Increase in serum 25-hydroxyvitamin-D3 in humans after sunbed exposures compared to previtamin D3 synthesis *in vitro*

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**ABSTRACT**

Ultraviolet (UV) radiation is liable to cause skin cancer but it is the main source of vitamin D. Vitamin D photosynthesis takes place in skin at sub-erythemogenic UV doses, while larger exposures destroy vitamin D and increase DNA damage. Proper UV dosimetry is needed to obtain an optimal vitamin D status when skin cancer risk is minimal. A simple approach to such dosimetry using physically measured accumulated UV dose cannot provide a satisfactory quantification of vitamin D because of the complexity of the processes involved in vitamin D synthesis. A biological dosimeter of vitamin D synthetic UV radiation (‘D-dosimeter’) has been introduced earlier on the basis of an in vitro model of previtamin D photosynthesis. In the present study in vivo generation of 25-hydroxyvitamin D (25(OH)D) in serum of healthy volunteers exposed to UV radiation from the sunbed was accompanied by in vitro measurements of vitamin D formation using ‘D-dosimeter’. It was found that the increase in serum 25(OH)D concentration depended both on the initial 25(OH)D level and on the cumulative sunbed exposure time. The observed linear correlation between in vivo and in vitro data can be used to estimate changes in vitamin D status after UV exposure using only one pre-exposure blood sample combined with further in vitro measurements.

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**1. Introduction**

Numerous studies of the vitamin D role for human health, undertaken during last two decades, indicate that vitamin D is more important for optimal health than previously assumed [1,2]. A sufficient vitamin D level is important for lowering the risk of different internal cancers, multiple sclerosis, diabetes types 1 and 2 together with the well-studied essential role for mineralization and maintenance of healthy skeleton and healthy bones [3–7].

Ultraviolet B (UVB) radiation (280–315 nm) converts 7-dehydrocholesterol (7-DHC) into previtamin D which further isomerizes to vitamin D1 by heat [8]. Afterwards vitamin D is metabolized to 25-hydroxyvitamin D (25(OH)D) in liver and in several other tissues [9]. Being the major circulating form of vitamin D in blood, 25(OH)D is metabolized to its active form 1,25-dihydroxyvitamin D (1,25(OH)2D) in kidneys and many nonrenal tissues, including bone, placenta, prostate, keratinocyes, etc. [9]. As steroid hormone, 1,25(OH)2D regulates calcium metabolism and bone health, a wide variety of genes in more than 30 different tissues, including brain, liver, kidney, prostate, and have important function in regulating cell growth, modulating immune system and cardiovascular health [2,7].

Selective sensitivity of vitamin D synthesis to the UVB part of solar spectrum makes almost impossible to give recommendations for effective solar exposure times because of daily, seasonal and latitudinal variability in the UVB intensity caused by changes in the ozone layer thickness, clouds, aerosols and air pollutions as well as personal sensitivity [10–12]. Insufficiency in solar UVB irradiation can lead to vitamin D deficiency in humans and thus create or increase already existing health problems.

On the other hand, indoor tanning became popular procedure, especially in high-latitude countries. Most of tanning devices have fluorescent lamps with erythemal-effective radiant exposure $H_{eq}$ (in the EU by law: $H_{eq} = 0.3 \text{ W/m}^2$) close to that in natural sunlight, but the ratio between UVA ($315–400$ nm) and UVB irradiances of these lamps is very different from the ratio in the midday summer sun [13]. Nevertheless, sunbed use may lead not only to cosmetic effects (tanning), but can also increase the human vitamin D level [14–16]. Thus the dosimetry of UV radiation is needed not only to
avoid the harmful effects of UV radiation, such as sunburn, photaging, and skin cancer, but because of the positive effect of UV radiation to synthesize vitamin D.

The estimation of harmful UV levels is traditionally carried out by a variety of commonly used broadband UV detectors which have an output in terms of sunburn units. Because of the complexity of the processes involved in vitamin D synthesis, a dosimetry using measured accumulated UV dose will not provide a satisfactory quantification of vitamin D synthesis, especially in view of significant difference between the CIE erythema and ‘Vitamin D synthetic capacity’ action spectra (Fig. 1) [12,17,18].

