analysis of an RCT of 92 Crohn's disease patients in remission (49% female, median age = 44). Participants were randomized to 2000 IU day^{-1} of vitamin D3 or placebo for 1 year, with 25-hydroxyvitamin D (25(OH)D) being measured at baseline and every 4 months. Based on participant's place of residence, daily ambient UVB dose at wavelengths that can induce vitamin D synthesis (D-UVB) was obtained. Cumulative and weighted ambient D-UVB (cw-D-UVB) exposure prior to each blood draw was calculated for each participant. Linear regression analysis and multilevel modeling were used to examine the association between UVB exposure, supplementation and 25(OH)D concentration. There was considerable annual variation in D-UVB, cw-D-UVB and 25(OH)D. Both supplementation and cw-D-UVB were found to be strongly associated with 25(OH)D: in multilevel model, an increase of approximately 6 nmol L^{-1} for every 100 kJ m^{-2} in cw-D-UVB was found, among those receiving placebo and supplementation ($P < 0.0001$). Treatment was associated with increase of 23 nmol L^{-1} ($P < 0.0001$). Sunshine is an important determinant of 25(OH)D concentration, even in those who are taking high-dose vitamin D supplements and reside at a higher mid-latitude location.

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; cw-D-UVB, Cumulative and weighted vitamin D producing UVB; TEMIS, Tropospheric Emission Monitoring Internet Service; UV, Ultraviolet.

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INTRODUCTION

Vitamin D synthesis occurs in skin following exposure to a very narrow band of wavelengths within the ultraviolet B (UVB) part of the solar spectrum (peak synthesis: 295–298 nm). These wavelengths induce photoconversion of 7-dehydrocholesterol to previtamin D, which is subsequently converted to vitamin D. Once vitamin D is synthesized or ingested, it is hydroxylated and predominantly stored as 25-hydroxyvitamin D (25(OH)D). This is tightly bound to vitamin D-binding protein in circulation. 25(OH)D is considered to be the best marker of vitamin D status at the time of blood draw.

Dietary sources of vitamin D are scarce: for example, the National Diet and Nutrition Survey in the UK found that the median dietary consumption of vitamin D in 2008/2009 was less than 120 IU in both males and females, much less than the recommended daily dose of 400 IU (1). Therefore, sunshine is the most important natural source of vitamin D for most humans (2–4), while supplements are known to improve the status of individuals when sunshine exposure is naturally or behaviorally low. Contrary to the expectations (5), sunshine is an important contributor to vitamin D status even at higher latitudes; studies have shown that year-round 25(OH)D sufficiency in the UK is possible following exposure to adequate UVB in late summer (6). However, this might not be the case for all individuals and many individuals may still be at risk of deficiency—particularly when exposure to sunshine is limited (7,8).

In contrast to the beneficial role of sunshine in vitamin D production, exposure to sunshine has been recognized as a significant contributor to skin cancer, skin aging and cataract. This is why sun avoidance and use of sun protection are widely advised: for example, even the Public Health Service in Ireland (higher mid-latitude country) actively recommends that people remain in the shade, wear sunglasses, cover-up and use sunscreen when outdoors (9). Similarly, existing sunshine exposure guidelines around the world mostly come from the agencies involved with skin cancer prevention (10–12). In recent years, the impact such recommendations may have on dermal exposure to UVB and subsequent vitamin D status has become a concern (13,14).

Internationally, vitamin D deficiency is widespread (15) and has been associated with numerous health outcomes such as bone health, and more recently some associations have been suggested for metabolic, cardiovascular, infectious and immunological diseases, as well as cancer occurrence and mortality (16). Therefore, it is evident that a conflict exists between limiting sun exposure for prevention of skin cancer risk and ensuring vitamin D sufficiency.

Limited data exist on the contribution of solar radiation on 25(OH)D concentration in free-living individuals or on the deficit that would occur when exposure is reduced. Interestingly, published studies report a smaller increase in 25(OH)D concentration with supplementation in those who have a higher baseline level, and that an initial linear increase in 25(OH)D with a fixed supplement dose slows down once higher levels are reached (17–19). This means that the effectiveness of vitamin D supplementation might be impacted by dermal production and vice versa. A negative feedback loop in vitamin D absorption and/or dermal synthesis at higher levels might provide a mechanism to explain this, although this is not well researched. Alternatively, this finding could be biased by modified behavior, such as noncompliance in supplement taking if one has abundant sun exposure, or by reduction in sun exposure once supplements are being taken. The aim of this article was to quantify the absolute and relative contribution of ambient UVB to 25(OH)D concentrations and examine whether this relationship is modified by high-dose vitamin D supplementation with 2000 IU day^{-1} (50 μg) in a cohort of Crohn's disease patients in remission in a higher mid-latitude region (53.14° N).

