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Vitamin D production after UVB exposure – A comparison of exposed skin regions ☆

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ABSTRACT

Background: Cholecalciferol is an essential steroid produced in the skin by solar ultraviolet B radiation (UVB 290–315 nm). Skin production of cholecalciferol depends on factors affecting UVB flux, age and exposed skin area.
Purpose: Serum cholecalciferol and 25-hydroxyvitamin D_3 [25(OH)D_3] concentrations were measured after UVB irradiation of 3 different skin areas to compare the skin capacity to produce vitamin D in different anatomic sites in the same individuals.
Method: Ten voluntary Caucasians (skin photo type II & III, aged 48 ± 12 years (±SD)) were exposed to broadband UVB (280–320 nm) between February and April. Hands and face, upper body and whole body were exposed to a suberythemic dose of UVB (median 101 mJ/cm^2 (min 66, max 143)) (for 3 subsequent days 24 h apart with a wash out period of about 3 weeks (median 18 days (min 11, max 25)) between the exposures of respective area. Serum concentrations of cholecalciferol and 25(OH)D_3, were measured immediately before the first and 24 h after the last dose of radiation.
Results: There was a significantly higher increase in serum cholecalciferol after UVB exposure of the two larger skin areas compared to face and hands, but no difference in increase was found between upper body and whole body exposures.
Conclusion: Exposure of a larger skin area was superior to small areas and gave greater increase in both serum cholecalciferol and serum 25(OH)D_3 concentrations. However, exposure of face and hands, i.e. only 5% of the body surface area, was capable of increasing serum concentrations of 25(OH)D_3.

1. Introduction

Vitamins D_3 (cholecalciferol) is naturally produced in the skin of animals, including humans, and vitamin D_2 (ergocalciferol) is produced in plants, fungus and yeast after exposure to sunlight and UVB radiation [1,2]. Vitamin D is fat soluble and primarily obtained through endogenous production after UVB (Ultraviolet B, 280–315 nm) irradiation of the skin [3]. Food intake is a minor source [1].

Vitamin D photoproduction in the skin starts by synthesis of 7-dehydrocholesterol (7-DHC, provitamin D_3), the final precursor in cholesterol biosynthesis. Large quantities of 7-DHC are produced in the skin of vertebrates and incorporated in the plasma membranes of cells in their epidermis and dermis [4–6]. During exposure to sunlight 7-DHC is converted to previtamin D_3 which is rapidly thermally isomerized in the plasma membrane to cholecalciferol. Produced cholecalciferol is ejected into the extracellular space and reaches by diffusion the capillary bed in the dermis. Cholecalciferol is transported to the liver by vitamin D binding protein (DBP) where it is transformed into 25-hydroxyvitamin D [25(OH)D_3, calcidiol]. 25(OH)D_3 is the major circulating metabolite which is further hydroxylated by 1-alpha-hydroxylase into its active form, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D, calcitriol] [1,6].

Since the half-life for serum 1,25$(OH)_2$D is ~4 h and tightly regulated by the kidneys, serum 25(OH)D_3 concentrations (half-life – 2 weeks) are used as an indicator of vitamin D status [1]. Vitamin D (D represents D_2 or D_3), on the other hand, is primarily stored in adipose tissue [7,8]. Toxic concentrations of vitamin D cannot be

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reached solely through sun exposure since excess UV irradiation transforms previtamin D_3 and cholecalciferol into inert products [5].

There is no consensus on optimal serum 25(OH)D_3 concentrations but a serum 25(OH)D_3 < 50 nmol/L is considered to be vitamin D deficiency [9,10]. The threshold values of insufficiency (i.e. the risk of developing illness over a longer period of time) vary even more, but several studies support the threshold value of 75 nmol/L at least [9,11,12]. This finding implies suboptimal concentrations worldwide including the Swedish inhabitants during winter [13,14].