With the aim to provide correct measurement of the vitamin D synthetic capacity of different UV sources, the original ‘dosimeter’ has been developed [20–22]. This method is based on UV exposing of 7-DHC solution, recording UV absorption spectra and further spectrophotometric analysis of formed vitamin D photoisomer mixture, i.e. on the first photochemical stage of vitamin D synthesis in vitro. As a result, the quantity of previtamin D (direct vitamin D precursor) is obtained which is the biologic measure of accepted ‘antirachitic’ UV dose. Earlier laboratory and field tests have revealed that the ‘Dosimeter’ is useful for in situ measurements of the vitamin D synthetic capacity of different UV sources [12,22–24]. Furthermore, thin film UV sensors (polymeric and liquid–crystalline) based on provitamin D photoconversions have been developed to avoid the inconvenience of liquid solvent use and to simplify the read-out [25,26].

The main goal of the present study is to perform direct measurements of the vitamin D level in blood of healthy volunteers exposed to artificial UV source (sunbed), in parallel with measurements of vitamin D generation in vitro using ‘Dosimeter’. Then, such study can provide a missing link between in vivo and in vitro measurements and will be useful for obtaining of adequate vitamin D status by sunbed exposure.

2. Materials and methods

2.1. UV source

The UV source was a commercially available and approved sunbed Wolff Suveren 53G equipped with 2 types of fluorescent lamps, ‘Brun og blid’ 100W and ‘Suveren S’ 25W spaghetti tubes (Wolff System, Basel, Switzerland). The UV radiation spectra of the sunbed were measured using portable spectrometer Avantes Ava-Spec-2048x14 Fiber Optic Spectrometers. The erythema weighted irradiance was calculated and exposure time for 1 standard erythema dose (1 SED, or 100 J/m²) was determined.

2.2. In vitro estimation of previtamin D accumulation using ‘Dosimeter’

As was mentioned, UV irradiation of 7-DHC leads to formation of previtamin D which further is thermally converted into vitamin D. In this manner the amount of previtamin D accumulated during UV exposure is a measure of the biologically active ‘antirachitic’ UV dose.

However, previtamin D absorbs at the same spectral region as provitamin D and thus undergoes a number of side photoconversions [27]. An important point is that the photoisomers mixture composition (the ratio between previtamin D and the side-products concentrations) strongly depends on the UV irradiation wavelength [21,27].

With the aim to measure the concentration of formed previtamin D in vitro, solutions of 7DHC in ethanol (C = (2.5 ± 0.3) × 10⁻⁵ mol/L) were placed in a rectangular quartz cuvettes (d = 1 cm), and further cuvettes were exposed inside the sunbed from two sides by upper and lower lamps. The UV absorption spectra were recorded before and after several exposures with a Perkin–Elmer Lambda 40 UV/VIS spectrophotometer (Norwalk, CT, USA). At last, the previtamin D concentration was determined from the recorded UV spectra using specially designed PC software [21,22].

2.3. In vivo study of 25(OH)D level in healthy volunteers

Thirty-two healthy volunteers living in Oslo (59°N) have participated in the study which was approved by Regional Ethical Committee, and each participant gave informed consent.

All the participants filled out the questionnaire with points about age, weigh, height and skin type. Age of volunteers ranged from 21 to 61 years, and the average age was 31.3 years. All the participants had normal weight with body mass index (BMI) from 20 to 25, and the average BMI in the group was 23.4. Most of the participants had Fitzpatrick skin Type II.

The study lasted 10 weeks. Each participant was exposed 2 times per week during irradiation time corresponded to erythema dose 0.74 MED (or 185 J/m², or 1.85 SED) in conditions of whole body exposure. To avoid any contribution from solar radiation, the study was conducted during the winter months (January to March) when no vitamin D is synthesized in skin under sunlight in Oslo [12].

Their blood was sampled before the start of the investigation, then before 4, 8, 12 and 16 exposure, and 3 days after all 20 sunbed sessions. Serum was separated from the blood cells by centrifugation and then frozen to −20 °C. All samples were analyzed at Haukeland University Hospital (Bergen, Norway) using a modified liquid chromatography-mass spectrometry method (LC/MSD SL; Agilent Technology, CA) [28].

3. Results

Measured sunbed irradiance spectra³ are shown in Fig. 1 in comparison with calculated solar spectra in Oslo (59°57'N, 10°45’E) using FASTRT program (http://nadir.nilu.no/~oelaeng/fastrt/fastrt.html, June, 22, GMT 11:00, ozone layer thickness 300 DU,

Fig. 1. The UV irradiance spectra from top (1) and bottom (2) sunbed parts and solar spectrum in Oslo (3) combined to CIE action spectra of erythema (4) and previtamin D synthesis in vivo (5) and with action spectrum of previtamin D formation in vitro (6).

