# Layer Thickness of SPF 30 Sunscreen and Formation of Pre-vitamin D

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**Abstract.** *Background: Most studies have demonstrated that sunscreens with lower sun protection factor (SPF) do not prevent the production of vitamin D because much lower amount of sunscreen (SPF<30) is applied than recommended (2 mg/cm$^2$) indicating that a significant amount of UV radiation can penetrate the skin. Since less sunscreen is applied, higher SPF sunscreens may be used to achieve the desired protection. However, there is little information regarding the application of high-SPF sunscreen and vitamin D formation. The aim of this study was to measure the influence of the amount of two SPF 30 sunscreens on pre-vitamin D formation in a cuvette with 7-dehydrocholesterol. Results: Sunscreen with physical (reflecting) or chemical (absorbing) UV filters exhibits different levels of protection in vitro even if the SPF is the same. The level of photoprotection is differentially reduced when less sunscreen than the recommended application thickness is applied. Conclusion: The usual application of 0.8-1 mg/cm$^2$ is below the recommended value of 2 mg/cm$^2$, and pre-vitamin D may be formed when lower amounts of SPF ≤30 sunscreen are applied, showing that a significant amount of UV radiation may enter the skin.*

Solar UV radiation is the main environmental skin carcinogen (1). Both total solar UV exposure and the UV exposure pattern influence the effect of UV on human health (2). The regular use of sunscreens prevents skin photodamage, sunburn and skin cancer (3). The application of sunscreen reduces the penetration of UVB (280-315 nm) radiation responsible for vitamin D production in human skin (4).

Vitamin D plays an essential role in mineral homeostasis and skeletal health. Vitamin D deficiency has been associated with increased risk of osteoporosis, hypertension, certain infectious diseases, diabetes, multiple sclerosis, cardiovascular diseases, and some types of cancer (5). Only a limited number of dietary sources contain vitamin D. The major source of vitamin D for most humans is the exposure to sunlight.

When the skin or a solution of the precursor of vitamin D, 7-dehydrocholesterol (7-DHC), is exposed to sunlight, the action of solar UVB causes the conversion of 7-DHC to pre-vitamin D. Once pre-vitamin D is formed (first step), it is further transformed by the rearrangement of its double bounds into vitamin D (second step, Figure 1).

A variety of factors influence cutaneous production of vitamin D, including latitude, season, time of the day, ozone amount, cloud cover, aerosols in the atmosphere, surface albedo, skin pigmentation, clothing and the usage of sunscreen (6, 7). The topical application of a sunscreen (2 mg/cm$^2$) may reduce the production of vitamin D by as much as 99% because less UVB photons are entering the skin and reaching the 7-DHC (7, 8). However, individuals apply less than 2 mg/cm$^2$ sunscreen (9-16), and some pre-vitamin D may be formed in the skin. On the other hand, this may be compensated by using sunscreens with high sun protection factor (SPF) (17). Faurschou and co-workers (18) demonstrated that vitamin D production increases exponentially with decreasing amount of applied sunscreen. Most studies demonstrate that sunscreens with low SPF do not prevent production of sufficient vitamin D because in real life since much lower than recommended (2 mg/cm$^2$) amounts of sunscreen (SPF<30) are applied and not all body parts are covered with sunscreen (19). Thus, it is necessary to test if sunscreen itself does allow some UVB to go through the upper layers of the skin and initiate pre-vitamin D production. This can be investigated by conducting an *in vitro* study by testing the impact of different sunscreen layer thickness on pre-vitamin D formation in a cuvette containing a 7-DHC solution.

5th International Symposium of Biologic Effects of Light that was held in Homburg, Germany, June 2015.

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*Key Words:* Sunscreen, skin protection, solar ultraviolet radiation, 7-dehydrocholesterol, vitamin D.

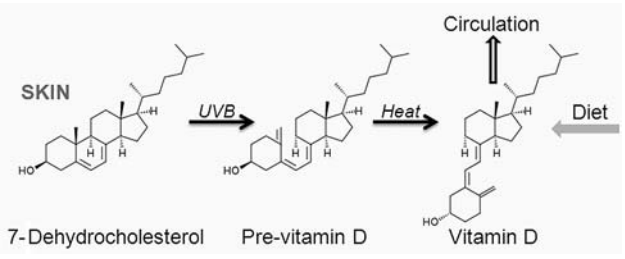


Figure 1. *Schematic representation of vitamin D production.*

The aim of this study was to measure the influence of the amount of two SPF 30 sunscreens on pre-vitamin D formation in a cuvette containing 7-DHC.