The aim of the study was to measure the changes in serum cholecalciferol and 25(OH)D_3 concentrations after UVB irradiation of three different skin areas in the same individuals. The capacity of vitamin D production in the skin of the hands and face was compared to that of other, larger skin regions.

2. Method

2.1. Participants

Ten voluntary subjects (8 women and 2 men, aged 48 ± 12 years [mean ± SD] (range 35–69 y), skin types II (n = 4) and III (n = 6) living in Gothenburg (57°N) were enrolled in the study between February and the beginning of April. The baseline data are depicted in Table 1. Information about weight, height, medical history, medication, visits to sunny countries and dietary intake was collected through a questionnaire. The participants were asked not to change their food habits, not to take cod-liver oil or vitamin D supplements, not to change their lifestyles or travel to any sunny country during the study time.

2.2. Ethics

The study was approved by the Ethics committee at the University of Gothenburg and the Swedish National Data Inspection Board. All participants gave written and verbal informed consent.

2.3. Intervention

The study was conducted in Gothenburg at the end of the winter when there is no sun-induced vitamin D synthesis and when serum concentrations of vitamin D and 25(OH)D_3 are at its lowest.[5,13] Three different skin areas (hands and face, upper body and whole body) were exposed to a suberythemic dose of UVB (median 99 mJ/cm^2, (min 66, max 143) for 3 subsequent days 24 h apart with a wash out period of about 3 weeks (median 24 days (min = 18, max = 25) between treatment of face and hands and upper body treatment, and median 17 days (min = 11, max = 18) between upper body treatment and full body treatment) between the exposures of respective area. The wash-out period has been shortened in two subjects due to the planned trip to a sunny country. The participants were in a standing position exposed to broadband UVB, 280–320 nm, from a Philips TL 12/Corona 4. This is a phototherapeutic device with 28 Philips TL12 tubes á 100 W mounted on the walls of the box, produced by ESSHÅ electricity agency in Värnamo, Sweden.

The source of radiation was placed 30 cm from body surface. The UVB dose was measured with a PUVA Combi light UV meter from ESSHÅ, Värnamo, Sweden. Measurement of the UVB dose was performed by a calibrated PUVA Combi light UV meter (model: DC0003, serial Nr: 31000917) at a certain distance (30 cm) from the tubes. The calibration of the sensor used in this measurement was performed using established test procedures and equipment with accuracy ±5% with respect to the European standard. The participants wore their own clothes, covered with an operation gown to avoid unwanted exposure as well as protective glasses. The individuals were exposed to a suberythemic dose of UVB a total of nine times, organized as follows. Face and hands (the whole face up to the hairline and the back of the hands) were exposed three times, each separated by a 24-hour period. A wash out period of three weeks followed this procedure. The process was then repeated by upper body exposure (from the waist up, including the face) and an additional wash out period of about three weeks was followed by whole body exposure (naked, with no clothes). Serum samples were collected just before the first, and 24 h after the last exposure of respectively body area (72 h after the first exposure), thus a total of six times during the study. Serum samples were frozen at −80 °C and stored for later analysis. The UVB doses given to each subject varied according to the skin phototype and the irradiated area. The median dose of UVB (mJ/cm^2) was 107 (min 66, max 127) for the face and hands; 99 (min 66, max 116) for the upper body and 99 (min 66, max 131) for the whole body. The presented doses are physical units and a weighting factor to calculate the physical broad band UVB dose as erythemally CIE weighted unit according to IEC 335-2-27 for broadband UVB (290–320 nm, peaks at 313 nm) is 0.074.

Participants were their own controls for measurement of cholecalciferol production of different skin areas since the same procedure was repeated in all subjects after wash out periods.

