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Major inter-personal variation in the increase and maximal level of 25-hydroxy vitamin D induced by UVB†

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Vitamin D influences skeletal health as well as other aspects of human health. Even when the most obvious sources of variation such as solar UVB exposure, latitude, season, clothing habits, skin pigmentation and ethnicity are selected for, variation in the serum 25-hydroxy vitamin D (25(OH)D) response to UVB remains extensive and unexplained. Our study assessed the inter-personal variation in 25(OH)D response to UVR and the maximal obtainable 25(OH)D level in 22 healthy participants (220 samples) with similar skin pigmentation during winter with negligible ambient UVB. Participants received identical UVB doses on identical body areas until a maximal level of 25(OH)D was reached. Major inter-personal variation in both the maximal obtainable UVB-induced 25(OH)D level (range 85–216 nmol l^{-1}, mean 134 nmol l^{-1}) and the total increase in 25(OH)D (range 3–139 nmol l^{-1}, mean 48 nmol l^{-1}) was found. A linear model including measured 25(OH)D baselines as personal intercepts explained 54.9% of the variation. By further including personal slopes in the model, as much as 90.8% of the variation could be explained. The explained variation constituted by personal differences in slopes thus represented 35.9%. Age, vitamin D receptor gene polymorphisms, height and constitutive skin pigmentation (a skin area not exposed to UVB) explained 15.1% of this variation. Despite elimination of most known external sources of variation, our study demonstrated inter-personal variation corresponding to an observed maximal difference of 136 nmol l^{-1} in the total increase of 25(OH)D and 131 nmol l^{-1} in the maximal level of 25(OH)D.

Introduction

Vitamin D plays an important role for bone health[1] and possibly several extra-skeletal diseases.[2] Serum 25-hydroxy vitamin D (25(OH)D) is a commonly used marker of vitamin D status. Assessment of personal vitamin D status displays considerable variation in addition to variation attributable to the assessment method. Much of this variation is thought to arise from variations in different aspects of vitamin D metabolism such as synthesis, storage capacity and degradation.[3] Furthermore, a substantial part of the inter-personal variation in the UVB-induced 25(OH)D synthesis in skin may be ascribed to differences in sun altitude, season, weather conditions, sun habits, clothing habits and skin pigmentation.[4,5] In ultraviolet radiation (UVR) treatment studies, much of the variation in vitamin D deriving from these mainly external parameters can be eliminated when identical UVB doses are given to identical body areas on participants with similar ethnic origin, skin pigmentation and body mass index (BMI) over a period with negligible ambient UVB.[6] In studies with uniform UVB doses and exposed body areas, a consistent relation between UVB dose and increase in 25(OH)D is usually found. Still, these studies also indicate that variation in the UVB-induced vitamin D synthesis continues to be considerable although the magnitude and background for this variation have not as yet been investigated as a primary objective.[6–9]

If this variation arises from internal non-modifiable parameters, it is possible that the recommended optimal 25(OH)D level displays considerable variation as well. It is therefore important to determine whether vitamin D variation is (1) predominantly due to external, modifiable parameters or (2) mainly caused by personal biology or (3) by a mixture of both.

Inter-personal variation in two aspects of vitamin D metabolism was investigated: (1) the maximal UVB-inducible level

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and (2) the course of experimental UVB-induced increase. These aspects were studied in a homogeneous population consisting of Caucasian participants with similar light skin pigmentation and similar BMI. Study-start was set to 1st October as 25(OH)D baselines are relatively high at that time of year in order to eliminate the influence of baseline 25(OH)D and ambient UVB on the inter-personal variation in 25(OH)D synthesis. Despite the restrictive selection of participants, major inter-personal variations in the maximal level and the UVB inducible increase of 25(OH)D were found.

Results and discussion

To determine the variation in the maximal UVB-induced 25(OH)D level and the UVB induced 25(OH)D increase, 22 healthy Danish sun worshippers with similar light skin colour and similar BMI were exposed to an identical UVR dose treatment regimen (Table 1). They were irradiated on approximately 80% of the total body area for nine weeks post-summer in a period when ambient UVB is negligible (1st October to mid-December) with concurrent weekly measurements of serum 25(OH)D (Fig. 1). Participants were instructed not to use vitamin D supplements one month prior up to and during the study period and by completing a questionnaire to record the number of fatty fish-meals consumed daily. Vitamin D fortified food was not available in Denmark during the study period.

Variation in UVB-induced maximal serum 25(OH)D level

The mean 25(OH)D level after ended UVB treatment was 134 nmol l^{-1} (N = 22, range 85–216 nmol l^{-1}, SD 33.7 nmol l^{-1}, Fig. 2 and 3).