SUBJECTS AND METHODS

Study population. A secondary data analysis of a double-blind randomized placebo-controlled trial that investigated the effect of vitamin D supplementation on clinical outcomes in 92 patients with established Crohn's disease who were in remission at time of recruitment was conducted (20,21). The vast majority of patients were Caucasian (99%). Briefly, patients were excluded if they were pregnant or lactating, alcohol dependent, had a history of hypercalcemia (corrected serum calcium >2.66 nmol L^{-1}), known hypersensitivity to vitamin D or had personal supplemental intakes of vitamin D > 800 IU day^{-1} (complete exclusion criteria are described in (20)). Patients were allowed to continue with prescribed vitamin D and calcium supplementation under 800 IU day^{-1} if they were taking it (42.7% of participants were taking calcium and vitamin D supplements with equal numbers in each randomized group). Participants were randomized to vitamin D_3 (2000 IU day^{-1}) or placebo for one year (Figure S1). No individual sunlight exposure or dietary information was taken in this study. Compliance was found to be >95% in this study and was determined by counting remaining pills in pill packets in both the placebo and treated patients at each subsequent visit.

The baseline blood samples (time-point 1; T1) were taken between March 2012 and July 2013. Serum 25(OH)D was measured four times during the study: at baseline and every 4 months thereafter, so that each individual had measurements which spanned an entire year. The study was approved by the St. James's Hospital and the Adelaide and Meath Hospital Research Ethics Committee and conducted at Tallaght Hospital and St James's Hospital in Dublin, Ireland. All participants provided informed, written consent.

Vitamin D measurement. Total 25(OH)D (25(OH)D_2 and 25(OH)D_3) was measured from serum samples by Liquid chromatography–mass spectrometry using a proprietary assay (MassChrom™ 25(OH)D, Chromsystems, Munich, Germany) at the Biochemistry Department of St James's Hospital, Dublin, Ireland. The laboratory is fully accredited to ISO 15189 standards. Quality assurance and accuracy of the assay were monitored by participation in the Vitamin D External Quality Assessment Scheme and use of National Institute of Standards and Technology traceable standards. All samples were assayed at the same laboratory to minimize technical assay variability. Due to drop out and late recruitment, not all four 25(OH)D measurements were available for all participants. 25(OH)D measurements were available at all time-points for 70 participants. 25(OH)D was missing for 10 individuals at baseline, seven at T2, six at T3 and one at T4. Measurements were carried out in four batches corresponding to time-point.

Ambient UVB data source. Daily ambient UV dose data from the TEMIS database (www.temis.nl/uvradiation/UVdose.html), version 2.0, was used to determine the ambient UVB levels relevant for cutaneous vitamin D production. Briefly, spectra of surface UV radiation are weighted by the vitamin D action spectrum (22), adopted by the CIE in 2006 as standard (CIE-174), to provide the daily UV dose (D-UVB, in kJ m^{-2}), which essentially depends on total ozone column and solar zenith angle; see the Supporting Information section for more detailed description of methods. The TEMIS UV data grid cells are 0.25° × 0.25° (longitude × latitude); over Ireland, these grid cells are about 28 km (north–south) × 17 km (east–west), and 256 such grid cells cover Ireland. Data used in this paper covers the time period January 2004 to June 2017.

Cumulative and weighted D-UVB (cw-D-UVB) estimation calculation. In order to investigate the relationship between 25(OH)D and ambient D-UVB, the estimate must account for the accumulation and diminution of 25(OH)D in the body. Therefore, we used a cumulative and weighted estimate of daily D-UVB (cw-D-UVB). Daily ambient D-UVB doses over 135 days preceding blood draw were retrieved; this period was found to be optimal in a previous study (23). The mean daily doses for each of the 135 days were weighted as per Eq. 1 and summed up, to give a 135-day cw-D-UVB estimate corresponding to the date of blood draw. This was the second, temporal, weighting of D-UVB. It was carried out so that exposures immediately preceding blood sampling contribute more to the estimate than exposures from a more distant past, to account for accumulation and diminution of vitamin D in the body: vitamin D synthesized in the past would mostly be used up. It has been observed that the half-life of vitamin D in the body is normally 2 months, while circulating 25(OH)D can get broken down after 15 days (24); therefore, a half-life of 35 days was chosen as optimal (23) (Eq. 1). The weighting equation is shown below; where x = days ago (starting day before and up to 135 days prior to sampling), y = rate of disappearance of effect of D-UVB in days (half-life set at 35 days) and e $(-\ln 2)(x/y)$ is the weighing formula applied. From this, it was possible to compare daily D-UVB doses and 135-day cw-D-UVB for each day of the year and investigate the relationship between them. More information about this method can be found at (25). The relationship between daily D-UVB doses and cw-D-UVB doses are shown in Fig. 1E and highlight the lag between the two estimates. This is similar to the lag which would be expected between daily D-UVB doses and 25(OH)D measurements in blood.