2.4. Serum sample analyses

Serum concentrations of cholecalciferol, 25(OH)D_3, 25(OH)D_2, 1,25(OH)$_2$D, PTH, calcium and creatinine were analyzed directly before (basal determination) and 24 h after the last exposure of an area (peak value). Serum samples were kept from light exposure and stored frozen at −80 °C. Analyzes were carried out at the Department of Clinical Chemistry, Sahlgrenska University Hospital (SU), Gothenburg, Sweden, at the Department of Clinical Chemistry, Labmedicin Skåne, Malmö, Sweden and at Core Assay Laboratory, Clinical Translational Science Institute, Boston University School of Medicine, Rm M-1022, 85 E. Newton St., Boston MA 02118, USA.

Serum 25(OH)D_3 was analyzed by high-performance liquid chromatography (HPLC) at Labmedicin Skåne, Malmö, Sweden. HPLC has been used in Malmö since 2005 for separating 25(OH)D_2 from 25(OH)D_3. The most 25(OH)D_2 values were very low, only results >5 nmol/L were recorded.

Serum cholecalciferol concentrations were analyzed with HPLC at Boston University School of Medicine. Serum total 1,25(OH)$_2$D was analyzed with ^{125}I-RIA (radioimmunoassay) at SU, Gothenburg. For 1,25(OH)$_2$D the reference range was 25–66 μg/L. S-PTH was analyzed with an immunochemical method (mass

Table 1
Anthropometric data including age, gender, Body Mass Index (BMI), body weight, skin phototype and Serum baseline concentrations of cholecalciferol (vitamin D_3), 25-hydroxyvitamin D_3 [25(OH)D_3], 25-hydroxyvitamin D_2 [25(OH)D_2]), 1,25-dihydroxyvitamin D [1,25(OH)$_2$D], calcium, parathyroid hormone (PTH) and creatinine, of study participants. (min–max (median)).

Age, years	35–69 (46)
Gender, M:F	8:2
BMI, kg/m^2	24–38 (27)
Body weight, kg	64–100 (73)
Skin type, II:III	4:6
S-cholecalciferol, ng/mL	0–3 (0.5)
S-25(OH)D_3, nmol/L	26–113 (48)
S-25(OH)D_2, nmol/L	<5–8 (5.5)
S-1,25(OH)$_2$D, pg/mL	61–203 (110)
S-Calcium, mmol/L	2.26–2.52 (2.37)
S-PTH, ng/L	10–64 (33)
S-Creatinine, μmol/L	55-91 (65)

concentration) and a reference interval of 15–68 ng/L. Photometry 600 nm was used to determine concentrations of S-calcium, reference value: 2.15–2.50 mmol/L. S-creatinine was analyzed with an enzymatic method, reference values: 45–90 μmol/L (women) and 60–105 μmol/L (men).

Mean BMI was 28 ± 5 kg/m^2.

2.5. Data analysis

The data were analyzed with R version 2.14.2 (The R Foundation for Statistical Computing Vienna, Austria).

Wilcoxons sign rank test was used for pairwise comparison and Spearmans correlation coefficient was used for testing correlations between variables. Multiple linear regression was used for modeling increase in 25(OH)D_3 versus baseline 25(OH)D_3 and increase in cholecalciferol. Any values considered as non-detectable were interpreted as zero.

All tests were two-tailed and $p < 0.05$ was considered statistically significant.

3. Results

The serum concentrations of 25(OH)D_3 increased after exposure to UVB radiation during the entire study period, from baseline to the measurement after the last exposure, ($p = 0.007$) (Fig. 1). The serum concentrations of cholecalciferol were restored during the wash out periods between treatments as seen in Fig. 1.

Mean increase in serum cholecalciferol after upper body exposure was 14.5 ng/mL (1208%, 95% CI: [880%, 1537%], $p = 0.002$), after whole body exposure 16.2 ng/mL (3256%, 95% CI: [2535%, 3953%], $p = 0.004$). No significant increase after exposure of face and hands occurred in serum cholecalciferol (0.9 ng/mL, $p = 0.34$). A significant difference in serum cholecalciferol increases were found when comparing exposure of face and hands with upper body exposure ($p = 0.002$) and whole body exposure ($p = 0.004$), respectively, but not when comparing upper body with whole body exposure ($p = 0.77$) (Fig. 2).