Table 1 UVB doses and 25(OH)D levels. Study Part 1 comprises the 25(OH)D responses to UVB treatment (N = 22). Study part 2 comprises the 25(OH)D responses to the sun holiday (N = 19 of the 22). Mean personal solar UVB doses were measured with personal, electronic UV dosimeters

Study part	Mean day	Weekly UVB dose (kJ m^{-2})	25(OH)D (nmol l^{-1} ± SD(range))
1.			
	0	—	85 ± 21 (46–120)
	7	1.9	90 ± 22 (49–143)
	13	1.9	97 ± 21 (59–143)[a,b]
	20	2.8	97 ± 25 (55–147)
	27	2.8	106 ± 26 (63–175)[b]
	34	2.8	112 ± 29 (66–179)[b]
	41	2.8	116 ± 27 (69–178)
	48	2.8	119 ± 27 (62–184)
	54	4.2	128 ± 32 (63–196)[b]
	61	4.2	134 ± 33 (85–216)[b]
2.			
	61		132 ± 28 (85–191)[b]
	72	66	129 ± 27 (69–176)

[a] Significant (P < 0.05) increase in 25(OH)D from this point and forward compared to day 0. [b] Significant (P < 0.05) increase in 25(OH)D compared to prior sample point.

To ensure that the maximal 25(OH)D levels were achieved after ended UVR treatment, 19 of the 22 participants were sent on a one-week sun holiday with good sunbathing opportunities. During the sun holiday personal time-stamped solar UVB doses (kJ m^{-2}) were measured as each participant wore a personal electronic UV dosimeter (Table 1). The maximal exposed body areas during UVB treatment (80%) and sun holiday (72%) were similar. All participants received a total UVB dose of 4.2 kJ m^{-2} during the last week of UVB treatment. In comparison, the 19 participants received a mean solar UVB dose to the skin of 66 kJ m^{-2} and a mean dose estimated at 22 kJ m^{-2} after correction for measured sunscreen use during the subsequent one week of sun holiday.[10] Despite this, the mean 25(OH)D level of 132 nmol l^{-1} (range 85–191 nmol l^{-1}) in the 19 participants after UVB treatment had not changed significantly at the conclusion of the sun holiday (mean 25(OH)D level: 129 nmol l^{-1}, range 69–176, P = 0.317). Thus, the maximal observed 25(OH)D level on a group basis had been reached after ended UVB treatment. The range in the 22 participants resulted in an observed maximal difference of 131 nmol l^{-1} (range 85–216 nmol l^{-1}) in the maximal UVB-induced 25(OH)D level.

UVB-induced 25(OH)D synthesis is higher at low 25(OH)D levels and gradually decreases with increasing 25(OH)D levels.[6] Hence, it is commonly believed that the 25(OH)D response to UVB is non-linear and with an upper maximal UV-inducible level of 25(OH)D that prevents vitamin D intoxication.[11–13] The fact that the mean 25(OH)D after UVB treatment did not increase further after a mean solar exposure of 22 kJ m^{-2} on around 72% of the body area strongly supports this notion. A prolonged UVB treatment period with further increasing doses would have been preferable to ensure with greater certainty that stable maximal 25(OH)D levels had been reached in all participants. However, this would have led to a high risk of skin burn and reduced compliance. The coming Christmas holidays would probably also have led to poorer compliance. A possible effect from solar UVA on 25(OH)D synthesis during the sun holiday could not be assessed as there was a strong linear relation between UVA and UVB (R^2 = 0.997, $P = 1.1 \times 10^{-22}$).

Parameters influencing the variation of maximal serum 25(OH)D

Twelve accessible parameters (Table 2), some of which were kept within a narrow range, were investigated for their influence on the maximal 25(OH)D level using general linear models (GLM). Baseline facultative PPF (range 5.2–9.1) decreased significantly (P = 0.005, range 5.2–8.3) after UVB treatment, confirming that the UVB doses had been kept at a non-erythematous level (Table S1, Fig. S3†). Both facultative (mean PPF of five measurement sites on upper body) and constitutive PPF (buttock) after UVB treatment increased significantly (P < 0.005) after the sun holiday. The skin area receiving the least UVB exposure is usually the buttocks. This site is therefore used to measure constitutive PPF. However, the area is nevertheless exposed to very slight UVB radiation that pene-