Equation 1. Cumulative and weighted D-UVB dose (cw-D-UVB).

$$\mathrm{cwDUVB}\ (x) = \sum_{x=1:135} \left(\mathrm{DUVB}\ (x) * e^{-\frac{\ln 2}{y}x}\right) \qquad (1)$$

Cw-D-UVB calculation for study participants. In order to calculate cw-D-UVB for each individual in the cohort, a TEMIS grid field was assigned to each participant in the study based on their residential location. Exact daily D-UVB doses over 135 days prior to blood draw were then extracted independently for each participant. Hence, input data were determined by a participant's place of residence and date of blood draw; this enabled us to account for both the seasonal and regional differences in D-UVB radiation.

These daily ambient D-UVB doses were then combined to give a cumulative and weighted estimate of D-UVB, with each daily dose being weighted as described above. As blood samples were taken four times (T1–T4), and 25(OH)D measured in each sample, four cw-D-UVB estimates were calculated for each individual prior to the date at which corresponding blood sample was drawn.

Statistical analysis. All analyses were performed in R (R Development Core Team, 2011). Seasons were defined by the meteorological seasons: winter (December 1st to February 28th), spring (March 1st to May 31th), summer (June 1st to August 31st) and autumn (September 1st to November 30st). Seasonal differences in 25(OH)D concentration, D-UVB and cw-D-UVB were assessed. Linear regression models were used to determine whether there was an association between