Mean increase of 25(OH) D_3 was, for face and hands 6.1 nmol/L (11.5%, 95% CI: [2.9–20.1%], $p = 0.03$), for upper body 12 nmol/L (20.0%, 95% CI: [13.8–26.2%], $p = 0.002$) and for whole body exposure 8.5 nmol/L (11.2%, 95% CI: [3.8–18.6%], $p = 0.03$). A significant difference in 25(OH)D_3 increases were found when comparing exposure of face and hands with upper body exposure ($p = 0.04$). The difference was not significant when comparing upper body with whole body or face/hands compared with whole body, respectively (Fig. 2).

All participants (except 1 person) were vitamin D insufficient (baseline serum 25(OH)D_3 < 75 nmol/L) [9] and five participants (50%) still remained insufficient after the end of study, although all subjects reached concentrations of >50 nmol/L.

The increase in serum 25(OH)D_3 during each treatment period (3 per patient) was negatively correlated with the baseline 25(OH)D_3 before the respective treatment ($p = 0.01$) when controlling for increase in serum cholecalciferol using multiple linear regression. A tendency for a positive correlation was seen between the increase in 25(OH)D_3 and increase in cholecalciferol, when controlling for 25(OH)D_3 at baseline ($p = 0.08$).

No significant correlations were found for respective skin area between radiation doses (mJ/cm^2) corrected for body surface area

