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Serum 25(OH)D levels after oral vitamin D_3 supplementation and UVB exposure correlate

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Summary Statement

Vitamin D_3 supplementation is the first-choice in the treatment of vitamin D deficiency. This study shows that there is a major and similar inter-individual variation in the 25(OH)D_3 response to vitamin D_3 supplementation as to UVB treatment. Correlated 25(OH)D start-, end-levels and total increase for the two interventions suggested an individual background. A common genetic background for variation in the two types of treatments was found. Accordingly, persons characterized by certain individual genetic traits may not respond sufficiently after receiving a recommended dose of vitamin D_3 supplementation.

Summary

Background: The inter-individual variation in 25(OH)D_3 increase (Δ25(OH)D_3) after vitamin D_3 supplementation was determined and compared the UVB irradiation.

Methods: Nineteen Danish participants received 85 µg vitamin D_3 (cholecalciferol) daily for nine weeks with regular serum 25(OH)D_3 measurements. These participants had three years earlier taken part in a 9-week controlled UVB study. The Δ25(OH)D_3 was not confounded by ambient UVB, BMI or ethnicity.

Results: Δ25(OH)D_3 was 53 nmol l^{-1} and almost identical to Δ25(OH)D_3 (52 nmol l^{-1}) after UVB. Δ25(OH)D_3 ranged from 17 to 91 nmol l^{-1} (span 74 nmol l^{-1}) and was about half of that observed after UVB irradiation (span 136 nmol l^{-1}). The interquartile ranges for vitamin D_3 supplementation (38.8-71.4 nmol l^{-1}, span: 32.6 nmol l^{-1}) and UVB irradiation (35.7-65.4 nmol l^{-1}, span: 29.7 nmol l^{-1}) were similar indicating a comparable response of the two interventions. As the 25(OH)D_3 start levels ($R^2 = 0.398$, $P = 3.8 \times 10^{-3}$), 25(OH)D_3 end levels

(R^2 = 0.457, P = 1.5 × 10^{-3}) and Δ25(OH)D_3 (R^2 = 0.253, P = 0.028) between both interventions were correlated, this suggested a possible common individual background for the variation. Four pigment SNPs influenced the variation in the vitamin D_3-induced and UVB-induced Δ25(OH)D_3. A combined model including the influence of these four SNPs and the 25(OH)D_3 start level explained 86.8% (P = 1.6×10^{-35}) of the individual variation after vitamin D_3 supplementation.

Conclusion: The inter-individual variation in the two interventions was comparable and had no common demographic but a partly common genetic background.

1 | INTRODUCTION

A serum 25-hydroxyvitamin D (25(OH)D) level of at least 50 nmol l^{-1} in healthy individuals is recommended primarily to prevent osteoporosis/loss of bone density and risk of fractures at older age.[1] The natural dietary supply of vitamin D is usually limited (less than 3 μg/day) [2] and a rise in UVR exposure may involve an increased risk of skin cancer. Consequently, vitamin D supplementation is the first-choice and a cost-effective treatment strategy to replenish vitamin D stores. We have previously found major inter-individual variation in the 25(OH)D response to long-term UVB irradiation suggesting that healthy persons require individualized UVB doses to obtain the same 25(OH)D level.[3] Whether the same inter-individual variation applies to the treatment with vitamin D supplementation is largely unknown. Out of the numerous randomized clinical trials conducted on the effect of oral vitamin D supplementation on serum 25(OH)D, only a few trials present data that allow a rough assessment of the variation of the individual 25(OH)D response to vitamin D_3 supplementation.[4-8] These trials suggest that the inter-individual variation is wide [9-14] and

may be confounded by additional ambient solar exposure (season, sun habits and clothing habits)[15,16], ethnicity[17], BMI[18] genetics[16], weight and physical activity[19]. Accordingly, subgroups of individuals characterized by certain individual traits may exhibit relatively low serum 25(OH)D responses to a recommended dose of vitamin D_3 supplementation. These subgroups may fail to maintain an optimal concentration of serum 25(OH)D on a yearly basis, which could increase the risk of osteoporosis and fragility fractures at older age. It is therefore important to increase our understanding of this fundamental aspect of treatment with vitamin D supplementation.