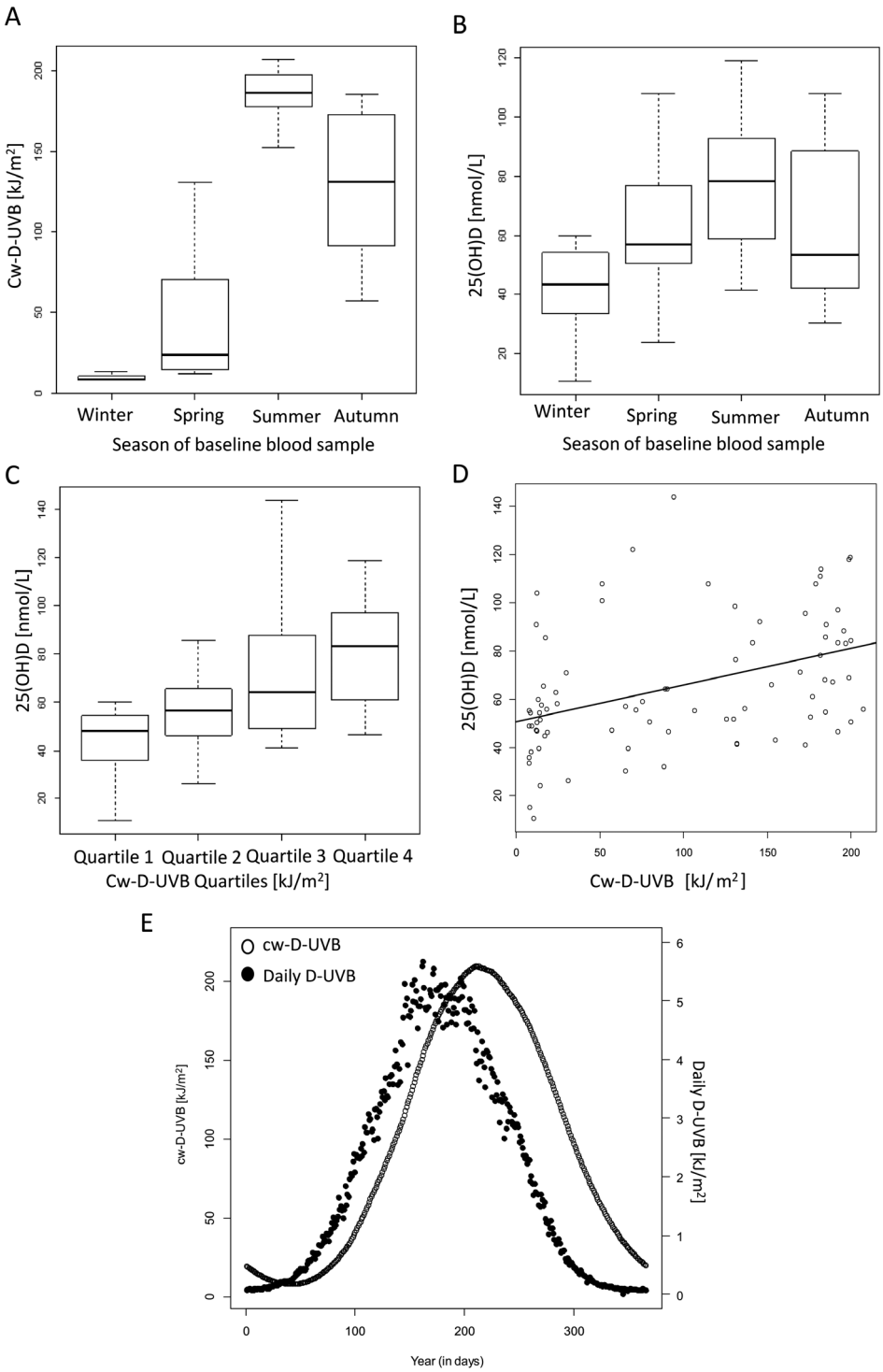

Figure 1. Relationship between 25(OH)D, cw-D-UVB and seasons. (A) baseline 25(OH)D concentration and season in all participants, (B) cw-D-UVB calculated at T1 (or baseline) and season, (C) box plot demonstrating the relationship between cw-D-UVB quantiles and 25(OH)D at baseline for all participants, (D) scatter plot of cw-D-UVB dose and 25(OH)D at baseline for all participants. Season definitions: winter [December 1st–February 28th], spring [March 1st–May 31st], summer [June 1st–August 31st], autumn [September 1st–November 30th]. (E) Average relationship between daily D-UVB dose and cw-D-UVB over a one-year period. Data were averaged from daily doses from 2004 to 2017 in Dublin.

cw-D-UVB dose and serum 25(OH)D at baseline and follow-up. Linear regression was deemed appropriate after examining residuals, R^2, and carrying out diagnostic plots. The model was initially adjusted for age, sex, smoking status, alcohol consumption, baseline (T1) 25(OH)D concentration [low: <50 nmol L^{-1}, medium: 50–74 nmol L^{-1}, high: ≥75 nmol L^{-1}] (except at baseline) and randomization group. Backwards stepwise regression was conducted, and the final model was determined by R^2 number (coefficient of determination), Akaike information criterion (AIC) and Bayesian information criterion (BIC). The model with the lowest AIC, BIC and highest R^2 was chosen (Table S1). Multiple testing correction was not carried out for this study. Multilevel regression models were used to determine whether there was an association between

cw-D-UVB dose and serum 25(OH)D longitudinally, over all time-points. This was calculated using the "nlme" packages in R (26). Stratification by treatment was also carried out to test associations in supplemented, and placebo patients separately. $P < 0.05$ was considered statistically significant.

RESULTS

Ninety-two Crohn's patients in remission at baseline were included in this study (Figure S1). Median (IQR) age of participants was 44 (33–52) years, and approximately half (49%) were female (Table 1). At baseline, median 25(OH)D concentration was 57.3 nmol L^{-1} (57.3 nmol L^{-1} in treatment and 57.2 nmol L^{-1} in placebo group). Baseline measurements (T1) were taken throughout the year, but predominantly in spring (38%) and summer (26%). More individuals in vitamin D group were recruited in winter (20%) compared to 10% in the placebo group; this is also reflected in the lower median cw-D-UVB at baseline in treatment group (72.34 *vs* 90.34 kJ m^{-2}).

Cw-D-UVB given in kJ m^{-2} differed considerably according to season (Fig. 1A): as expected, it was the highest in summer (mean: 185, IQR: 181.5–196.6) and lowest in winter (12.3, 8.4–14.0). Similarly, 25(OH)D concentrations were found to be the highest in samples taken in summer and lowest in winter (Fig. 1B). Finally, there was a linear correlation between 25(OH)D_{T1} and cw-D-UVB_{T1} at baseline (Fig. 1C and D) ($R^2 = 0.16$).