## Materials and Methods

*Experimental setup*. Pre-vitamin D formation *in vitro* was investigated using an experimental arrangement consisting of a solar simulator (emitting 0.11 mW/cm$^2$ of UVB), a quartz cuvette containing 0.1 mM 7-DHC dissolved in ethanol, and two quartz plates with different amounts of sunscreen with SPF 30 between them. Quartz plates and the cuvette were ventilated by air flow at room temperature. Five milliliters of 7-DHC solution in a quartz cuvette having a path length of 1 cm was exposed to simulated solar radiation filtered by different amounts of sunscreen. A cuvette with a sample was placed at a distance of 35 cm from the solar simulator in the path of the beam. This system served as an approximation of the first step of vitamin D production in the skin influenced by topical sunscreen application.

*Simulation of solar spectrum*. Solar spectrum in the Canary Islands (28.1˚N, 15.4˚E; Figure 2) was calculated using Coupled Ocean Atmosphere Radiative Transfer (COART) model (parameters used: June 21, GMT 13:00, ozone layer thickness 300 DU, cloud-free conditions) (20).

*Preparation of 7-DHC solution*. Forty milliliters of 7-DHC stock solution was prepared by dissolving 15.4 mg of 7-DHC (Sigma-Aldrich, Oslo, Norway) in ethanol. Five milliliter samples of 0.1 mM 7-DHC were prepared by diluting the stock solution.

*Sunscreen application*. Two different commercially available sunscreens (A and B) with SPF 30 from different manufacturers were chosen to examine their capability to absorb UV radiation and so to prevent pre-vitamin D formation. Sunscreen A was a cream which contains two active ingredients: Methylene-*bis*-benzotriazolyl tetramethylbutylphenol (nano) and titanium dioxide (nano). Methylene-*bis*-benzotriazolyl tetramethylbutylphenol (nano) is a hybrid type sun-blocking agent acting both as chemical and physical UV filter. It covers both UVA and UVB ranges. Titanium dioxide is a physical UV filter protecting against UVB and short UVA (280-350 nm). The main protection mechanism of this sunscreen is due to physical filters which mostly reflect, scatter and block UV radiation. Sunscreen B was a lotion which contains six chemical UV filters: Octocrylene, butyl methoxy-dibenzoyl methane (avobenzone), ethylhexyl salicylate (octisalate), homosalate, *bis*-ethylhexyloxyphenol methoxyphenyl triazine and phenylbenzimidazole sulfonic acid (ensolizole). Octocrylene absorbs UVB and shortwave UVA (covering 290-350 nm). Butyl methoxy-dibenzoyl methane absorbs UVA radiation. Ethylhexyl salicylate and homosalate absorb only UVB radiation, and do not protect against UVA. Bis-ethylhexyloxyphenol methoxyphenyl triazine covers both UVB and UVA ranges; peak protection at 348 nm. Phenylbenzimidazole sulfonic acid absorbs UVB and part of UVA radiation (290-340 nm). The photoprotection mechanism of this sunscreen is due to chemical filters which absorb UV radiation.

Sunscreens were spread between two 2.5×5.0 cm quartz plates. The necessary amount of the sunscreen between the plates was obtained by pressing them together and wiping-off the excess sunscreen. The remaining sunscreen was weighed every time after wiping on a calibrated scale with 0.1 mg resolution. The selected amounts were 0, 0.5, 1, and 2 mg/cm$^2$. For reflectance measurements, 9 cm$^2$ surface area of forearm was covered by 18 mg of sunscreen (2 mg/cm$^2$).

*Action spectra*. The data for pre-vitamin D formation in the skin (Figure 2) were extracted from the work of MacLaughlin and co-workers (21). Normalized absorption spectrum of 7-DHC in ethanol was used as a reference spectrum for pre-vitamin D formation *in vitro* (Figure 2).