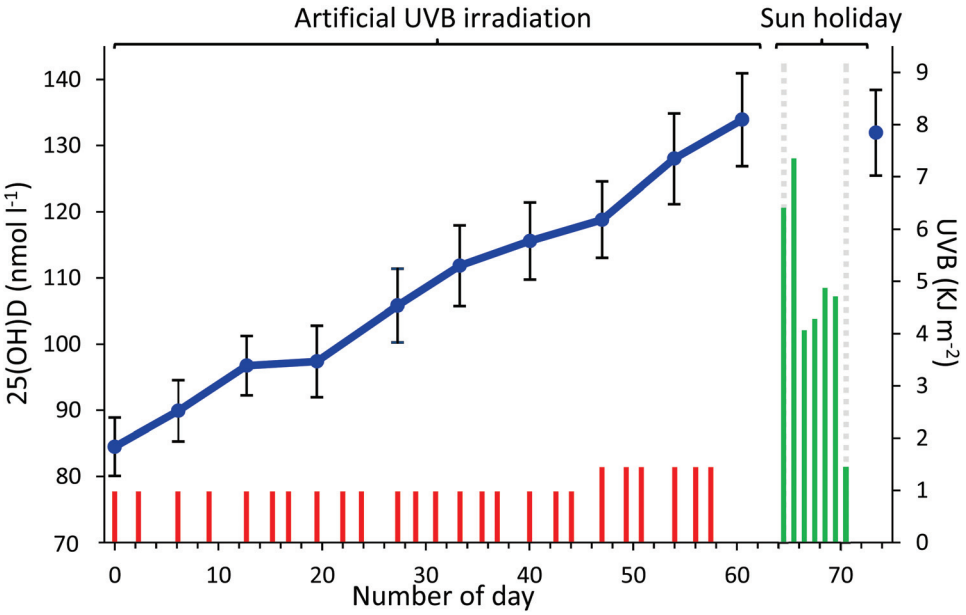

Fig. 1 Mean 25(OH)D levels, UVB treatment and solar UVB doses during study period. The dark blue curve shows mean 25(OH)D with 1 standard error of the mean bars (left *y*-axis) over time in days (*x*-axis) for the 22 participants. Artificial UVB doses given (right *y*-axis) over time are shown with red bars. Mean daily solar UVB doses for the 19 persons participating in one week of sun holiday (N = 19) are indicated with green bars. Each bar represents one day with UVB exposure. Solar doses are not corrected for the use of sunscreen and UVB-exposed body areas. For these 19 participants, the mean 25(OH)D level (132 nmol l^{-1}) before the sun holiday did not change significantly (paired t-test, two-tailed, P = 0.317) after the sun holiday (129 nmol l^{-1}).

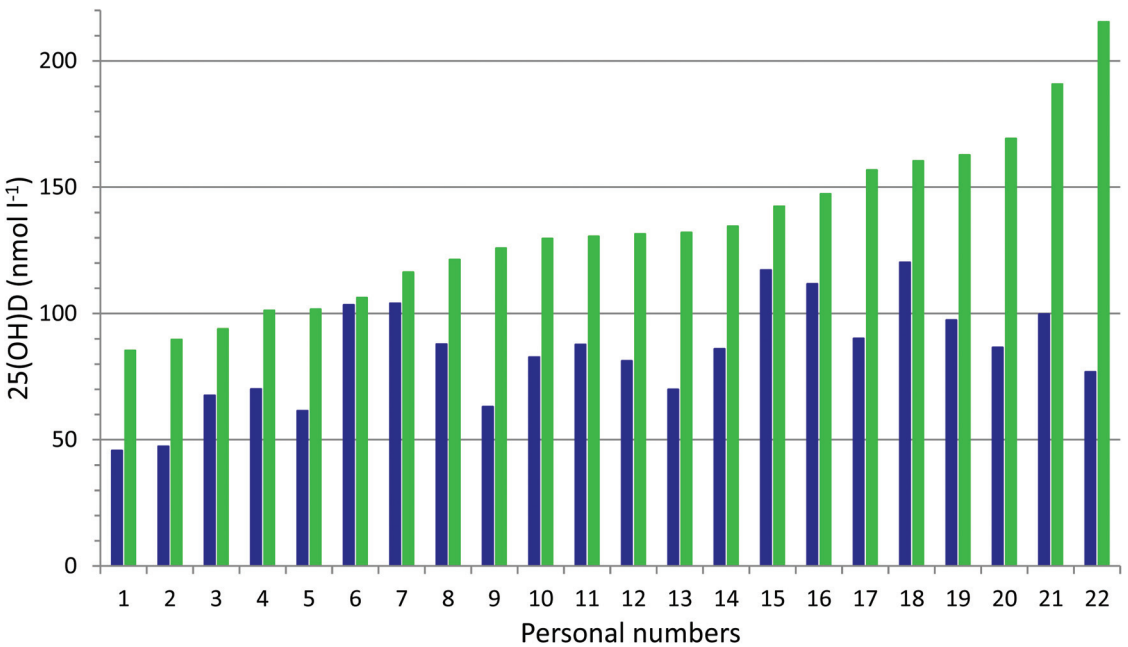

Fig. 2 Personal variation in maximal 25(OH)D compared with baseline 25(OH)D. Data from each of the 22 persons (*x*-axis) with corresponding 25(OH)D levels (*y*-axis) are shown. The blue bars indicate the 25(OH)D baseline levels. The green bars indicate maximal UVB-induced 25(OH)D level after identical UVB exposure for nine weeks. The relative total increase of 25(OH)D is seen as the difference between maximal and baseline 25(OH)D levels.