The purpose of this study was to assess the extent of the inter-individual variation in the 25(OH)D response to long-term vitamin D_3 supplementation. This investigation was performed in the same group of healthy individuals, as those taking part in a previous UVB study allowing a direct comparison between the two interventions. The investigated 25(OH)D_3 response was not confounded by ambient UVB, latitude, ethnicity and BMI. Therefore, a possible common demographic and genetic background for the inter-individual variation in 25(OH)D_3 response to the two interventions could be investigated.

2 | MATERIALS AND METHODS

2.1 | Study design

This was a single-centre, open and non-blinded clinical trial conducted at Bispebjerg Hospital, University of Copenhagen, Denmark (56°N) from December 2013 to March 2014. In Denmark, ambient UVB radiation[20,21] and solar-exposed body areas are negligible during winter season, and there is a significant decrease in 25(OH)D at this time of year.[22,23] Hence, a placebo group was not included. Written, informed consent was obtained from all

participants. Study protocols (H-4-2013-175 and H-2-2010-097) were approved by the Committees for Biomedical Research Ethics for the Capital Region in Denmark and completed in accordance with the Declaration of Helsinki.

2.2 | Participants

All participants were of Danish origin. Only participants that had previously participated in a UVB study for nine weeks could be included.[3] The exclusion criteria were (1) supplementary vitamin D intake exceeding 10 μg per day one month prior to study start; (2) use of other supplementary vitamin D than given during the study period; (3) sun holiday south of latitude 45°N less than one month prior to or during the study period; (4) use of solarium less than one month prior to or during the study period; (5) chronic disease; (6) skin disease; (7) intake of cholesterol-lowering medication; (8) pregnancy; (9) drug addiction; (10) psychiatric disorder; (11) physical disabilities; (12) serious adverse events such as hypercalcaemia and nephrolithiasis. Nineteen out of the 22 participants in the previous UVB study were included in this study. There were no drop-outs. Food fortified with vitamin D is not available in Denmark. The number of daily consumed fatty fish meals was registered via a questionnaire.

2.3 | Intervention and ambient UVB

Participants received a bottle containing sufficient vitamin D_3 tablets (Cholecalciferol, Apovit®, Takeda Pharma A/S, Roskilde, Denmark) to cover the entire study period. They were instructed to consume one vitamin D_3 tablet per day corresponding to 85 μg (3400 IE). Tablets were counted at each visit with 25(OH)D_3 sampling to control for compliance and adverse events registered. Visits for 25(OH)D_3 sampling were at study start, weekly the first three weeks, hereafter every second week. In total, there were seven 25(OH)D_3 sample time

points corresponding to 133 samples in all. If the intake of vitamin D_3 tablets deviated from the study plan, participants were instructed to regulate this by taking one extra or one less tablet the following days and before the next sample time. The intake of vitamin D_3 tablets was allowed to deviate with a maximum of two tablets in the periods between two blood samples and a maximum of two consecutive study periods without exclusion of participants.

During the study period, the mean daily ambient UV dose was 0.78 standard erythema dose (SED) and represented a mean of 0.12 minimal erythema dose (MED). Mean outdoor temperature was 3.0° C. At such circumstances, sun exposed body areas are usually mostly limited to face and sometimes hands. Mean hours of sunshine per day was 1.2. Furthermore, all participants were full-time indoor workers. This supports that ambient UVB during winter in Denmark is negligible in terms of cutaneous vitamin D synthesis.[20,21]

Three years earlier, from October to December, the same participants participated in a controlled UVB study. They received identical and artificial UVB irradiation on around 80% of their body surface area for nine weeks as part of a larger study group.[3] UVR cabinets from Waldmann, Willingen-Schwenningen, Germany with 26 F85/100W UV6 tubes (broadband UVB: 290-360 nm) were used. Of the vitamin D weighed UV6 spectrum, 90 % (295-360 nm) is present in daylight during a summer day in Denmark. During UV irradiation, the participants wore a UV protective helmet covering head/face and underwear covering buttocks. Irradiation time was determined and regulated by measuring UV intensity with a Sola-Hazard spectroradiometer (Solatell, Cornwall, UK). The UVB intervention consisted of 0.94 kJ m^{-2} (2 SEDs) bi-weekly the first two weeks, tri-weekly for the following five weeks and 1.4 kJ m^{-2} (3 SEDs) tri-weekly the last two weeks of the study (total UVB dose: 26 kJ m^{-2} (56 SEDs)).[3]

2.4 | Blood analysis

All blood samples were collected prior to the participant's daily dose of vitamin D. Serum $25(OH)D_3$ was used as a parameter of vitamin D status and analysed in-house on a liquid chromatography tandem mass spectrometer (LC-MS/MS).[20,24] To minimise analysis variance, at least triplet analyses (technical replicates) were performed and all $25(OH)D_3$ samples from each participant were analysed in one batch. The total relative standard deviation (SD) varied between 4.9% at 20 nmol l^{-1} and 14.1% at 222 nmol l^{-1} reflecting experimental variability.