*Reflectance spectroscopy*. Reflectance measurements of the skin (Figure 3) were performed using Perkin-Elmer LS45 luminescence spectrometer (Perkin Elmer, Waltham, MA, USA) equipped with a Hamamatsu R3896 photomultiplier (Hamamatsu Photonics, Toyooka, Iwata-Gun, Shizuoka, Japan). The instrument was fitted with an in-house-built integrated sphere coated with barium sulfate connected to the instrument with two optical fibre bundles. The reflectance spectrum is given by R(λ):

$$R(\lambda)=(S(\lambda) - S_{DARK}(\lambda))/[S_{REF}(\lambda) - S_{DARK}(\lambda)],$$

where S(λ) is the recorded signal on a skin, $S_{DARK}(\lambda)$ is a spectrum recorded in the absence of a sample, and $S_{REF}(\lambda)$ represents the spectrum of a white reference standard defining 100% of reflectance. Labsphere USRS-99-010 (Labsphere Inc., North Sutton, NH, USA) was the reference standard.

*Transmission and absorbance measurements*. Transmission of UV radiation through the sunscreen layer (Figure 4) and the absorption of irradiated samples (Figure 5) were measured using a Lambda 40 UV/VIS spectrophotometer (Perkin-Elmer, Norwalk, CT, USA). A scanning speed of 60 nm/min, slit size of 1 nm, and 2 nm smoothing were selected in order to obtain sharp absorption peaks and to maximize the signal-to-noise ratio.

*High-performance liquid chromatography (HPLC)*. Twenty microliter samples of irradiated 7-DHC solution were injected by an autosampling injector (Gilson 234, Gilson, France) into a Hichrom Hypersil H5ODS-250A 250×4.6 mm column (Hichrom Ltd., Theale, Reading Berkshire, UK). The mobile phase of the isocratic method consisted of HPLC-grade methanol and water (95:5 v/v) purchased from Fisher Chemicals (Västra Frölunda, Sweden). A low-pressure gradient pump system (P680A LPG, Dionex, Germering, Germany) with integrated degasser was used to generate a flow rate of 1 ml/min. The column effluent was

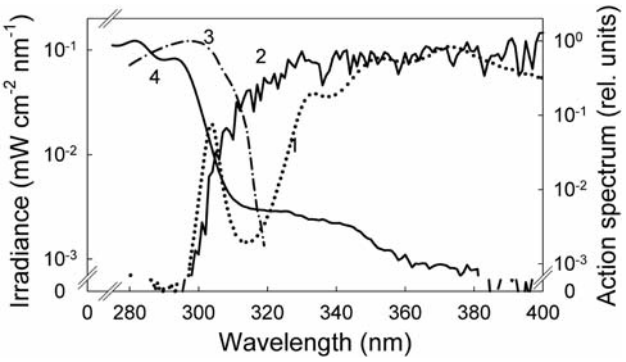


Figure 2. *The spectra of the solar simulator (1) and sun in the Canary Islands (2), the action spectra of pre-vitamin D formation in vivo (3) and 7-dehydrocholesterol absorption in ethanol (4) as the reference spectrum for pre-vitamin D formation in vitro.*

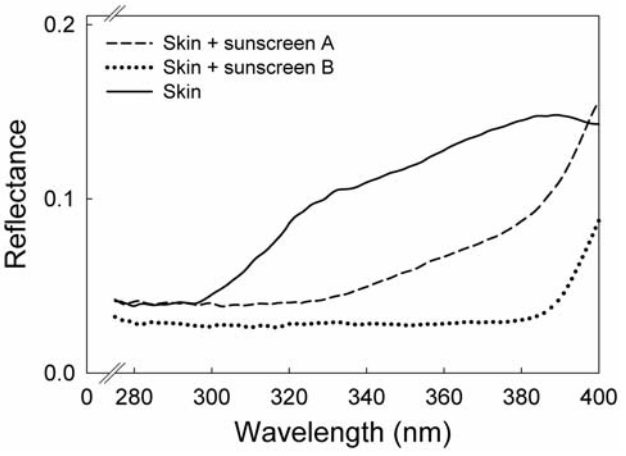


Figure 3. *Reflectance spectra of the skin (forearm) without and with sunscreen application. Reflectance spectra were measured 3 h after sunscreen A (2 mg/cm$^2$) and B (2 mg/cm$^2$) application.*

monitored at 281 nm by an UV detector Spectra 200 (Newport Spectra-Physics, Darmstadt, Germany) connected to the chromatography interface UCI-100 (Dionex). All the chromatographic separations were carried out at ambient temperature. 7-DHC standards were created at four calibration levels (100, 50, 25, and 10%), where the highest concentration of 100% corresponds to 0.1 mM. Chromeleon software (version 6.50, Dionex) was used for data acquisition and for calculation of the peak areas.