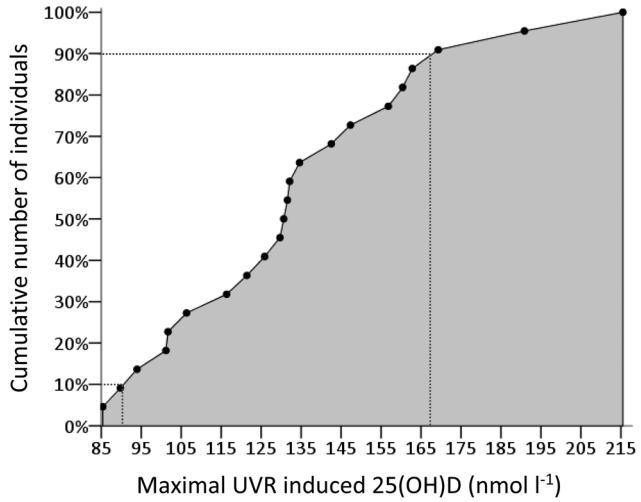

Fig. 3 The cumulative distribution of maximal 25(OH)D level in 22 participants. The maximal 25(OH)D level (nmol l^{-1}) is shown on the *x*-axis and the cumulative number of participants (%) is shown on the *y*-axis. Boundaries indicating 10% and 90% of the total number of participants are marked with dotted lines.

Table 2 Demographic data. Skin pigmentation protection factor (PPF) is an objective measurement of skin pigmentation with a measurement range of 1–25. Measuring sites were buttock (constitutive) and a mean of chest, midriff, back of shoulder, medial and lateral sides of arm (facultative)

Participants – no.	22
Gender – no. female/male	11/11
Age – years[a]	45 ± 9 (22–62)
Weight – kg[a]	77 ± 10 (60–100)
Height – cm[a]	174 ± 9.0 (160–190)
Body mass index – kg/m^{-2} [a]	25.2 ± 2.4 (21.0–29.7)
Fatty fish meals per week[a,b]	2.7 ± 1.4 (0–5.2)
Vitamin D receptor markers	
rs1544410 genotypes – no. AA/GG/AG	2/7/13
rs2228570 genotypes – no. TT/CC/TC	3/11/8
Fitzpatrick skin type – no. I/II/III/IV	0/6/11/5
Constitutive PPF[a]	3.7 ± 0.9 (2.0–6.0)
Facultative PPF[a]	7.2 ± 1.1 (5.2–9.1)
25(OH)D baseline level – nmol l^{-1} [a]	85 ± 21 (46–120)

[a] Mean ± 1 SD (range). [b] Maximal possible number of fish meals per week was 14.

trates swimwear during sunbathing and explains the small increase in constitutive PPF.[14]

Parathyroid hormone (PTH) (mean range 3.2–4.0 pmol l^{-1} at all sample time points) did not change significantly over the study period (Fig. S2,† linear regression analysis, $P = 0.323$) and was therefore not investigated as a parameter with possible influence on 25(OH)D. As 25(OH)D baselines were relatively high this was to be expected.

The highly variable maximal 25(OH)D (range 85–216 nmol l^{-1}) was found to be influenced only by baseline 25(OH)D ($P = 0.018$, $R^2 = 0.249$). Baseline 25(OH)D may partly reflect differences in prior summer solar UVB exposure, which was not assessed. Baseline 25(OH)D may also reflect inter-personal differences in 25(OH)D reactivity to previous sun exposure, *i.e.* an influence from both internal and external parameters.

Variation of the linear UVB-induced 25(OH)D increase

The UVB treatment-induced 25(OH)D increase over time was best described by a linear model. However, this response was from relatively high 25(OH)D baselines and to a nonlinear increasing UVB treatment dose regimen indicating a nonlinear dose–response relationship in accordance with previous findings.[12,13]

The average increase in 25(OH)D after nine weeks of UVB treatment was 48 nmol l^{-1} (SD 29 nmol l^{-1}) exhibiting substantial inter-personal variation (range 3–139 nmol l^{-1}). The observed maximal difference in the increase of 25(OH)D was thus 136 nmol l^{-1}. This is exemplified by the comparison of participant no. 6 with no. 21 in Fig. 2. Despite similar 25(OH)D baselines for participant no. 6 (103.4 nmol l^{-1}) and no. 21 (99.7 nmol l^{-1}) the difference in total 25(OH)D increase was 85 nmol l^{-1}. The mean increase in 25(OH)D was 0.79 nmol l^{-1} per day (slope) corresponding to a mean weekly increase of 5.5 nmol l^{-1} (range 0.32–15.4). Thus the slope of increase displayed major variation as well.

The proportion of explainable variation in slope during the course of increase was explored by comparing different GLMs. A GLM with common slope and common baseline (intercept) described a modest part of the observed variation corresponding to 25.8% ($R^2 = 0.258$). When measured personal baselines were included as personal intercepts in the GLM, the proportion of explainable variation increased from 29.1% to 54.9% ($R^2 = 0.549$, Table 3). Still, a considerable part of the variation of about 45% (100–54.9) remained unexplained by this model. To assess the proportion of the maximal explainable observed variation, personal intercepts as well as personal slopes were included in the model. This model indicated that as much as 90.8% ($R^2 = 0.908$) of the variation was in principle explainable, if all the relevant parameters were known and investigated. It also indicated that around 36% (90.8–54.9) of the variation could be accounted for by the influence of internal and some external personal parameters on the slope of the increase.