To monitor for possible adverse effects serum ionised calcium, alkaline phosphatase and parathyroid hormone (PTH) were analysed by the Department of Clinical Biochemistry, Bispebjerg Hospital using methods described previously.[24] Serum ionised calcium (Ca^{2+}) and PTH were measured at study start, after three weeks and at study end and alkaline phosphatase was measured at study start and study end.

Genetic analyses were performed in relation to the UVB study[25] In the UVB study, 14 SNPs located in pigment genes had separate significant influence on the UVB-induced $25(OH)D_3$ increase and were selected for investigation in this study. SNPs with genotype subgroups containing less than four participants were merged with other allele-sharing subgroups (e.g. genotype AA with AG and not with GG). Heterozygote subgroups with less than four participants were only merged with an allele-sharing subgroup displaying insignificant ($P > 0.05$) difference in influence on the slope of the $25(OH)D_3$ increase. SNPs displaying no allele dose effect or no dominant allele effect on $25(OH)D_3$ were excluded. For SNPs with dominant allele effect, subgroups with no significant differences in influence on the slope of the $25(OH)D_3$ increase were merged according to allele sharing.

2.5 | Statistics

Individual data were tested with the Kolmogorov-Smirnov test to assess whether the data were normally distributed. Normally distributed data were tested by Student's t-test. Pair-wise comparison of ionised Ca^{2+} levels at different time points was performed using Wilcoxon Signed Ranks test. Levene's test were used to compare variance of the two interventions.

The $25(OH)D_3$ increase over time was investigated. The following models were explored separately: linear, inverse, quadratic, cubic, power, sigmoid and exponential. Determination of the best suitable model (power model) was based on R^2 values and the accordance between the individual investigations and group investigation of the $25(OH)D_3$ increase over time. The inter-individual variations of the linearised $25(OH)D_3$ increase were explored by comparing general linear models (GLMs) with: (1) $25(OH)D_3$ start-level-influenced slope and individual intercepts (i.e. measured $25(OH)D_3$ start levels) and (2) individual slopes (i.e. an individual constant) and measured $25(OH)D_3$ start levels.

Initially, the influence of parameters on the inter-individual variation of the $25(OH)D_3$ slope was examined separately. The influence of separate significant parameters was not independent and therefore subsequently investigated by a stepwise forward selection of separate significant parameters deployed in a combined GLM according to *P*-value.[26]

According to the previous UVB study the difference in $25(OH)D_3$ from study start to study end was 48 nmol l^{-1} with an SD of 29 nmol l^{-1}.[3] In the present study with vitamin D_3 supplementation, the detectable difference in $25(OH)D_3$ was expected to be half of that, i.e. 24 nmol l^{-1}. Similarly, after a daily vitamin D_3 dose of 80 µg, Gallagher et al. found a total

$25(OH)D_3$ increase of 68 nmol l^{-1} after 26 weeks, corresponding to an increase of 24 nmol l^{-1}after 9 weeks.[27] Given a significance level of 5% and a power of 80 %, 13 participants were required. As the inclusion criterion was participation in a prior UVB study for nine weeks, an attempt was made to include all 22 participants. Nine-teen out of the 22 participants were included, allowing six drop-outs during this relatively long study period.

Sample size calculation was performed using the program *Power and Sample Size Calculation version 3.1.2.2014* available online on the website: http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize

Data were statistically analysed using SPSS 24.0 for Windows (SPSS Inc., Chicago, IL, U.S.A.). $P < 0.05$ was considered significant. All tests were 2-tailed.

3 | RESULTS

3.1 | Study start characteristics

Demographic data are summarised in Table 1. The BMI span was relatively narrow as 16 participants had a BMI within normal range or slightly above (<25.6 kg m^{-2}) but was nevertheless tested for possible influence on the inter-individual variation in the 25(OH)D response to vitamin D_3 supplementation.