*UVB sources*. Air Mass 1.5 solar simulator model 16S-300-002 (Solar Light Co., Inc., Glenside, PA, USA) equipped with a 150 W xenon arc lamp and Air Mass filters was generating 260 mW/cm$^2$ of total irradiance at the chosen current of 18 A. The UVB intensity was calculated by integrating the spectral irradiance in the range of 280-315 nm. The spectral irradiance was measured using a portable Avantes AvaSpec 2048×14 FiberOptic spectrometer (Azpect Photonics AS, Södertälje, Sweden). Total irradiance was measured using Gentec Solo2 power meter equipped with a Gentec solid state thermopile (Laser Components Nordic AS, Göteborg, Sweden).

Twenty-minutes irradiation to a broad-band UVB source (TL12 fluorescence tubes, Philips, Eindhoven, the Netherlands) following 18-hour incubation at 37˚C was used to produce all the 7-DHC photoproducts in ethanol (Figure 6A) for the representation of chromatographic peaks.

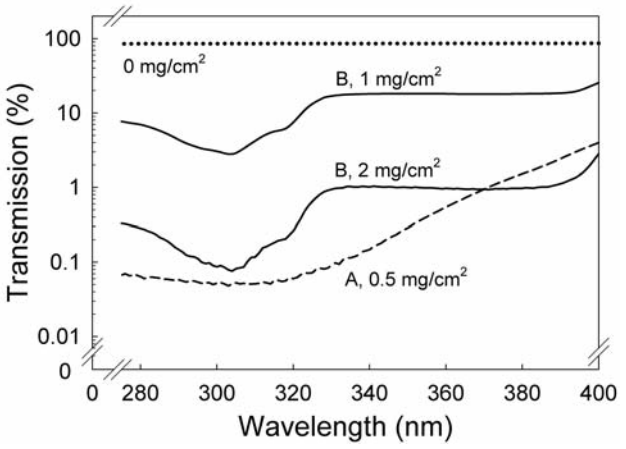


Figure 4. *Transmission spectra of two sunscreens used in the experiment. The dotted line represents transmission through two quartz plates without sunscreen between them. The dashed and solid lines represent the transmission spectra of sunscreens A and B, respectively.*

## Results

*Vitamin D-effective UV radiation*. The integrated UVB irradiances of the solar simulator and summer sunlight in the Canary Islands were calculated to be 0.11 mW/cm$^2$ and 0.26 mW/cm$^2$, respectively. The corresponding pre-vitamin D (*in vitro*) weighted irradiances were 0.015 mW/cm$^2$ and 0.017 mW/cm$^2$.

*The impact of sunscreen on UV reflectance from the skin*. Sunscreen A worked more like physical UV filter, while chemical absorption was more important for sunscreen B (Figure 3). The highest difference in reflected radiation appeared to be in UVA1 region (340-400 nm), which has no influence on pre-vitamin D production.

*Transmission of light through sunscreen*. The exposure to the solar simulator for 3 h did not change the transmission spectra of either sunscreen (data not shown). Eighty-five percent of the incident UVB was able to penetrate two quartz plates of 3 mm thickness each. The UVB transmission decreased to 3% when 1 mg/cm$^2$ of sunscreen B was spread between the plates, while the corresponding value for 0.5 mg/cm$^2$ of sunscreen A was less

Table I. *The concentrations of 7-dehydrocholesterol (DHC) and its photoproducts measured using high-performance liquid chromatography after 3-h exposure to radiation emitted by a solar simulator (0.11 mW/cm$^2$).*

| Sunscreen and thickness | Concentration of 7 DHC (mM) | Increase in pre-vitamin D (%) | Increase in lumisterol and tachysterol (%) |
|---|---|---|---|
| A, 2 mg/cm$^2$ | 0.100 | 0 | 0 |
| A, 0.5 mg/cm$^2$ | 0.100 | 0 | 0 |
| B, 2 mg/cm$^2$ | 0.098 | 0 | 0 |
| B, 1 mg/cm$^2$ | 0.086 | 22.1 | 5.4 |
| Without sunscreen | 0.031 | 100 | 100 |