Parameters with separate influence on the slope of the 25(OH)D increase

The separate influence of 12 available parameters (Table 2) on the slope of increase with GLM was investigated. The GLM included personally measured baselines as intercepts. Eight parameters had separate significant effects (Tables 2 and 3) on the inter-personal variation of the slope: age, a genetic vitamin D receptor (VDR) marker (rs1544410), height, BMI, constitutive and facultative PPF, gender and intake of fatty fish. 25(OH)D baseline level did not influence the slope significantly ($P = 0.67$, Table 3). This was expected as baselines were relatively high and only lower 25(OH)D baseline levels have been shown to impact UVB-induced increase.[6]

Table 3 Personal parameter influence on the variation of the slope in the UVB treatment-induced increase of 25(OH)D over time. A total of 54.9% of the variation in the increase was explained by a linear model comprising personal measured baselines as intercepts and common slope. Additional explanation of the variation was obtained by investigating the influence of different parameters on the variation of the slope. Constitutive skin pigmentation protection factor (PPF) was measured on buttock and facultative PPF was defined as a mean of measurements on chest, midriff, back of shoulder, medial and lateral sides of arm. R^2 is squared correlation coefficient

Parameters	R^2	P value	Power[b]
Model with common slope and personal intercepts	0.549	2.99×10^{-3}	1.000
25(OH)D baseline level	—	0.67	—
Gender[a]	0.567	3.1×10^{-3}	0.846
Age[a]	0.596	1.1×10^{-6}	0.999
Weight	—	0.29	—
Height[a]	0.589	7.2×10^{-6}	0.996
Body mass index	0.569	1.7×10^{-3}	0.883
Fatty fish intake[a]	0.527	4.2×10^{-4}	0.946
rs1544410 (AA/GG/AG)[a]	0.636	1.1×10^{-10}	1.000
rs2228570 (TT/CC/TC)	—	0.13	—
Constitutive PPF[a]	0.566	3.7×10^{-3}	0.832
Facultative PPF at baseline[a]	0.559	3.2×10^{-2}	0.573
Fitzpatrick skin type (II, III, IV)	—	0.38	—

[a] Single significant ($P < 0.05$) parameters that remained significant in a subsequent combined stepwise backward elimination of a general linear model (results not shown here). [b] Power is the probability of confirming the given result in a new material with similar size and uncertainties as in this material.

Backward elimination of separate influential parameters in a combined GLM

Parameters with separate influence on the slope were deployed in a combined GLM and eliminated according to P values in a stepwise backward analysis. Age, rs1544410, height, constitutive PPF, gender and intake of fatty fish combined influenced the slope of the increase significantly ($R^2 = 0.733$, $P = 2.6 \times 10^{-55}$). As power is an estimate of the probability of confirming the given result in a similar new material, a power of at least 0.750 was retained. Consequently, intake of fatty fish and gender were eliminated from the combined GLM as well (data not shown).

The genetic VDR marker, rs1544410, did not exhibit an allele dosage effect possibly due to there being only two participants with the AA genotype (Table 2). Therefore, in the final step of the backward GLM analysis participants with AA and AG genotypes were combined and compared with the GG genotype group. A GLM comprising the four parameters (age, height, constitutive PPF and rs1544410) influencing the slope of the 25(OH)D increase explained 70.0% of the observed variation ($R^2 = 0.700$, $P = 5.4 \times 10^{-53}$). This final GLM of 25(OH)D increase over time (t) could be expressed as:

$$\begin{aligned} 25(\mathrm{OH})D(t) = {} & 1.6 + 0.98 \times 25(\mathrm{OH})D(t_0) + t(\mathrm{days}) \\ & \times [-0.031 \times \mathrm{age(years)} + 0.027 \times \mathrm{height(cm)} \\ & - 0.11 \times \mathrm{constitutive\ PPF} \\ & + 0.26\ (\mathrm{if\ rs1544410\ genotype\ is\ GG})] \end{aligned}$$

If a participant has the rs1544410 genotype of AG or AA instead of GG, the coefficient of 0.26 is replaced by zero. 25(OH)D(t0) is the measured personal baseline level.

From this model the independent influence of these four parameters on the slope can be calculated (Table 4). Apart from the impact of obvious external influential parameters on variation, these results suggest an important contribution from internal non-modifiable parameters as well. An influence of age on 25(OH)D synthesis has previously been identified and had the largest impact on 25(OH)D within our age span of 22–62. The amount of the precursor 7-dehydrocholesterol in the skin cells has been found to decrease with age, thereby reducing the capacity to synthesise 25(OH)D.[15,16] The activated VDR is a transcription factor active in regulating several steps in vitamin D metabolism and thus constitutes an obvious influential candidate gene in synthesis and regulation.[17,18] Inconsistent reports of VDRs' influence on vitamin D may be due to the confounding effect of external parameters and use of different populations, which was selected for in the present study.[19–21] BMI did not ultimately exert an influence on the 25(OH)D increase most likely due to a relatively narrow range (21.0–29.7 kg m^{-2}). There was a positive effect of height on the slope of the 25(OH)D increase. This presumably represents a relative increase in UVB-exposed body area as there was a relation between height and body area ($R^2 = 0.711$, $P < 0.001$) and not between height and BMI ($P = 0.12$). Despite conflicting results, skin pigmentation is often presumed to reduce UVB-induced 25(OH)D synthesis[6,8,9,22–25] as melanin absorbs UVB.[26] A narrow PPF span was therefore intentionally selected to minimise the influence of skin pigmentation as our study was not designed to elucidate the influence of this factor. Consistent with this, neither facultative PPF representing the