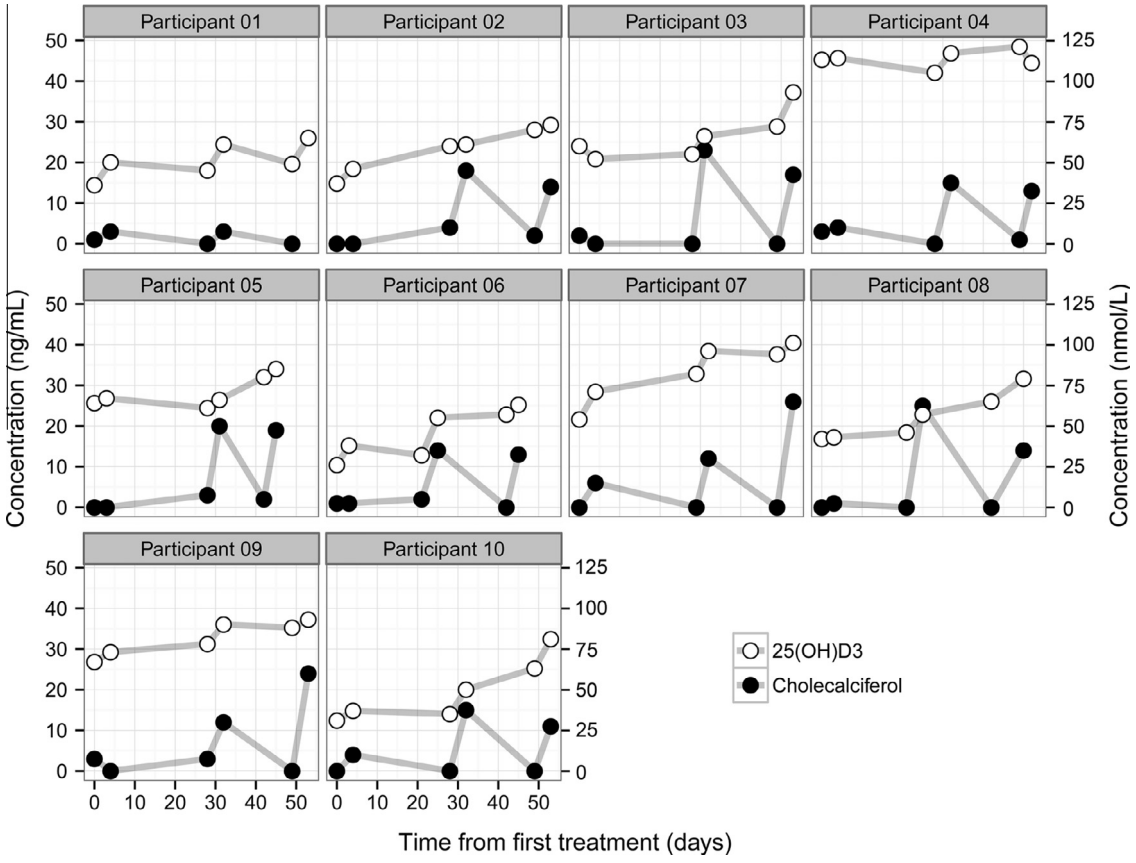

Fig. 1. Serum concentrations of cholecalciferol (vitamin D_3) and 25-hydroxyvitamin D_3 [25(OH)D_3] of each participant before and after the entire UVB treatment session. Neighboring points in time are measurements from before and after respective treatments. The treatments in chronological order were face and hands, upper body and last whole body. The scale on the left is ng/mL and is valid for both metabolites. The nmol/L scale on the right is only accurate for 25-hydroxyvitamin D_3 due to a slightly different molecular weight of cholecalciferol.

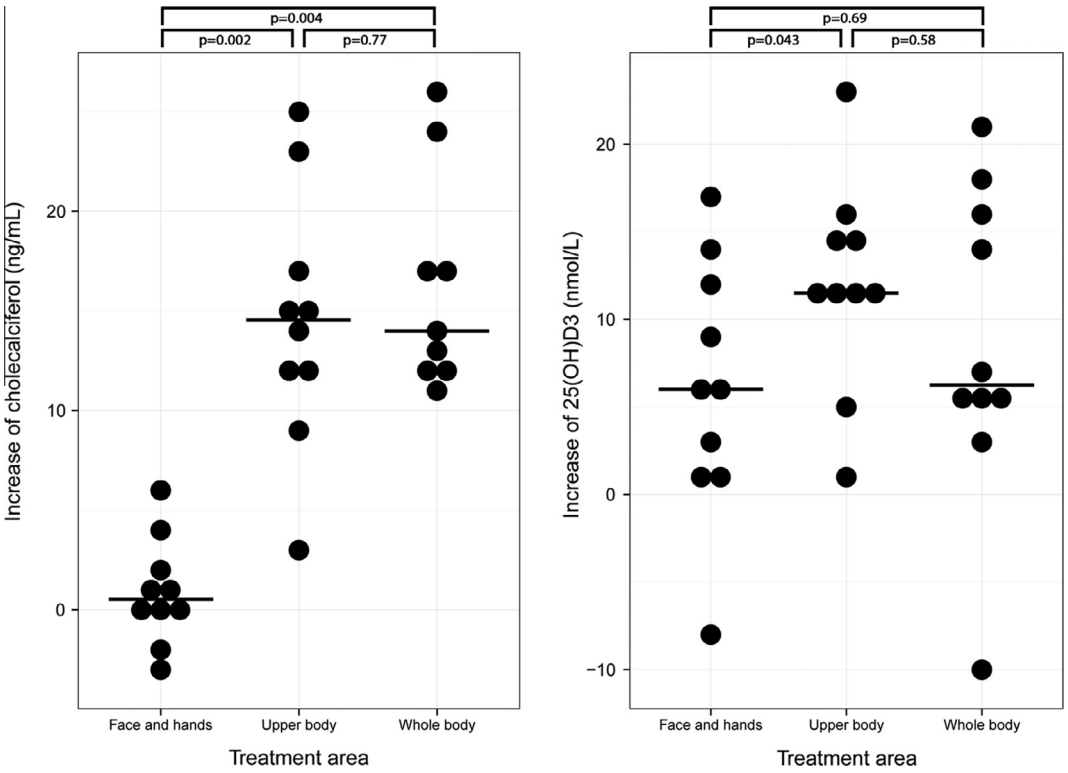

Fig. 2. The figure shows the increase in cholecalciferol (vitamin D_3) and 25-hydroxyvitamin D_3 [25(OH)D_3] during the UVB exposures of 3 different skin areas. Each point depicts one participant during the UVB exposures (before and 24 h after the 3 exposures for one skin region). Horizontal bars are median values.