Table II. *The reported densities of applied sunscreen in previous studies.*

| Author (Ref) | Number of participants | Density of sunscreen (mg/cm$^2$) |
|---|---|---|
| Stenberg and Larko (9) | 50 | 1.00 |
| Bech-Thomsen and Wulf (10) | 42 | 0.50 |
| Szepietowski, Nowicka, *et al*. (12) | 49 | 0.94 |
| Reich, Harupa, *et al*. (13) | 52 | 0.68 |
| Petersen, Datta, *et al*. (14) | 20 | 0.79 |
| | Sum: 213 | Average: 0.8 |

than 0.1%. Transmission measurement of the 2 mg/cm$^2$ layer of sunscreen A was below the sensitivity of the spectrophotometer.

*Pre-vitamin D formation in vitro*. The absorption spectra of irradiated 7-DHC solution in the absence of sunscreen continuously decreased at the two highest peaks (272 and 281 nm) showing 7-DHC depletion. Absorption at wavelengths below 260 nm indicates mainly the formation of pre-vitamin D (Figure 5A). The recommended amount (2 mg/cm$^2$) of both sunscreens completely inhibited any formation of 7-DHC photoproducts (Figure 5B, Table I). A 1 mg/cm$^2$ layer of sunscreen B transmitted a sufficient amount of UVB for pre-vitamin D production (Figure 5B). A 0.5 mg/cm$^2$ layer of sunscreen A did not allow 7-DHC photoconversion even after 3 hours of irradiation.

*HPLC separation*. HPLC chromatogram showed four peaks (Figure 6A). The peaks of 7-DHC and vitamin D were identified by injecting the corresponding standards. The peak of pre-vitamin D was identified by following the pre-vitamin D → vitamin D channel and by spectrophotometric analysis (Figure 6B), where both the effluents in peak-1 and peak-2 had no signature of lumisterol or tachysterol absorption. The conclusion that lumisterol and tachysterol had the same retention times had also been checked spectrophotometrically (Figure 6B). The coincidence of the tachysterol and lumisterol peaks using methanol as a mobile phase is in agreement with the chromatographic results reported by Terenetskaya and co-workers (22).

Sixty nine percent of the initial 7-DHC in the absence of sunscreen was photoconverted to other products after 3-h exposure to the simulated solar radiation, while 14% of this vitamin D precursor was converted under the presence of 1 mg/cm$^2$ sunscreen B (Table I). In the latter case, 22% of pre-vitamin D was formed compared to that which had been produced without sunscreen. The corresponding formation percentage for lumisterol and tachysterol was lower and amounted for only 5%. The detector response for pre-vitamin D and lumisterol together with tachysterol was assumed to be linear. No pre-vitamin D formation was observed when 2 mg/cm$^2$ of sunscreens A or B were applied.

## Discussion

The reported median densities of sunscreens applied by the general public start from as little as 0.4 mg/cm$^2$ (15). A review of the literature involving 213 participants in total revealed that the average quantity of sunscreen in use is around 0.8 mg/cm$^2$ (Table II). In this table, all included investigations had similar number of participants. Each study was weighted according to the number of participants. One large prevention trial study that included 595 participants reporting a mean value of 1 mg/cm$^2$ (11) is not presented in Table II.

The solar simulator used in the experiment had similar pre-vitamin D-producing capacity *in vitro* as the sun being close to the zenith. One may expect that simulated solar light would have a similar effect on the skin even though the action spectrum for pre-vitamin D formation *in vivo* is