Table 4 Model estimated parameter influence for the four parameters identified as being of importance in this study. Estimates, with influence on the variation of the slope in the UVB treatment-induced linear increase of 25(OH)D, are given within full parameter range and without accounting for interactions ($N = 22$). Pigmentation protection factor (PPF) is an objective measurement of skin pigmentation (range of 1–25). Rs1544410 is a genetic vitamin D receptor gene marker

Common course/parameter	P value	Power	Range/category	Per day (nmol l^{-1})	Per week (nmol l^{-1})
Mean change			—	0.79	5.5
Age	1.1×10^{-13}	1.000	22–62 years	−1.24	−7.68
Rs1544410	7.2×10^{-4}	0.927	GG *versus* AG + AA	0.26	1.8
Height	7.2×10^{-8}	1.000	160–190 cm	0.81	5.7
Constitutive PPF (buttock)	6.7×10^{-3}	0.779	2.0–6.0	−0.45	−3.2

pigmentation on the UVB-exposed body area, nor Fitzpatrick skin type classification had any final significant effect on the slope of the 25(OH)D increase. Unexpectedly, constitutive PPF, representing a body area not exposed to UVB, had a significant negative effect on the slope of the 25(OH)D increase. However, the observed influence of constitutive PPF may reflect other underlying parameters, possibly genetic. Still, in the final combined GLM constitutive PPF had a somewhat lower power of 0.780 compared to the other parameters, age, rs1544410 and height, which each had a power of at least 0.920 in the final GLM (Table 4). So the influence of constitutive PPF has not been conclusively determined in this study.

General discussion

We demonstrated a major inter-personal variation in the 25(OH)D response to UVB as well as maximal UVB-induced 25(OH)D level mostly explained by internal non-modifiable parameters when the influence of most external modifiable parameters were eliminated. This should perhaps have been expected, as variation arising from different aspects of vitamin D metabolism, such as personal capacity for skin synthesis, uptake of dietary supplement, storage, supplementary daily consumption, degradation and excretion, contributes the overall variation of vitamin D status. The 25(OH)D level, at which bone health is optimally preserved indicated by maximal suppressed PTH, has been shown to display wide variation as well.[27] Despite the negative relation between 25(OH)D and PTH, elevated PTH may only be present in around 30–40% of patients with insufficient 25(OH)D.[28]

Our findings suggest that each individual may have an inborn profile composed by internal and non-modifiable parameters determining the personal 25(OH)D responsiveness to UVB and the maximal obtainable 25(OH)D level. In addition to this, a study by Aloia *et al.*, found that a group of African Americans had lower total 25(OH)D, higher serum PTH but higher bone density compared to a white control group, thus demonstrating a possible race based variation.[29] Is it therefore possible that the adequate 25(OH)D level presently defined as a single cut-off value may display major inter-personal variation as well? From a physiological perspective it could be unreasonable to require an optimal level of 50 nmol l^{-1} throughout the year[1] from a person with a maximal 25(OH)D level of 85 nmol l^{-1}, only obtainable after 9 weeks of intensive full body UVB irradiation. Conversely, it could be insufficient to require an optimal level of 50 nmol l^{-1} when the highest obtainable level is 216 nmol l^{-1}. Hence, our results challenges the wide use of recommended single cut-off values or fixed reference levels of 25(OH)D as a measure of adequate vitamin D status. Other aspects of the suitability of using 25(OH)D as a biomarker of vitamin D status are currently being debated as free 25(OH)D or bioavailable (vitamin D binding protein and albumin bound) 25(OH)D may provide better correlates to bone mineral density.[29,30]

In the present study, the influence of most external and behavioural confounding parameters on vitamin D variation was effectively eliminated. As participants with a narrow span of skin pigmentation were selected, the UVB dose penetration into the skin could be kept nearly identical for all persons. All these selected conditions allowed an assessment of the contribution of some internal and non-modifiable parameters to the variation of two aspects of vitamin D metabolism. The influence of VDBP gene polymorphism on inter-personal variation was not investigated. However, as our participant group consisted of healthy Caucasians with pale skin, the influence of VDBP gene polymorphism was slight.[31,32] Our study was limited by the relatively small number of participants. This applies especially to the investigation of personal parameters influencing the maximal 25(OH)D level, for which only one sample per participant was available. Consequently, only one influential parameter was identified. In the evaluation of the course of 25(OH)D increase sufficient sampling over a relatively long investigation period was accessible to show personal responses, and many more influential parameters were identified.