3.2 | Compliance

The 25(OH)D_3 sampling and intervention compliance was 100%. Four participants had five incidents (3.8%) out of the 133 study visits consuming one less and up to two tablets more than scheduled (over a period of 1 or 2 weeks). Deviations in intake of study medication were successfully corrected in between each blood sample period according to tablet counting.

3.3 | Side effects, adverse effect and safety

Three participants reported mild temporary side effects (nausea, dizziness and flu symptoms). No serious adverse events occurred during the study period. Paraclinical parameters are presented in Table 2**.** There were no incidents of hypercalcemia. Ionised Ca^{2+} decreased significantly (P = 0.003) from study start to study end, most likely due to the lack of concurrent calcium supplementation. One participant had unexplained and elevated alkaline phosphatase level at study start decreasing from 178 Units l^{-1} to 130 Units l^{-1} at study end.

3.4 | The course of the 25(OH)D_3 increase over time after vitamin D_3 supplementation

The course of 25(OH)D_3 increase over time is shown in Figure 1. Visual inspection of Figure 1 showed there was an initial and temporary elevated increase at Day 7 and 14 from the 25(OH)D_3 start level. This was followed by an insignificant (compared to Day 14) lowering of the 25(OH)D_3 level at Day 21. Despite this lowering, the 25(OH)D_3 level at Day 21 was significantly increased compared to the 25(OH)D_3 start level and was followed by a point-to-point significant increase in 25(OH)D_3 at Day 35. This initial temporary peak of 25(OH)D_3 increase was individually present in 14 out of the 19 participants.

3.5 | Model of vitamin D_3 supplementation-induced 25(OH)D increase

As the purpose of this study was to investigate the long-term effect of vitamin D_3 supplementation, investigation of the best model fit was performed on time courses including: (1) all time points; (2) all time points except Day 7 and 14 and (3) all time points except Day 21 (Table 3).

Based on this investigation, the increase in 25(OH)D_3 over time was best described by a power model ($R^2 = 0.433$, $P = 4.2\times10^{-13}$) in a time course excluding the temporary peak (i.e. Day 7 and 14) and was individually significant in 15 out of 19 participants. Similarly, in the time course including all time points, the 25(OH)D_3 increase was also best described by a power model ($R^2 = 0.332$, $P = 4.0\times10^{-13}$) and was individually significant in 11 out of 19 participants. The strongest model excluding the temporary peak was therefore selected for further analysis. The equation for the power model is: $Y = Y_0 \times t^{\alpha}$ (Y is the 25(OH)D_3 level at the time (t), Y_0 is the 25(OH)D_3 start level and α is the power coefficient). To obtain a linear relation of 25(OH)D_3 over time, natural logarithm was applied and the linearised model could be expressed as: $\ln(Y) = \ln(Y_0) + (\alpha \times \ln(t))$, where α is the slope and ln(25(OH)D_3 start level) is the intercept.

3.6 | Comparison of the inter-individual variation in vitamin D_3- and UVB-induced 25(OH)D_3 response

There was considerable inter-individual variation in the mean 25(OH)D_3 increase (mean Δ25(OH)D_3 = 53 nmol l^{-1}, range 17 - 91 nmol l^{-1}, span 74 nmol l^{-1}) induced by vitamin D_3 supplementation. The mean Δ25(OH)D_3 was similar ($P = 0.66$) to the mean Δ25(OH)D_3 of 52 nmol l^{-1} after 9 weeks of UVB exposure. The inter-individual variation in the vitamin D_3-

induced Δ25(OH)D_3 was about half of what was observed after UVB irradiation (range 2.9 - 139 nmol l^{-1}, span 136 nmol l^{-1}). Therefore, the interquartile ranges were compared. After vitamin D_3 supplementation the interquartile range was 38.8 to 71.4 nmol l^{-1} (span: 32.6 nmol l^{-1}) and similar to the interquartile range after the prior UVB irradiation (35.7 to 65.4 nmol l^{-1}, span: 29.7 nmol l^{-1}). The variances were not significantly different in both interventions (P = 0.57). The inter-individual variation in the 25(OH)D response to vitamin D_3 supplementation and UVB is shown in Figure 2.