and change in serum concentrations of cholecalciferol. All values of serum 25(OH)D_2 concentrations received from the lab were of negligible change (baseline values had a median of 5.5 nmol/L, see Table 1).

Serum 1,25(OH)$_2$D median baseline concentration prior to all treatments was 110 pg/mL (min = 61, max = 203). Median concentration in 1,25(OH)$_2$D after all three treatments was 84 pg/mL (min = 63, max = 209), (p = 0.56).Median PTH concentration before all treatments was 33 ng/L (min = 9.7, max = 64) and there was no change after all treatments had been given (p = 0.64).

Median calcium concentration before any treatment was 2.37 mmol/L (min = 2.26, max = 2.52) and median concentration post treatments was 2.35 mmol/L (min = 2.16, max = 2.39), (p = 0.07).

Median creatinine concentration prior to all treatments was 65 μmol/L (min = 55, max = 91) and did not change post treatments (p = 0.62).

The participants did not change their food habits, their lifestyles or traveled to any sunny country during the study time. One participant (number 4) had higher serum 25(OH)D_3 concentrations due to the regular vacations in sunny countries (3–4 times per year) though the last trip ended more than 2 months before the study start.

There was no correlation between BMI and changes in the serum concentrations of cholecalciferol or 25(OH)D_3, respectively. The mean BMI was 28 ± 5 kg/m^2 (median 27 kg/m^2). Two subjects had a BMI above 30 kg/m^2 (see Table 1).

4. Discussion

UVB irradiation resulted in higher concentrations of serum cholecalciferol when exposing the upper body or whole body compared with smaller areas. There are a few studies on how the area of the exposed body surface determines the concentrations of serum cholecalciferol produced during UVB exposures [15–17]. In a previous study the cholecalciferol synthetic response to the fixed UVB dose reached a plateau when more than 33% of body surface area was irradiated [15]. According to results from a Danish study the size of the exposed body surface area was the most important factor when the skin was irradiated with the smallest UVB dose of 0.75 SED (standard erythema dose) [17]. Exposure of small skin regions such as face and hands (~5% of the body surface) was less effective in increasing serum concentrations of cholecalciferol compared to the exposure of larger skin regions (upper and whole body) (Fig. 2). There was a trend for increased serum cholecalciferol after exposure of face and hands although not significant (p = 0.34, Fig. 2). In concordance with other studies these data strongly suggest that skin region and surface area exposed are important determinants of the cutaneous cholecalciferol response to short term i.e. 3 UVB exposures [15,17]. However, these few exposures improve vitamin D status of the subjects.

No linear correlation between exposure of larger areas and serum concentrations of 25(OH)D_3 (Fig. 2) was found in the present study, which is in concurrence with results from earlier studies [15,17]. There is a direct relationship with increasing blood concentrations of cholecalciferol with increased irradiated area even though the 25(OH)D_3 concentrations did not increase to the same extent. This finding was expected since cholecalciferol initially enters the fat and is slowly released into the circulation to be converted to 25(OH)D_3 in the liver.

Data are sparse on individual differences in vitamin D production between different anatomic sites in humans [17]. It has been shown, in chicken, that different anatomic sites differ in their capacity to produce D_3. The highest concentration of 7-DHC was found in the skin of the legs which are exposed to sunlight. This concentration was about 30 times greater than that in the back of chickens. The feathers covering the back of the chickens

preserve the cutaneous production of cholecalciferol. Whole body exposures of chickens to UVB resulted in the pre D_3 production in the skin of the legs and feet, whereas no pre D_3 was detected in the back skin [18]. There are no data on the variation in the 7-DHC concentrations and thereby no data on the difference in ability to produce cholecalciferol in different skin regions in humans.