Nevertheless, it was shown that the individual UV-erythemal sensitivity is a good marker of the individual efficiency of the resulting 25(OH)D₃ in blood serum after solar or solar-simulated UV exposure [19]. Besides, for several sunbeds correlation was found between exposures determined on the basis of erythema dose and increase of 25(OH)D₃ in serum [14–16].

³ The bottom of the sunbed contains only ‘Brun og blid’ 100W fluorescent lamps, whereas the top part includes both ‘Brun og blid’ 100W and ‘Suveren S’ 25W tubes.
clouds- and aerosol-free conditions). In addition, CIE action spectra of erythema [29] and previtamin D synthesis in vivo [30] are shown in Fig. 1 as well as previtamin D absorption spectrum which represents the action spectrum of initial vitamin D synthesis in vitro [31] in accordance with the first law of photochemistry [32].

The physical irradiance (unweighted) of the UVB part of the sunbed radiation was 1.3 ± 0.15 W/m² which is close to the UVB intensity of summer sunlight in Oslo (1.4 W/m²). The physical irradiance (unweighted) of UVA in the radiation of the sunbed was 212 ± 13 W/m², which is almost 4 times larger than that in solar radiation (54 W/m²). However, the erythemal effective irradiance of sunbed and solar radiation differ only by a factor of two (0.31 ± 0.03 W/m² comparing with 0.18 W/m²). Thus, the exposure times for obtaining 1 SED are 5.4 min and 9.3 min, respectively.

Initial absorption spectrum of previtamin D solution is presented in Fig. 2 together with the spectra recorded after 2-h exposure to sunlight (Oslo, June 29, clouds-free day) and to sunbed; and more remarkable transformation of the initial absorption spectrum under sunbed irradiation indicates more effective previtamin D formation [21].

The concentration analysis using the 7-DHC absorption spectra recorded after fixed sunbed exposures (Fig. 3a) shows that 7-DHC and previtamin D are the main components of the photoisomer mixture whereas tachysterol and lumisterol are detected in much lower concentrations (Fig. 3b). Decrease of total photoisomers concentration (Sum) demonstrates remarkable photodegradation upon prolonged exposures. Note that 0.74 MED (1.85 SED) achieved after ~10 min of sunbed exposure corresponds to only (3 ± 1)% of previtamin D accumulated in vitro.

The volunteers were divided into three groups based on the results of 25(OH)D measurements at the start of the investigation: 11 participants had insufficient levels (25–50 nmol/L), 13 ones had marginal (50–75 nmol/L) and the last 8 persons had sufficient levels (75–150 nmol/L) in blood (Fig. 4a) [7]. Overall changes of vitamin D status of volunteers after all 20 sunbed exposures are presented in Fig. 4b. As one can see, only 2 participants remained with insufficient levels, 11 had marginal and 19 had sufficient 25(OH)D levels that demonstrates the efficiency of sunbed irradiation to improve vitamin D status.

Statistical analysis for the three groups of volunteers before and after all sunbed exposures is shown in Fig. 5. As expected [19], the median value increased in all three groups but the 25(OH)D data spread after sunbed exposures became wider comparing to initial.

More detailed changes of 25(OH)D distribution median from initial concentration of 7-DHC (0.3 mol/L) under sunbed irradiation; Pro – provitamin D, Pre – previtamin D, T – tachysterol, L – lumisterol, Sum – sum of mentioned photoisomers.

A comparison of the above data reveals the linear correlation with high correlation coefficients R between in vivo changes of 25(OH)D distribution median in the three volunteer groups and in vitro accumulated previtamin D (Table 1, Fig. 7).

Two scales of previtamin D accumulation in vitro are shown in Fig. 7 that is the scale in previtamin D percentage in relation to initial 100% concentration of 7-DHC is of more general usage whereas the scale in absolute numbers mmol/L is related to specific initial concentration of 7-DHC (C = 24 μmol/L) only.

The coefficient A represents the median of the initial 25(OH)D level whereas coefficient B (the slope of linear dependences) represents the efficiency of 25(OH)D photobiogenesis in each group. The observed decrease in the slope from 0.7 to 0.46 with increasing of initial 25(OH)D value from insufficient up to sufficient level means that humans with vitamin D-deficiency are more sensitive and responsive to UV exposure than humans with normal vitamin D status that is valid for UV exposure of whole body by 0.7 MED or higher [19].