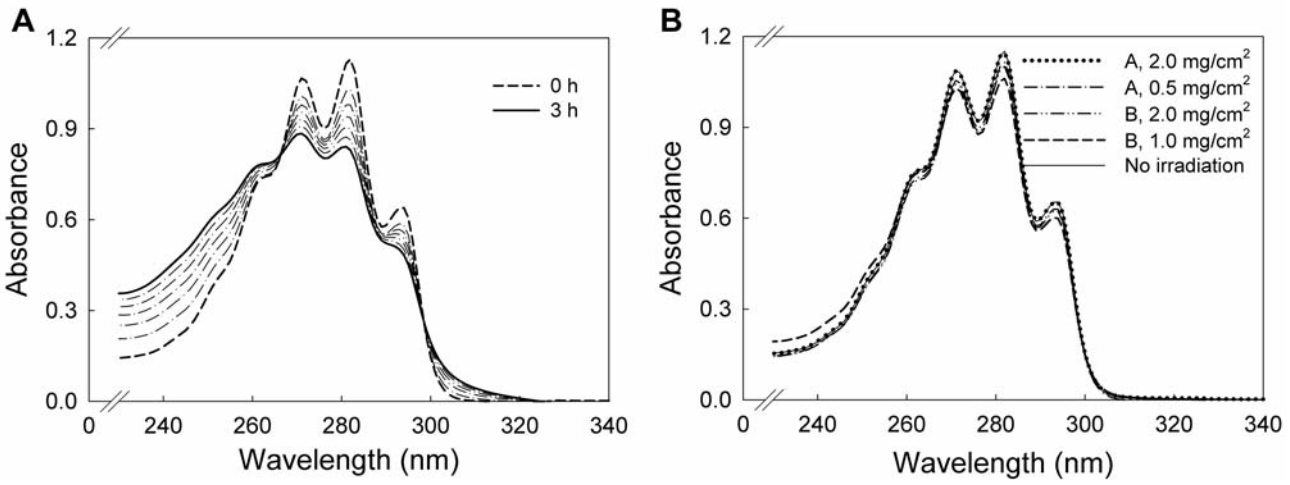


Figure 5. *The spectrum of 0.1 mM 7-dehydrocholesterol in ethanol (dashed line) and its photoproducts (dashed-dot lines) as a result of 3-h (solid line) exposure to the solar simulator measuring every 30 min (A) and the absorption spectrum after 3-h exposure to the simulated solar radiation that was filtered by different layers of the sunscreens (B).*

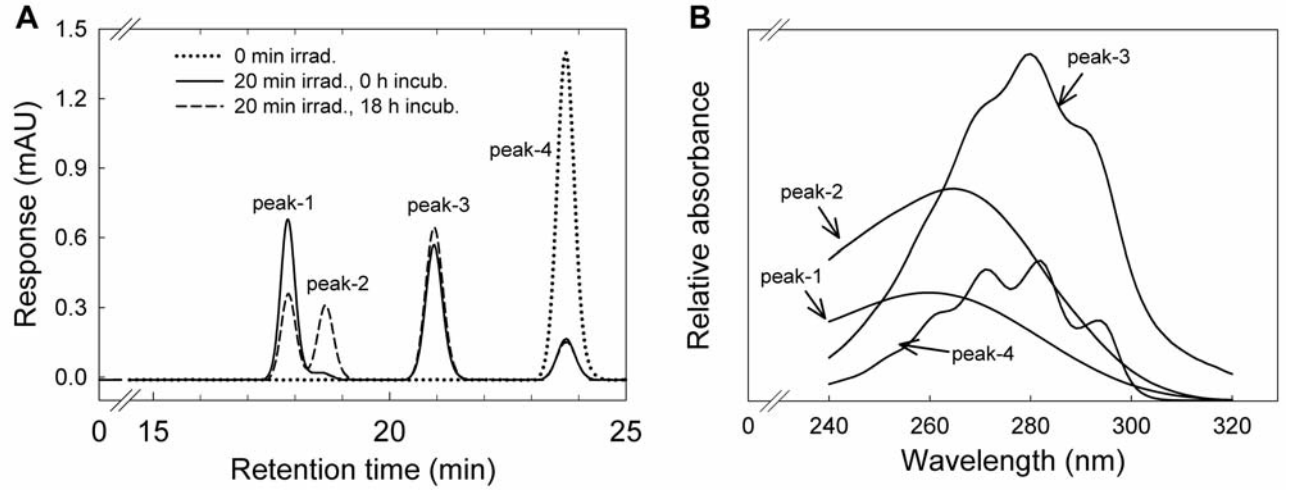


Figure 6. *The peaks of 0.1 mM 7-dehydrocholesterol and its photoproducts, as well as vitamin D, are shown in the chromatogram (A). Absorption spectra of pre-vitamin D (peak-1, ), vitamin D (peak-2), lumisterol together with tachysterol (peak-3) and 7-dehydrocholesterol (peak-4) are shown in panel (B).*

shifted to longer wavelengths by about 10 nm (Figure 2). A further analysis of the exact effect of spectral character on the modulation of pre-vitamin D photosynthesis is beyond the scope of this study.