However, a previous study on effects of skin thickness, age and body fat on serum 25(OH)D_3 demonstrated that serum 25(OH)D_3 concentrations in postmenopausal women were significantly related to skin thickness and, by inference, to the mass of skin tissues available for synthesis of cholecalciferol [19].

Most studies today solely use 25(OH)D_3 to analyze effects of UVB on circulating vitamin D. There are very few studies in which measurements of both cholecalciferol and 25(OH)D_3 after UVB exposures are reported [20,21].

In the present study, cholecalciferol and 25(OH)D_3 tended to correlate positively after UVB irradiation of face and hands (Fig. 3). This tendency was not seen when larger skin regions were irradiated indicating complexity in the regulation of cholecalciferol metabolism.

Cholecalciferol as a fat-soluble molecule is stored in the fat tissue rather than metabolizes into 25(OH)D_3 [8,20,22]. Once cholecalciferol is produced in the skin it enters the circulation and then into the body fat. Therefore it is slowly released back into the circulation where it is converted to 25(OH)D_3 in the liver. Thus serum 25(OH)D_3 concentrations do not significantly change within a few days after taking vitamin D orally or being exposed to sunlight [20,23]. The lifetime of cholecalciferol in serum is very short 24–72 h (peak 18–24 h) while it is 2–4 weeks for 25(OH)D_3. Hence, the peak of 25(OH)D_3 is not linearly correlated to the peak of cholecalciferol. It has been shown that after exposure to UVB radiation the 25(OH)D_3 concentrations increased gradually, reaching a plateau 7–14 days after UVB exposures [20]. In our study the serum concentrations of 25(OH)D_3 were measured 24 h after the UVB exposures. These findings are the likely explanations for why there was a direct correlation with increased serum concentrations of cholecalciferol with increased surface area exposed even though the serum 25(OH)D_3 concentrations did not reflect the higher blood concentrations of cholecalciferol.

The obtained UVB dose is the major determinant of cholecalciferol production in the skin [17,20]. There was no correlation between radiation dose and serum concentrations of cholecalciferol after correcting for skin region in the present report. This result was expected since subjects were exposed to roughly the same intensity of UVB, the only difference was that altered skin regions were irradiated. An interdependence between UVB dose and body surface area was discussed in the work of Bogh et al. [17]. Increase in 25(OH)D_3 depends mainly on the UVB dose; however, for small UVB doses the area of the irradiated skin surface is of certain importance [17].

Very slight facial erythema was observed after the first series of exposures which was reduced in continued exposures. No persistent tanning was seen. This consequence could influence on UVB penetration in continued exposures even if a new skin area was introduced to UVB for each new exposure period.

Over the study period we have seen an accumulation of 25(OH)D_3 (Fig. 1). Previous studies show a half-life of 25(OH)D_3 between 10-40 days with an individual metabolism rate [7]. However, the half-life of 25(OH)D_3 also depends on the 25(OH)D_3 concentration. Because of these variations the serum concentrations might not have returned to baseline concentrations during the wash-out periods. Cholecalciferol can be released from storage in the fat tissue and converted in the case of decreasing concentrations of 25(OH)D_3, like a buffer effect. The serum concentrations of cholecalciferol, on the other hand, decreased during the wash-out periods, indicating a conversion into 25(OH)D_3 or a storage in other tissues. All subjects did not reach desired concentrations of serum 25(OH)D_3 > 75 nmol/L. This finding might indicate that only 3 UVB exposures 3 weeks apart are hardly enough to induce sufficient production of 25(OH)D_3 after a long winter period when the majority of the population at northern latitudes declined in their vitamin D status [13,14]. However, all participants in our study reached 25(OH)D concentrations of >50 nmol/L, which is sufficient according to the recommendations of Institute of Medicine (2011) and covers the need of vitamin D in 97,5% of the Americans [24]. Nevertheless, results from other studies showed that the minimal desirable serum level of 25(OH)D_3 should be 75 nmol/L (30 ng/mL) [9,25,26].