3.7 | Vitamin D_3 supplement and UVB irradiation: 25(OH)D_3 start level and end level correlation

In the present study, the mean 25(OH)D start level was 62 nmol l^{-1} (range 33 – 92 nmol l^{-1}) and the mean end level was 115 nmol l^{-1} (range 68 – 166 nmol l^{-1}). In the prior UVB study there was a mean 25(OH)D_3 start level of 87 nmol l^{-1} (range 46 – 120 nmol l^{-1}) and a mean 25(OH)D_3 end level of 139 nmol l^{-1} (range 85 – 216 nmol l^{-1}).[3] The 25(OH)D_3 start levels (R^2 = 0.398, $P = 3.8 \times 10^{-3}$), 25(OH)D_3 end levels (R^2 = 0.457, $P = 1.5 \times 10^{-3}$) and Δ25(OH)D_3 (R^2 = 0.253, P =0.028) in both interventions were correlated as shown in Figure 2.Due to these relatively close correlations, it was hypothesised that the parameters influencing the UVB-induced 25(OH)D_3 increase might also influence the vitamin D_3 induced 25(OH)D_3 increase. The UVB induced 25(OH)D_3 increase was a linear ($P = 6.1 \times 10^{-15}$, Figure 1).

3.8 | General linear models used to explain the inter-individual variation in the 25(OH)D response to vitamin D_3 supplementation

The part of the inter-individual variation in the vitamin D_3-induced 25(OH)D_3 increase that could be explained was assessed using general linear models (GLMs). There was a significant influence of 25(OH)D_3 start level (P = 0.00094) on the slope of the vitamin D_3-induced 25(OH)D increase over time. This influence was included in the model. This GLM explained 72.0% (R^2 = 0.720, Table 4) of the observed variation and was used to investigate the influence of different parameters on the variation of the slope in the following. A GLM including individual intercepts and individual slopes explained 91.5% (the maximal part of the variation that could be explained) of the observed variation from vitamin D_3 supplementation. Hence, the unexplained variation of the slope represented 19.5% (the difference between 91.5% and 72.0%).

3.9 | Common background for inter-individual variation in 25(OH)D_3 response to vitamin D_3 supplementation and UVB

In the prior UVB study, 8 demographic parameters (sex, age, height, BMI, body surface area (BSA), fatty fish intake, objectively measured facultative and constitutive skin pigmentation) and 14 SNPs had separate significant influence on the 25(OH)D_3 variation.[25] The possible influence of these selected parameters on the vitamin D_3 supplementation-induced 25(OH)D_3 increase was therefore investigated. BSA, facultative and constitutive skin pigmentation was not investigated, as an influence of these parameters is unlikely in this context. None of the remaining 5 demographic parameters had separate, significant influence on the slope of the time course excluding the temporary peak (Table 4). Eight out of the 14 selected SNPs displayed separate significant influence on the vitamin D_3-induced 25(OH)D

increase (Table 4). Due to expected interaction between the influences of these SNPs, a forward stepwise selection of the SNPs in a combined GLM and according to *P*-value (<0.05) and power (>0.750) was performed. This analysis resulted in a combined GLM including 4 SNPs (rs12896399, rs2031526, rs4911442 and rs6475555) influencing the slope ($R^2 = 0.868$, $P = 1.6 \times 10^{-35}$, Table 5).

4 | DISCUSSION

The temporary peak in the $25(OH)D_3$ increase over time found after vitamin D_3 supplementation has not been reported by others, possibly due to less frequent 25(OH)D sampling.[28-32] BMI did not influence the 25(OH)D peak and therefore the effect cannot be explained by an interaction with fatty tissue. It will require further analysis with more frequent sampling to describe this initial enhanced increase more detailed.

We chose the time course excluding the temporary peak observed at day seven to 14 for further investigation, as this time course provided the strongest model. Furthermore, this was consistent with our aim to investigate the long-term effect of vitamin D_3 supplementation. There was a strong overlap in the results from both time courses confirming a concordance between the two time-courses. For the time course including all time points none of the five demographic parameters displayed separate significant influence on the slope. There was a complete overlap in the separate significant SNPs identified in both time courses. After a forward stepwise selection of the SNPs in a combined GLM, rs12896399 remained significant ($R^2 = 0.639$, $P = 2.4 \times 10^{-6}$) which was also the strongest SNP in the combined GLM in the time course excluding the temporary peak. These overlaps in results between the two time-courses could indicate, that the temporary initial peak does not influence the long-term effect of vitamin D_3 supplementation in an important way.

Despite, an almost half as wide inter-individual variation in the Δ25(OH)D_3 after vitamin D_3 supplementation than after UVB irradiation the span of the interquartile ranges were similar in both interventions. This suggests that the individual efficacy of vitamin D_3 supplementation on serum 25(OH)D_3 is, if not better, at least close to that of UVB.