The distribution of applied sunscreens on the skin influences the level of photoprotection, and the secondary information, such as reflectance (Figure 3) or transmission (Figure 4), may be easily misinterpreted (23). It is more difficult to define the real effect of physical sunscreen when not accounting for enhancement phenomenon of nanoparticles absorbed by the basal layers of the epidermis (24).

As expected, transparent sunscreen B showed a higher potential to transmit UV radiation than sunscreen A with physical filters, when both were applied between non-matte quartz plates (Figure 4). In order to avoid an additional loss of UV entering the 7-DHC solution due to scattering by sunscreen A, these plates were placed in contact with a cuvette having a front wall of dimensions 1×5 cm. However, even 0.5 mg/cm$^2$ of sunscreen A did not allow any pre-vitamin D production (Figure 5B, Table I). The SPF for 1 mg/cm$^2$ layer of the sunscreen B calculated using the model proposed by Sayre *et al*. (25) was 29 which is higher than the expected value of 15:

$$PF = \sum_{290}^{320} \varphi(\lambda) I(\lambda)\, \Delta\lambda / \sum_{290}^{320} \varphi(\lambda) I(\lambda)\, T(\lambda) \Delta\lambda = 29$$

where $\phi(\lambda)$ is the erythema action spectrum; $I(\lambda)$ is the spectral irradiance of the solar simulator; $\Delta\lambda$ is the integration interval taken to be 1 nm; and $T(\lambda)$ is the measured transmission.

In theory, a 1 mg/cm$^2$ layer should diminish the SPF even more than to 15 (26). Nevertheless, pre-vitamin D production was observed even at the lower transmission obtained theoretically (Figure 5B, Table I), which strengthens the conclusion that for longer UV exposures, a smaller amount of sunscreen than recommended is not sufficient for skin protection. Several important reasons do not allow us to state that the obtained relative numbers for pre-vitamin D production *in vitro* are valid in real life situations. Firstly, because of the difference in wavelength composition of UV sources (sun *versus* solar simulator), the data presented in Figure 5A may not follow photoconversion of 7-DHC in skin. Secondly, the ratio between photoproducts is also modulated by cutaneous optical properties. Finally, the concentration of 7-DHC (0.1 mM, or 38 μg/ml) was much higher than the concentration found in human epidermis (27). Self-absorption had only little impact on the results because the irradiance at the back of the cuvette was more than $10^{-0.15}$ or 70% of that at the front at the wavelengths longer than 300 nm where solar simulator was most efficient. Despite all other possible discrepancies between *in vitro* and *in vivo* models described in (28), pre-vitamin D formation in solution is comparable to pre-vitamin D production in skin for the same UVB dose (29). The 3-h exposure used in the experiment was close to a real-life sunbathing situation because the unweighted UVB irradiance emitted by the solar simulator was only about half of that of intensive solar radiation. The relatively high pre-vitamin D formation in the case of 1 mg/cm$^2$ of sunscreen B compared to production in the absence of sunscreen is due to the high initial rate of the pre-vitamin D increase, while accumulation of lumisterol and tachysterol takes place during prolonged exposure (30). However, the 14% decrease in 7-DHC concentration confirms that sunscreens even with as high SPFs as 30 may allow some pre-vitamin D formation in skin if the applied thickness is like that used by the general population (1 mg/cm$^2$, Table II).

This study confirms that sufficient UV may be received through the sunscreen itself, in addition to the lack of total skin cover (31) and UV exposure before application (32), to induce pre-vitamin D and, consequently, vitamin D production.

## Conclusion

Physical (reflecting) and chemical (absorbing) sunscreens have different levels of protection *in vitro* even if the SPF is the same. The level of protection is differentially reduced when less sunscreen than the recommended application thickness is applied. The usual application of 0.8-1 mg/cm$^2$ is far below the recommended value of 2 mg/cm$^2$, and pre-vitamin D may be formed *in vitro* when such lower amounts of sunscreen with SPF≤30 are applied, showing that a significant amount UV radiation may still enter the skin.

## Acknowledgements

Technical assistance given by Ghassan Shabat was a great help in performing the experiments. Fruitful discussions with Professor Irina Terenetskaya (Ukraine) are gratefully acknowledged.

The present work was financed by the South-Eastern Norway Regional Health Authority and by the Oslo University Hospital.

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*Received November 30, 2015*
*Revised January 13, 2016*
*Accepted January 14, 2016*