Unsurprisingly, there were considerable differences in serum 25(OH)D concentration at follow-up in response to treatment. 25(OH)$D_{T2\text{-}4}$ concentration was 64, 59 and 64 nmol L^{-1} among the placebo and 100, 99 and 98 nmol L^{-1} among the supplemented patients. A seasonal fluctuation of 25(OH)D and cw-D-UVB was clearly observed in placebo patients, and in some participants among the supplemented group (Figure S2).

Strong associations were observed between cw-D-UVB and serum 25(OH)D concentration in a linear regression model (Table 2). Individual cw-D-UVB dose was highly associated with the 25(OH)D concentrations at most time-points (Time-point 1: *P*-value: 4.9×10^{-4}, $R^2 = 0.18$; Time-point 2: *P*-value: 4.9×10^{-4}, $R^2 = 0.59$; Time-point 3: *P*-value: 6.3×10^{-4}, $R^2 = 0.57$; Time-point 4: *P*-value: 0.30, $R^2 = 0.55$) (Table 2). Association between cw-D-UVB and serum 25(OH)D concentration was confirmed in a multilevel regression model and after stratification by treatment (Table 3) (*P*-value: <0.0001). For every 100 kJ m^{-2} increase in cw-D-UVB dose, an average increase of 6 nmol L^{-1} in 25(OH)D concentration was observed (SE: 1 nmol L^{-1}) in the multilevel model (Table 3). Similar trends were noted when this association was restricted by treatment group (β vitamin D treated: 0.069; β placebo: 0.058).

As expected, a significant association between treatment group and 25(OH)D concentration was observed. Treatment with 2000 IU day^{-1} of vitamin D was associated with an average of 30 nmol L^{-1} increase in serum 25(OH)D in the treated group.

DISCUSSION

In this longitudinal study, we used data from a randomized controlled trial and found that 25(OH)D concentration is strongly associated with the ambient UVB radiation preceding blood draw, both among individuals randomized to placebo and those receiving 2000 IU day^{-1} of vitamin D. We have hence demonstrated that sunlight is a significant contributor to vitamin D status even in individuals taking high doses of vitamin D supplement who reside in a higher mid-latitude region.

The relationship between solar radiation and vitamin D status is well known (27–29), and results reported here are comparable to previous studies that focused on ambient UVB radiation

Table 1. Characteristics of the Crohn's disease patients in remission cohort.

Characteristic	All *N* (%)	Vitamin D *N* (%)	Placebo *N* (%)
No. of patients	92	50 (54)	42 (46)
Age*	44 (33–52)	44 (32–52)	44 (38–53)
Age at diagnosis*	31 (22–42)	27 (20–37)	32 (27–44)
Sex (female)	45 (49)	22 (45)	23 (55)
25-OHD (nmol L^{-1})*			
T1	57.3 (47–84)	57.3 (46–89)	57.2 (51–80)
T2	87.5 (63–109)	100.0 (85–128)	63.7 (64–84)
T3	84.8 (55–108)	99.0 (79–124)	58.5 (40–77)
T4	82.0 (57–105)	97.5 (81–116)	64.0 (41–77)
Cw-D-UVB (kJ m^{-2})*			
T1	88.2 (14–174)	72.34 (14–181)	90.34 (18–155)
T2	115.50 (34–197)	116.30 (34–196)	105.3 (41–198)
T3	34.91 (13–154)	35.52 (16–155)	32.82 (10–139)
T4	57.02 (15–172)	45.86 (14–180)	63.53 (17–166)
Alcohol (NA = 6)‡			
Yes	46 (50)	29 (62)	17 (44)
No	40 (43)	18 (38)	22 (56)
Smoking Status (NA = 6)			
Never	38 (41)	23 (49)	15 (38)
Past§	39 (42)	18 (38)	21 (54)
Current	9 (9)	6 (13)	3 (8)
Season of T1 blood draw (NA = 1)			
Winter	14 (15)	10 (20)	4 (10)
Spring	35 (38)	18 (36)	17 (41)
Summer	24 (26)	13 (26)	11 (27)
Autumn	18 (20)	9 (18)	9 (22)

*Values represent median and IQR; ‡NA, Not available; §Quit over a year ago.

Table 2. Associations between cw-D-UVB and 25(OH)D concentration at each time-point.