Going from 25(OH)D value to increment of 25(OH)D (depending on in vivo initial 25(OH)D and in vitro accumulated previtamin D), it is possible to describe all the experimental data simultaneously using 3D fit: $C_{25(OH)D, mmol/L} = (A + B/C_{25(OH)D, mmol/L}(initial))^{C_{PrevD,3}}$. The corresponding parameters are presented in Table 2.
4. Discussion and conclusions

We note that for the first time in vivo 25(OH)D changes in human blood under sunbed UV radiation was linked to previtamin D accumulation in vitro. As one can see, sunbed is effective for improving of vitamin D status (Fig. 5). Participants from group with insufficient 25(OH)D levels have turned into marginal and sufficient groups whereas most of participants from marginal group have elevated the vitamin D status up to sufficient. As for participants with sufficient vitamin D status at the start of investigation, they remained within the group, but the 25(OH)D levels have remarkably increased. Summing up, the overall growth of 25(OH)D level under sunbed exposures is more noticeable for people with initially lower vitamin-D status that is in excellent agreement with previous investigations of vitamin D metabolism in vitamin-D deficient subjects [28,33].

Besides, detailed analysis of growth of 25(OH)D levels shows that around 15 sunbed sessions per 10 min leads to maximal 25(OH)D levels of ~64 nmol/L for insufficient group, ~81 nmol/L for marginal group and ~104 nmol/L for sufficient group (Fig. 6). Further UV radiation up to 20 sunbed sessions almost doesn’t increase 25(OH)D level and thus has no effect on vitamin D status.

In conclusion we note that linear correlations between in vivo changes of 25(OH)D distribution median in three volunteer groups and in vitro photosynthesis of previtamin D. For detailed parameters of linear fits see Table 1.

### Table 1
Parameters of linear fit $C_{25(OH)D, nmol/L} = A + BC_{PreD, %}$ for three volunteer groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>$A$</th>
<th>$A$ error</th>
<th>$B$</th>
<th>$B$ error</th>
<th>Correlation coefficient $R$</th>
<th>Standard deviation $SD$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insufficient</td>
<td>38.8</td>
<td>2.2</td>
<td>0.70</td>
<td>0.07</td>
<td>0.979</td>
<td>2.6</td>
</tr>
<tr>
<td>Marginal</td>
<td>61.5</td>
<td>1.4</td>
<td>0.54</td>
<td>0.05</td>
<td>0.985</td>
<td>1.7</td>
</tr>
<tr>
<td>Sufficient</td>
<td>85.4</td>
<td>1.5</td>
<td>0.46</td>
<td>0.05</td>
<td>0.977</td>
<td>1.8</td>
</tr>
</tbody>
</table>

### Table 2
Parameters of 3D fit $A_{25(OH)D, nmol/L} = (A + B/C_{25(OH)D, nmol/L(initial)})C_{PreD, %}$ for all the experimental data.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Estimate</th>
<th>Standard error SE</th>
<th>t-Statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$</td>
<td>0.21</td>
<td>0.05</td>
<td>3.9</td>
<td>0.0013</td>
</tr>
<tr>
<td>$B$</td>
<td>20.7</td>
<td>2.7</td>
<td>7.6</td>
<td>$1.1 \times 10^{-8}$</td>
</tr>
</tbody>
</table>

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**Fig. 4.** Number of participants with insufficient (25–50 nmol/L), marginal (50–75 nmol/L) and sufficient (75–150 nmol/L) levels of 25(OH)D before (a) and after (b) all 20 sunbed exposures.

**Fig. 5.** Box plots of 25(OH)D distributions before and after all sunbed exposures for three volunteer groups: boxes represent interquartile range around median (also depicted), whiskers show 1.5 interquartile ranges above and below the boxes, stars indicate outliers outside the whiskers.

**Fig. 6.** Detailed changes of 25(OH)D distribution median in three volunteer groups on sunbed cumulative irradiation time (20 sessions of 10 min).

**Fig. 7.** Linear correlations between in vivo changes of 25(OH)D distribution median in three volunteer groups and in vitro photosynthesis of previtamin D. For detailed parameters of linear fits see Table 1.
for predictions of vitamin D status based on the measurements of previtamin D accumulation in vitro. Certainly, obtained in this study correlations should be re-measured for sunbed equipped with other fluorescent lamps because previtamin D photosynthesis strongly depends on the irradiation spectrum of an UV source [21].

Taking into account the non-linear dependence of previtamin D photoinduced accumulation correlating with the results of different UV exposures [19], the lower sunbed exposures would be preferable from the point of view of the safety requirements especially for the lamps with elevated ratio UVB/UVA.

Furthermore these data need to be detailed depending on age, BMI, Fitzpatrick skin type, presence or absence of regular vitamin D intake, etc., that requires large epidemiological studies for completeness statistics.

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