Furthermore, no effects of the short UVB exposures were seen on serum calcium or PTH in the present study.

Serum concentrations of 25(OH)D_2 did not change during the present study period which implies that the dietary intake of ergocalciferol remained unchanged and had no influence on variations in serum concentrations of 25(OH)D_3.

Many factors other than food intake or UVB exposure play a role in determining 25(OH)D_3 concentrations in serum such as BMI and age [5,19,27]. The BMI did not seem to have an impact on the cholecalciferol production in this study. One limitation was the small sample size. However, each subject was its own control and all had normal creatinine indicative of normal renal function.

There is an increasing interest in the role of vitamin D in various diseases. This finding has triggered discussion on threshold values as well as official and treatment recommendations to reach sufficient 25(OH)D_3 concentrations taking into consideration the risk

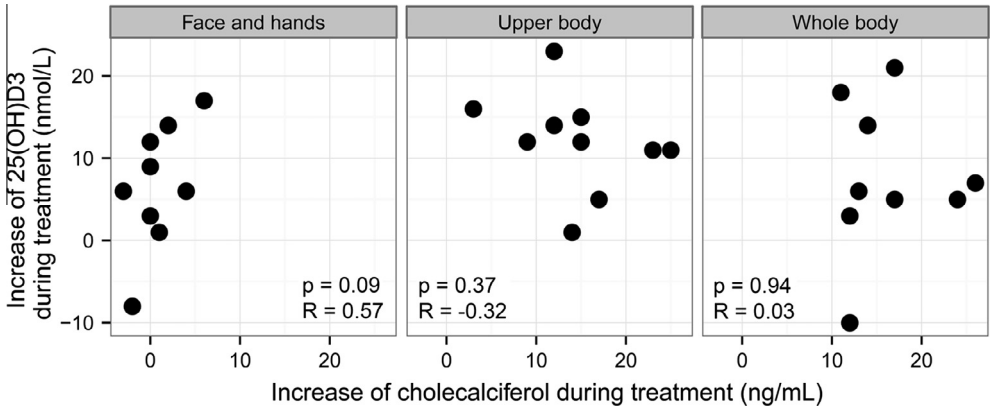

Fig. 3. The figure shows the correlation between the change in 25-hydroxyvitamin D_3 [25(OH)D_3]) versus the change in cholecalciferol (vitamin D_3) in serum after UVB exposure of different skin areas.

of sun exposure [28–30]. In this study radiation from phototherapy unit was used. This radiation differs from the UV radiation of solar origin where the highest irradiation is delivered to the anterior and posterior sides of trunk and legs; in the solar irradiation it is highest on face and arms [7,15]. Furthermore UVA (320–400 nm), present in the solar radiation, can destroy already produced D_3 in the skin which makes study results difficult to apply to real life situations [31]. More facts on this discrepancy are needed to understand and use the information we have today. A substantial amount of future research is required to gain insight into this relatively unexplored field.

5. Conclusion

UVB exposure resulted in higher concentrations of serum cholecalciferol and 25(OH)D_3 when exposing a larger body surface, such as upper body or whole body compared to smaller areas. However, exposure of only face and hands, i.e. 5% of the body surface, was capable of increasing serum concentrations of 25(OH)D_3.

6. Abbreviations

UVB	ultraviolet radiation B;
PTH	intact parathyroid hormone;
25(OH)D_3	25-hydroxyvitamin D(calcidiol)
1,25(OH)$_2$D	1,25-dihydroxyvitamin D (calcitriol)
Vitamin D_3	(cholecalciferol)
Vitamin D_2	(ergocalciferol)

Conflict of interest

All authors declare no conflict of interest.

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