On the basis of our findings concerning major inter-personal variation in 25(OH)D metabolism, we hope to contribute to the overall discussion of the suitability of using 25(OH)D as a biomarker of vitamin D status and bone health.

Experimental

Study design

This single-centre, open and non-blinded clinical trial was conducted at Bispebjerg University Hospital, Denmark (56° N) from 1st October to December in 2010, a period during which ambient UVB is insignificant.[33] The participants were irradiated repeatedly with an identical UVB dose regimen on approximately 80% of the total body area for nine weeks with concurrent weekly measurements of 25(OH)D (220 samples). This was followed by one week of sun holiday (Hurghada, Egypt, latitude: 27° N), with sunbathing to ensure that maximal 25(OH)D had been reached.

Participants

22 healthy Caucasians sun worshippers originating from northern countries (Denmark, the Faroe Islands and England) with similar light skin pigmentation and similar BMI participated in the study. The inclusion criteria were: (1) age 18–70; and (2) parents of Caucasian origin. Exclusion criteria were (1) supplementary vitamin D intake exceeding 10 μg per day one month prior to study start; (2) use of supplementary vitamin D during study period; (3) sun holiday south of latitude 45° N one month prior to or during the study period (except the sun holiday that was a part of the study); (4) use of solarium one month prior to or during the study period; (5) chronic disease; (6) skin disease; (7) intake of cholesterol-lowering or photosensitising medication; (8) pregnancy; (9) drug addiction; (10) psychiatric disorder; (11) physical disabilities. Three participants did not participate in the sun holiday for personal reasons. Demographic data are shown in Table 2. Written, informed

consent was obtained from all participants. Study protocols (H-2-2010-097, H-C-2008-072 and H-B-2007-100) were approved by the Danish Medical Ethics Committee and completed in accordance with the Declaration of Helsinki.

UVR exposure

UVB treatment. UVR cabinets (Waldmann, Willingen-Schwenningen, Germany) with 26 F85/100W UV6 tubes (290–350 nm, broadband) covering the vitamin D action spectrum were used to irradiate approximately 80% of the participants' total body area[34] with an identical and gradually increasing UVR dose treatment regimen (Table 1) for nine weeks from October to December. Ambient UVB radiation and solar-exposed body areas during this period are negligible in regard to vitamin D synthesis.[33] UVB doses were given as physical doses in kilojoules (kJ) per m^2. UVB treatments, each of 0.94 kJ m^{-2}, were given bi-weekly in the first two weeks. The frequency was then increased to three sessions per week, each of 0.94 kJ m^{-2}, for a further five weeks. In the last two weeks, 3 weekly sessions, each of 1.4 kJ m^{-2}, were given. This corresponded to a total UVB dose of 26 kJ m^{-2} per participant. The UVA and UVB fractions were 63% and 37%, respectively, corresponding to a UVA/UVB ratio of 0.587. Irradiation time was determined and regulated by measuring UV intensity with a Sola-Hazard spectroradiometer (Solatell, Cornwall, UK) at baseline, after five weeks and at the end of the exposure period.

Solar UVR exposure. The UVB treatment dose was limited to avoid erythema. To examine if the maximal 25(OH)D level had been reached the participants were subsequently sent on a one-week sun holiday in Hurghada, Egypt (latitude: 27° N). Previous studies have shown that during sun holidays participants expose their skin on their own initiative to solar UVB to a higher degree than acceptable under laboratory conditions.[35] The purpose of the holiday was to make sure that maximal 25(OH)D levels had been reached by UVB exposure. During the sun holiday personal time-stamped doses of UVB and UVA (kJ m^{-2}) were measured by personal electronic UV dosimeters (SunSavers)[36] and solar exposed body areas registered. There was a close linear relation between the UVB and UVA doses received (R^2 = 0.997, $P = 1.1 \times 10^{-22}$), which meant that the possible influence of UVA on 25(OH)D increase could not be investigated. It has previously been demonstrated that the mean wrist dose is 50% of the dose received on the top of the head.[37] As solar exposure is usually on one side of the body at a time, the wrist dose provides a suitable estimate of the UVB dose received. The SunSavers were an updated versions of the personal electronic UVR dosimeter described elsewhere.[36] Participants used a mean sunscreen factor of SPF15 with an average application thickness of 0.79 mg cm^{-2}. This corresponded to an average effective protection factor of approximately three.[10]

Skin pigmentation

Self-reported skin photo-type according to Fitzpatrick's criteria[38] was assessed along with objective constitutive (buttocks) and facultative (mean of chest, midriff, back of shoulder, medial and lateral sides of arm) pigment protection factor (PPF, measuring range 1–25) measured with a skin reflectance meter (UV-Optimize Scientific, Chromo-light, Espergaerde, Denmark)[39] at baseline and after $4\frac{1}{2}$, 9, 10 and 18 weeks. Constitutive PPF is a measure of the innate skin pigmentation on a body area not exposed to UVB. Facultative PPF represents body areas influenced by prior history of solar exposure and in this study the UVB-exposed areas.