Eight common pigment SNPs displayed separate significant influence on the variation of the 25(OH)D_3 increase in both interventions while there were no common influential demographic parameters. BMI had a *P*-value relatively close to significance (0.09) and did not confirm previous findings of an influence of BMI.[2,12,17,18] Probably, a larger span in BMI is required than represented in this study. Thus, these common findings support a partially common genetic background for the inter-individual variation in 25(OH)D_3 response to intervention. After accounting for interaction, 4 SNPs combined remained to influence the vitamin D_3 supplementation-induced 25(OH)D_3 increase and explained 14.8% of the variation of the slope. Thus, 4.7% of the variation of the slope remains to be explained by other parameters. The 4 significant SNPs were located in the genes: *Solute Carrier Family 24, Member 4, Methylthioadenosine Phosphorylase, Dopachrome tautomerase* and *Agouti signaling protein*. It seems likely that these genes may exert an influence on 25(OH)D_3 metabolism through a mechanism other than one related to pigmentation. In fact, many genes associated with the pigmentation pathway also have several other functions.[33] None of the identified SNPs in this study have previously been linked to 25(OH)D_3 concentration or 25(OH)D_3 increase. However, only few studies have investigated this so far.[34,35] Overall, the GLM including the influence of measured 25(OH)D_3 start levels and the 4 SNPs explained 86.8% of the variation.

There was a stronger correlation between 25(OH)D_3 end levels for the two interventions than between 25(OH)D_3 start levels and Δ25(OH)D_3. As the 25(OH)D_3 start levels were individually different this results in a weaker correlation for Δ25(OH)D_3. We have previously found a major inter-individual variation in the UVB induced maximal 25(OH)D_3 level.[3] If a similar individual maximal 25(OH)D_3 level exist for vitamin D_3 supplementation, this could explain the strongest correlation found between 25(OH)D_3 end levels. Only few studies have compared vitamin D supplementation with controlled UVB irradiation.[36,37] These two studies have aimed to compare efficacy in treatment or effect on cholesterol and transcription. Furthermore, it is also difficult to perform a study-to-study comparison of a dose efficacy between both interventions, as the UVB doses were increasing with time in this study and not identical for each individual in the studies by Bogh and Ponda.

The mean Δ25(OH)D_3 was 53 nmol l^{-1} after a daily vitamin D_3 supplement dose of 85 µg. This equals to an increase of 0.6 nmol l^{-1} 25(OH)D_3 per µg vitamin D_3 supplement. The reported slopes from other studies varies and ranges from 0.3 to as much as 5.5 nmol l^{-1} increase per µg vitamin D_3 supplement administered orally.[25,27,38-44] For the daily dose of 80 µg very similar to ours, Gallagher et al. found a slightly higher increase rate of 0.85 nmol l^{-1} 25(OH)D per µg vitamin D_3 supplement.[27] As the mean 25(OH)D_3 start level was around 20 nmol l^{-1} higher in this study, this could explain the difference in efficacy.

Gallagher also treated healthy individuals with six other different doses of µg vitamin D_3 supplement ranging between 10 µg to 120 µg for one year. After six months treatment each treatment arm had reached its maximal 25(OH)D level showing a mean overall plateau of 112 nmol l^{-1}. Thus, with increasing daily doses of supplement, the 25(OH)D increase per µg vitamin D_3 supplement decreases.[27] This is consistent with the low to moderate vitamin D_3

supplement dose (5 to 20 µg per day) studies that tend to yield higher efficiency of treatment.[38-40,43,44]

The $25(OH)D_3$ end level in this study of 115 nmol l^{-1} is very similar to the plateau level found by Gallagher. At study end there was an insignificant but mean increase of 6 nmol l^{-1}. It is therefore uncertain if the therapeutic effect perhaps was maximised at the end of this study. Heaney et al. reported a plateau level of about 150 nmol l^{-1} after a daily dose of 125 µg.[41] Therefore, there is some uncertainty about the exact level of such an upper plateau. Also, there was wide dispersion of the distribution of the individual plateau level (about 60 nmol l^{-1} to 160 nmol $^{-1}$) in the study by Gallagher.