Time-point	Association with	*N*	Cw-D-UVB Beta§	SE	*P*-value
All participants					
Cw-D-UVB_{T1}†	25(OH)D_{T1}	82	0.13	0.04	4.9×10^{-4}***
Cw-D-UVB_{T2}‡	25(OH)D_{T2}	84	0.11	0.03	4.9×10^{-4}***
Cw-D-UVB $_{T3}$‡	25(OH)D_{T3}	85	0.15	0.04	6.3×10^{-4}***
Cw-D-UVB $_{T4}$‡	25(OH)D_{T4}	91	-0.03	0.03	0.30
Vitamin D supplemented					
Cw-D-UVB_{T1}†	25(OH)D_{T1}	44	0.17	0.05	1.7×10^{-3}**
Cw-D-UVB_{T2}¶	25(OH)D_{T2}	49	0.13	0.05	6.7×10^{-3}**
Cw-D-UVB $_{T3}$¶	25(OH)D_{T3}	47	0.15	0.07	0.039*
Cw-D-UVB $_{T4}$¶	25(OH)D_{T4}	50	-0.08	0.05	0.14
Placebo					
Cw-D-UVB_{T1}†	25(OH)D_{T1}	38	0.08	0.05	0.14
Cw-D-UVB_{T2}¶	25(OH)D_{T2}	35	0.08	0.04	0.07
Cw-D-UVB $_{T3}$¶	25(OH)D_{T3}	38	0.28	0.05	8.6×10^{-4}***
Cw-D-UVB $_{T4}$¶	25(OH)D_{T4}	41	0.01	0.04	0.73

†Model adjusted for age and sex; ‡Model adjusted for age, sex and baseline 25(OH)D concentration [Low: <50 nmol L^{-1}, Medium: 50–74 nmol L^{-1}, High ≥75 nmol L^{-1}] and randomization group; §Beta refers to the change in 25(OH)D per kJ m^{-2} increase in Cw-D-UVB dose; ¶Model adjusted for age, sex and baseline 25(OH)D concentration [Low: <50 nmol L^{-1}, Medium: 50–74 nmol L^{-1}, High ≥75 nmol L^{-1}]; *indicates *P*-value <0.05, **$P \leq 0.01$ and ***$P < 0.001$.

Table 3. Multilevel associations between cw-D-UVB and 25(OH)D concentration

	All participants[†]			Placebo[‡]			Vitamin D supplemented[§]		
Variable	Beta[§]	SE	*P*-value	Beta	SE	*P*-value	Beta	SE	*P*-value
Sex: Female *vs* Male	1.77	5.50	0.75	1.49	6.69	0.83	1.82	8.55	0.83
Age	0.59	0.23	0.01**	0.62	0.28	0.03*	0.56	0.36	0.12
Vitamin D supplementation	23.00	5.52	<0.0001***	*NA*	*NA*	*NA*	29.82	3.23	<0.0001***
Cw-D-UVB dose (kJ m^{-2})	0.060	0.01	<0.0001***	0.058	0.017	0.002**	0.069	0.017	<0.0001***

[†]Model adjusted for age, sex, randomization group; [‡]Model adjusted for age, sex; [§]Beta refers to the change in 25(OH)D per kJ m^{-2} increase in sex, age, vitamin D supplementation or Cw-D-UVB dose; *indicates *P*-value <0.05, **$P \leq 0.01$ and ***$P < 0.001$.

(23,30). Interestingly, we found a comparable contribution of UVB radiation in both placebo and vitamin D-treated group. This is in contrast to some previous studies that failed to demonstrate a change in 25(OH)D given the seasonal fluctuation in solar radiation in individuals taking vitamin D supplements (31,32). The lack of the association in those studies might be due to the seasonal vitamin D supplementation, characterized by supplement taking during the winter months only; in this case, varied supplement use could mask seasonality in dermal production. The RCT setting used for the study, most notably the high vitamin D dose and randomization process, provided a strong design for testing the impact of UVB on vitamin D status since the bias introduced by "as-needed" supplement taking is minimized. In addition, the use of ambient UVB radiation which has been adjusted for various factors, in our study likely led to an increased precision of the exposure measure and thereby increased power to detect the associations.

The effect of cw-D-UVB seems to be largely linear. An increase of 100 kJ m^{-2} in ambient cw-D-UVB was associated with an *average* increase in 25(OH)D concentration by about 6 nmol L^{-1} throughout the year. The impact on 25(OH)D reported here is an *average* increase, given the *average* exposure to ambient UVB in terms of time (length of time and time of day) and surface of exposed skin. It is reasonable to expect that individuals who do not spend time outdoors will not benefit from any increase in D-UVB; equally, a greater impact of same D-UVB dose may be found in individuals who spend more time in the sun with more skin exposed; however, this will have to be examined in detail in a cohort with sun exposure information. Additionally, baseline 25(OH)D concentration also has an impact on the benefit of D-UVB exposure (33). In the summer when solar radiation is the strongest (cw-D-UVB over 200 kJ m^{-2} in Ireland), we noted an *average* increase of over 20 nmol L^{-1} in 25(OH)D compared to the winter time.