Blood analysis

Serum 25(OH)D. Vitamin D_2 is scarce in common diet, the main source in Europe being mushrooms. Therefore, serum 25(OH)D_3 (25OH)D was used as a parameter of vitamin D_3 status and analysed on a liquid chromatography tandem mass spectrometer (LC-MS/MS, Agilent 1100 HPLC & Micromass Quattro Ultima mass spectrometer).[6,40] Samples were pretreated with acetonitrile containing the internal standard hexadeuterium labeled 25-OH D_3 (Synthetica, Oslo, Norway) to release vitamin D binding protein bound 25(OH)D. A minimum of three analyses were performed to minimise analysis variance (technical replicates). All 25(OH)D samples from the same participant were analysed in one batch. Quantification was performed using calibrators from Chromsystems (Gräfelfing, Germany) and controls from Recipe (Munich, Germany) and a low QC blood sample from an employee in the department.

The total relative standard deviation (SD) varied between 4.9% and 14.1% at 20–222 nmol l^{-1} reflecting experimental variability. Serum 25(OH)D was measured at baseline (*i.e.* study start) and from after that weekly at least two days after the last UVB treatment as 25(OH)D production is sustained for around two to three days after UVB exposure.[41] 25(OH)D was also measured after the conclusion of the sun holiday in the 19 participants in the holiday.

Serum parathyroid hormone (PTH). Serum PTH was analysed using an Immulite 2500 Biochemistry Analyzer (Diagnostic Products Corporation, Los Angeles, CA). This measurement was based on a chemiluminescence-immunometric assay with a detection limit of 0.3 pmol l^{-1}. Intra-series variance was 15% at 2.4 pmol l^{-1}, 10% at 6.3 pmol l^{-1} and 12% at 22.8 pmol l^{-1}. PTH status was evaluated at baseline and, approximately, on days 13, 27, 41, 61, 72, 95 and 123.

Vitamin D receptor (VDR) gene SNP. The influence of the vitamin D receptor gene was investigated by genotyping the two single nucleotide polymorphisms (SNP), rs1544410 (BsmI) and rs2228570 (FokI), located in the gene (ENSG00000111424, Chromosome 12q13). These two SNPs were selected due to their association with bone diseases as well as many other diseases thereby indicating that polymorphisms in those regions of VDR are important for the functioning of VDR. DNA purification and method for SNP typing are described in ESI.†

Statistics

Personal data were tested with the Kolmogorov-Smirnov test to assess whether the data were normally distributed. Normally distributed data were tested by paired *t*-test (two-tailed) to

determine if maximal steady state level of 25(OH)D had been reached after ended UVB treatment. The influence of 12 parameters (Table 3) on the variation of maximal level of 25(OH)D was then examined.

To describe the increase in 25(OH)D over time in days (220 samples), the following models were investigated: linear, inverse, quadratic, cubic, power, sigmoid and exponential. The derivate function was defined as the average daily change in 25(OH)D between two sample time points (Δ25(OH)D per day) and prior successive 25(OH)D level. Determination of the best suitable model was based on the accordance between the investigations of the 25(OH)D increase over time and the derivate function.

The inter-personal variations in the linear increase of 25(OH)D were explored by determining the variation of the slope. This was performed by comparing general linear models (GLM) with: (1) common slope and common baseline values as intercepts, (2) common slopes and personally measured baseline values as intercepts and (3) personal slopes, *i.e.* personal constant and personally measured baseline values as intercepts.

The influence of parameters with independent significant influence on the slope variation in the increase of 25(OH)D over time was not independent and was therefore investigated *via* a stepwise backward elimination of a combined GLM.[42] Only parameters with a power of at least 0.750 were retained in the final GLM explaining the variation of the increase.

Comparison of 25(OH)D levels at different time points was performed using paired *t*-test (two-tailed) and the relation between solar UVA and UVB by GLM.

P-value < 0.05 was considered significant. Data were statistically analysed using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA).

Sample size calculation. SD was 14.1 nmol l^{-1} for high-end 25(OH)D samples.[40] The expected detectable difference was 15 nmol l^{-1} based on a previous study of two weeks.[6] The sample size was estimated to be 16 participants, given a significance level of 5% and 80% power to detect a difference of 15 nmol l^{-1}. Due to the relatively long study period, 22 participants were included. This allowed for a drop out of six participants corresponding to 27%. However, there was no drop out.

Conclusions

The inter-personal variation primarily due to internal non-modifiable parameters was investigated in two different aspects of vitamin D metabolism: the maximal 25(OH)D level induced by UVB and the 25(OH)D response to experimentally given UVB.

Despite the restrictive selection of participants, substantial inter-personal differences were present in both the maximal 25(OH)D level and the 25(OH)D response to UVB. Age, vitamin D receptor gene polymorphisms in the SNPs rs1544410, height and constitutive skin pigmentation explained 15% of the variation in the slope. The remaining unexplained 21% of the variation due to the slope is most likely constituted by other internal and non-modifiable parameters such as genetic factors.

It is our view that the observed inter-personal variation should be taken into account in the use 25(OH)D as a biomarker of vitamin D status and bone health.

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