Testing possible influential parameters, we chose to present raw *P*-values instead of performing a correction for multiple testing. This enables the reader to perform such corrections according to their own preferences. The commonly used Bonferroni method, although simple, is a relative conservative method. The *P*-values of the final significant genetic parameters presented were robust enough even for a Bonferroni correction.

As the participants in this study had previously received identical UVB irradiation with complete compliance over the same course of time, this provided an excellent background for comparing inter-individual variation in $25(OH)D_3$ response to the two intervention types. However, the difference in $25(OH)D_3$ start levels in the two studies was an important limitation causing some uncertainty in the assessment of range of variation. The relatively small sample size partly compensated by the relatively high number of samples per participant to minimise the intra-individual variation. Due to the relatively small sample size, it was possible to ensure complete intervention and sampling compliance. The ethnic

homogeneity of the study group limits the possibility of generalising our results to populations of other ethnic origin. But at the same time, it reduces the genetic inter-individual variation and increases the possibility of identifying putative influential parameters in a relatively small study group. As this study took place in Denmark during winter, this study was not confounded by ambient UVR.

In conclusion, there was a major inter-individual variation in the $25(OH)D_3$ increase after vitamin D_3 supplementation and UVB irradiation suggesting an equal individual efficacy of both interventions. There was no common demographic but a partially common genetic background for the inter-individual variation in both interventions. Consequently, persons characterized by certain individual genetic traits may not respond sufficiently to recommended doses of vitamin D_3 supplementation.

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CONFLICTS OF INTEREST

None of the authors reported any conflict of interest.

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TABLE 1 Demographic data	
Sex (women/men)	9 / 10
Age (years)	47 ± 8.1 (25-58)
Weight (kg)	76 ± 9.1 (60-95)
Height (cm)	174 ± 8.0 (160-186)
BMI (kg/m^2)	25 ± 2.3 (21-30)
Fatty fish meals per week	3.1 ± 1.5 (0-6.0)
25(OH)D start level ($nmol\ l^{-1}$)	62 ± 17 (33-92)

Values are mean ± SD (range). A maximum of 14 fish meals per week was possible.

TABLE 2 Serum 25(OH)D_3 levels and laboratory assessments after vitamin D_3 supplementation in 19 participants

	25(OH)D_3	Ionised Ca^{2+}	PTH	Alkaline phosphatase
Normal range	50-200 nmol l^{-1}	1.18-1.32 mmol l^{-1}	1.1-7.1 pmol l^{-1}	35-105 Units/l
Study day				
0	62 ± 17 (33-92)	1.20 ± 0.043 (1.1-1.3)	4.1 ± 1.2 (2.5-6.3)	70 ± 30 (41-178)
7	94 ± 24 (57-140) [ab]	-	-	-
14	98 ± 26 (68-175)	-	-	-
21	89 ± 25 (57-162)	1.20 ± 0.044 (1.0-1.3)	3.3 ± 0.89 (1.2-4.5) [b]	-
35	103 ± 24 (58-150) [b]	-	-	-
49	109 ± 26 (66-170)	-	-	-
63	115 ± 28 (68-166)	1.14 ± 0.080 (1.0-1.3) [ab]	3.5 ± 0.084 (1.9-5.2)	67 ± 24 (39-130)

Values are given in mean ± SD. Study days are mean values. Samples are from all 19 participants at all sample time points, except for study end (Study day 63), for ionised Ca^{2+} (N=16), PTH (N=17) and alkaline phosphatase (N=17) due to haemolysis of blood samples. a: Significant ($P < 0.05$) increase in paraclinical parameter from this point and forward compared to Day 0. b: Significant ($P < 0.05$) increase in paraclinical parameter compared to prior sample point.

TABLE 3 Selection of best model fit for the 25(OH)D_3 increase over time

	Time-points included	Best model fit	R^2	N individually significant
(1)	All time points	Power	0.332	11 out of 19
(2)	All time points except Day 7 and 14	Power	0.433	15 out of 19
(3)	All time points except Day 21	Power	0.378	12 out of 19

The best model fit was determined based on the R^2-value after testing the following models: linear, inverse, quadratic, cubic, power, sigmoid and exponential. N is the number of participants in which the best model was individually significant.