As expected, we noted a significant impact of supplementation on vitamin D status: supplementation with 2000 IU day^{-1} was associated with 30 nmol L^{-1} increase in 25(OH)D concentration in the treated group. Unsurprisingly, supplementation with vitamin D had the greatest impact on 25(OH)D. Although we found that cw-D-UVB dose was an important factor, the impact of supplementation on 25(OH)D should not be neglected. However, historically no vitamin D supplementation was recommended for adults, except for those over 65, pregnant and/or lactating women or those with darker skin (supplementation with 400–600 IU day^{-1} was recommended (34)), and currently, it is still the case that vitamin D supplementation is most often used by certain population groups. Due to this, cutaneous synthesis of vitamin D is still an important source of vitamin D for individuals who are not taking supplements, and this study has demonstrated that cw-D-UVB is still associated with 25(OH)D even in those taking supplements and should also be taken into account as a source of vitamin D in these individuals. If linear relationships are assumed, supplementation with 500 IU day^{-1} could be expected to be associated with a 5 nmol L^{-1} increase in 25(OH)D. Previous studies report similar effect size: for example, Brooks and Greene-Finestone report 11 nmol L^{-1} increase among Canadians (typical supplementation with 400–1000 IU day^{-1}) (35) and similarly Holick *et al.* found that an 24 nmol L^{-1} increase among participants who had been prescribed 1000 IU day^{-1} supplements over 11 weeks (36). The increase of 100 kJ m^{-2} in ambient cw-D-UVB would be associated with an average increase in 25(OH)D concentration by about 6 nmol L^{-1}, which would be equivalent to taking 400 IU of supplement daily. Therefore, we can conclude that the impact of D-UVB at levels in high mid-latitude region on vitamin D status is of the same order of magnitude as supplementation.

Previously, a smaller increase with supplementation was noted in those with higher baseline levels (17,18), suggesting a nonadditive effect. However, in this study we found a similar effect size for UVB in those randomized to placebo or treatment; equally, we noted the comparable effect of supplementation at the various levels of UVB radiation, consistent with Farrar *et al.* (37). Therefore, our results suggest an additive effect of two main sources of vitamin D for humans, that is, supplements and solar radiation. This is important for practice, because continued behavioral modification recommendations to decrease exposure to sunlight need to be evaluated in terms of the impact on vitamin D status and compensated via adequate supplementation recommendations.

The use of individually calculated ambient UVB dose is a superior to grouping individuals by descriptive variables, such as season, which is not quantifiable; actual UVB dose will depend on geographical region (altitude and microclimate) and day within the season (UVB dose at the beginning and end of a season is often dramatically different; Fig. 1). Therefore, D-UVB is measurable and unambiguous (hence comparable across individuals and cohorts). Previous work has demonstrated the impact of differing UVB on 25(OH)D (38) and supports investment in more granular metrics of UVB exposure. While dosimeters have the benefit of capturing the actual intensity of radiation, surface of exposed skin is still unknown, it is difficult to ensure compliance over a longer period of time (ideally >3 months) and collecting and analyzing the data for a large cohort would make it expensive, and therefore not appropriate for all studies. In contrast to this, ambient UVB dose can be easily obtained for even a very large (or historic) cohort at no cost, and the use of it as a proxy for UVB dose could be improved by questionnaires on time spent outdoors.

One-size-fits-all recommendations are inappropriate for sun exposure, as this is highly dependent on personal characteristics

and level of ambient UVB radiation (39). For example, those with darker skin require much longer exposure to ensure sufficiency compared to fairer skinned individuals. Age is another factor which is important, as those who are older produce less vitamin D (40–42). Optimal sun exposure combines level of exposure where risk of skin cancer is low and but vitamin D production can occur. If there is no overlap, there is no safe level of exposure that would allow for vitamin D production, and sun avoidance is the best recommendation—this may be the case individuals with fairer-skin types (skin types I and II). In such cases, adequate supplementation dose with vitamin D should be ensured. However, for individuals with darker skin (skin type IV–VI), particularly those living in nonextreme solar radiation regions, "safe and meaningful" level of sun exposure might offer the opportunity for natural phototherapy during parts of the year; however, supplementation might be necessary to ensure sufficiency year-round (43). Determining the "optimal level" will be highly individual, and accurately measuring and understanding the contribution of ambient UVB to 25(OH)D is the key step to achieving this. Consequently, one-size-fits-all recommendations for supplement use also cannot be assumed, as the ability and opportunity for dermal synthesis vary widely and high inter-individual variation has been reported for the same supplement dose (44,45). Interestingly, artificial phototherapy may be another way of vitamin D supplementation. Some previous research has demonstrated a beneficial effect of phototherapy on disease and 25(OH)D concentrations (46,47). This approach may be particularly relevant for individuals with impaired absorption of lipids (and hence lipid-soluble vitamins), but further research is needed.