TABLE 4 The separate influence of demographic and genetic pigment parameters on the slope of vitamin D_3-induced 25(OH)D_3 increase

Parameters / General linear model (GLM)	N	*P*-value	R^2	Power	Gene
GLM with individual measured intercepts and 25(OH)D_3 influenced slope		4.5×10^{-25}	0.720	1.000	-
Demographic parameters					
BMI	-	0.090	-	-	-
Age	-	0.15	-	-	-
Height	-	0.29	-	-	-
Fatty fish meals per week	-	0.44	-	-	-
Sex	-	0.93	-	-	-
Genetic parameters					
rs12896399 — GG/TG+TT	7 / 10 + 2	8.3×10^{-10}	0.816	1	Solute Carrier Family 24, Member 4
rs1042522 — CC+CG/GG	2 + 6 / 11	4.3×10^{-6}	0.779	0.998	Tumour protein p53
rs3733542 — CC+CG/GG	1 + 4 / 14	1.9×10^{-3}	0.749	0.886	Proto-oncogene receptor tyrosine kinase
rs11614913 — CC+CT/TT	6 + 9 / 4	2.7×10^{-3}	0.747	0.864	MIR196A29
rs6475555 — AG+GG/AA	9 + 6 / 4	3.9×10^{-3}	0.745	0.833	Methylthioadenosine phosphorylase
rs4911442 — AG/GG/AA	8 / 0 / 11	4.0×10^{-3}	0.745	0.833	Agouti signaling protein
rs2031526 — AA+AG/GG	1 + 5 / 13	9.4×10^{-3}	0.740	0.748	Dopachrome tautomerase
rs2276288 — AT+AA/TT	11 +2/ 6	0.035	0.734	0.564	Myosin family 7, member A
rs2284063 — AG+GG/AA	9 + 2 / 8	0.059	-	-	Phospholipase A2, Group VI
rs4821767 — AC/CC/AA	11 / 4 / 4	0.25	-	-	Phospholipase A2, Group VI
rs4911414 — GG/TG+TT	9 / 8 + 2	0.16	-	-	Agouti signaling protein
rs28777 — AC/CC/AA	1 / 0 / 18	-	-	-	Solute Carrier Family 45, Member 2
rs16891982 — CC/CG/GG	0 / 1 / 18	-	-	-	Solute Carrier Family 45, Member 2
rs26722 — CC/CT/TT	19 / 1 / 0	-	-	-	Solute Carrier Family 45, Member 2

A total of 72% of the variation in the $25(OH)D_3$ increase was explained by a GLM comprising individual intercepts (i.e. measured) and a slope influenced by $25(OH)D_3$ start levels. Additional explanation of the variation in slope was assessed by investigating the influence of demographic parameters and SNPs located in pigment genes. R^2 is squared correlation coefficient. Power is the probability of confirming the given result in a new material with similar size and uncertainties as this material.

TABLE 5 Parameters influencing the slope of vitamin D_3-induced 25(OH)D_3 increase in a combined general linear model

Parameters	*P*-value	Power
rs12896399	8.9×10^{-8}	1.000
rs2031526	5.2×10^{-5}	0.998
rs4911442	2.6×10^{-5}	0.856
rs6475555	1.9×10^{-3}	0.886

Separate significant SNPs were deployed in a combined general linear model (GLM) through a forward stepwise selection of SNPs according to *P*-value. The final GLM included 4 SNPs with significant influence on the slope of 25(OH)D increase (R^2 = 0.868, *P* = 1.6×10^{-35}, Power = 1.000). A power of at least 0.750 was retained.

FIGURE 1 Mean 25(OH)D_3 levels during study period. The curve (blue) of the mean 25(OH)D_3 response to vitamin D_3 supplementation (85 µg/3400 IU per day, N=19) over time with one SEM bars had a temporary peak observed at Study days 7 and 14 (excluded from analysis, dotted blue line) followed by a decrease at Study day 21. The same 19 participants' 25(OH)D_3 received UVB irradiation three years earlier (red curve)[25]. In the UVB study (26 kJ m^{-2}/56 SEDs in total).

FIGURE 2 Inter-individual variations in 25(OH)D_3 start levels and end levels in 19 participants after 9 weeks of vitamin D_3 supplementation (85 µg/3400 IU per day) (A). The same 19 participants had previously received 9 weeks of regular UVB irradiation (Total dose: 26.2 kJ m^{-2}) on around 80% of the body area. 25(OH)D_3 start levels (blue circles), 25(OH)D_3 end levels (red circles) and Δ25(OH)D_3 (green circles) correlation between the two interventions, vitamin D_3 supplementation (x-axis) and UVB irradiation (y-axis), are shown (B).