Strengths of this study include RCT setting, multilevel modeling and the longitudinal design, repeated-measures design that is less sensitive to variation within the study population since it focuses on changes within each study participant. The major strength of the study is the use of the "gold standard" method for 25(OH)D measurement and a detailed measure of ambient UVB at wavelengths that can induce vitamin D synthesis with excellent spatial and temporal resolution and adjustment for all major factors that affect ambient UVB dose. It has previously been shown that without the adjustment of cloud cover, ozone or altitude, UVB level can be altered substantially, and therefore limit the interpretability of such results (48). Furthermore, we individually calculated cw-D-UVB for each participant, based on their date of blood draw and place of residence, taking into account the accumulation and diminution of vitamin D. This is important as our estimate was created to act in a similar manner to that of 25(OH)D in the body.

This study was the use of precollected data so some important vitamin D-related information such as dietary sources of vitamin D and detailed information on personal supplementation dose are missing. The inclusion of those who use personal supplements (of up to 800 IU) is also a limitation of this study as it could reduce the differential between the treated and placebo group and increase heterogeneity within the groups. However, because of the randomized design, it is expected that there is no difference in personal vitamin D use between two arms. While availability of ambient UVB is a prerequisite for dermal synthesis to occur, behavioral factors play a major role in the "utilization" of ambient UVB (most notably time spent outdoors and proportion of skin that is exposed to sunlight) but this information is missing. Another weakness of this study was that it was carried out in a group of Crohn's disease patients, which might not be representative of the general population limiting the generalizability of the findings. 25(OH)D concentrations we observed are comparable to what was previously reported for the general population in Ireland (49–52) although it is thought that the prevalence of deficiency might be higher in Crohn's disease, due to reduced dietary vitamin D, reduced absorption of vitamin D or sun avoidance (53). Additionally, this study was carried out at one location, and therefore, there was little geographical variability (73% of participants were from the same D-UVB grid); therefore, most of the variation in UVB levels was temporal, due to different times of blood draw. Although the intended interval between blood measurements in this group was 4 months, this was not always achieved; irrespectively, the cw-D-UVB was calculated based on the actual date of blood draw so the relationship between cw-D-UVB and 25(OH)D is unlikely to be biased by this. An additional limitation is that this study used ambient D-UVB doses as we were unable to calculate personal UVB doses as we did not have access to information on clothing or sun use levels; therefore, an overestimation of UVB dose may have occurred.

CONCLUSION

We found a strong relationship between ambient UVB at a place of residence and vitamin D status, even in those who are taking 2000 IU day^{-1} of vitamin D supplement. Hence, we conclude that sunshine-induced dermal synthesis is an important determinant of 25(OH)D concentration even among those who reside at a higher mid-latitude location.

To our knowledge, this is the first study of its kind that utilized a randomized controlled trial setting to address this question. A longitudinal design, coupled with a detailed estimate of ambient UVB dose at a place of residence and limited to wavelengths that can induce vitamin D synthesis, enabled us to account for a pronounced seasonality of vitamin D production in the skin, a common and major confounder in the field of vitamin D research.

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COMPETING INTERESTS

Authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Fiona O'Sullivan: analyzed data, performed statistical analysis and wrote paper; *Tara Raftery:* designed and conducted research; *Michiel van Weele*: designed and conducted research; *Jos van Geffen*: designed and conducted research; *Deirdre McNamara*: designed and conducted research; *Colm O'Morain*: designed and conducted research; *Nasir Mahmud*: designed and conducted research; *Dervla Kelly*: analyzed data and performed statistical analysis; *Martin Healy*: conducted research and analyzed samples; *Maria O'Sullivan*: designed and conducted research; *Lina Zgaga*: designed study, wrote paper and had primary responsibility for final content.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Figure S1. Patient flow chart for this study design.

Figure S2. Distribution of 25(OH)D and season at T2 in (A) all participants (B) Placebo patients, C) Vitamin D supplement patients

Table S1. Model selection for manuscript Table 2.

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