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A computational model for previtamin D₃ production in skin

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Low levels of vitamin D have been implicated in a wide variety of health issues from calcemic diseases to cancer, diabetes and cardiovascular disease. For most humans, the majority of vitamin D₃ is derived from sunlight. How much vitamin D is produced under given exposure conditions is still widely discussed. We present a computational model for the production of (pre-)vitamin D within the skin. It accounts for spectral irradiance, optical properties of the skin and concentration profile of provitamin D. Results are computed for various sets of these parameters yielding the distribution of produced previtamin D in the skin.

Introduction

Low levels of vitamin D† have been implicated in a wide variety of health issues including cancer, diabetes and cardiovascular disease.[1–3] For most humans, the majority of vitamin D₃ is derived from sunlight. Dietary intake of vitamin D is usually low as natural foods containing a significant amount of vitamin D are rare. In the skin, solar ultraviolet B (UVB) photons are absorbed by 7-dehydrocholesterol (provitamin D₃), leading to its transformation to previtamin D₃, which is rapidly converted thermally to vitamin D₃. Once formed, vitamin D₃ is metabolized in the liver to 25-hydroxyvitamin D₃ and then in the kidney, and elsewhere intracellularly to its biologically active form, 1,25-dihydroxyvitamin D₃. As ultraviolet radiation also causes skin damage leading to skin cancer, erythema and photoageing,[4] excessive sunbathing in order to achieve or maintain high levels of vitamin D₃ is very inadvisable. As with a lot of health issues, finding if there is a golden balance in this dilemma is one of the key questions in current photodermatology.[5–7] Bearing in mind that UV radiation is always connected with skin damage – which may be repaired to some extent – UV exposure should be minimized. Thus the question is how much vitamin D is needed for a good health status, and how much UV radiation would we need to expose ourselves to in order to get it? The latter part of this question will be addressed here by introducing a computational model predicting UV derived vitamin D production in human skin. The effect of variations in the different input parameters are shown and discussed to give an idea on the capabilities and restrictions of the model. This will lead to quantitative calculations of vitamin D production in real irradiation scenarios in the future.

The computational model for vitamin D₃ formation in skin

Taking the photosynthesis of previtamin D as a simple photochemical process, the amount of previtamin D formed depends on effective spectral irradiance and available educt provitamin D within the skin. The UV component of the spectral irradiance is strongly attenuated within the skin. On its way through the first hundred microns of the skin, it is diminished by about two orders of magnitude in the UVB wavelength range.[8] Given this strong dependence of available radiation on depth within the skin, the total amount and distribution of the educt provitamin D within this small range is crucial for the total amount of previtamin D₃ produced. A valid dose–response function applied to calculate the induced amount of vitamin D₃ should purely account for the photochemical reaction parameters, *i.e.* it must not contain matrix effects from skin optics *etc.* Ideally, data should be available from a transparent *in vitro* model providing a chemical environment similar to that in the skin, *i.e.* allowing for the previtamin D₃ molecules to be present in a physiological conformation. This is desirable as reaction kinetics partly depend on the conformation of the educt for both photo- and thermal reaction.[9] However, a physiological lipid–water environment has not been used for previtamin D₃ action spectroscopy so far, probably because of a considerable number of new parameters introduced into such an *in vitro* model which are difficult to account for, *e.g.* additional chemical processes such as lipid peroxidation by UV which cannot be counteracted in such an otherwise simple model, or a change of optical properties (scattering on vesicles). The next best substitute probably are *in vitro* models of 7-DHC in solution, for example in ethanol.

Based on these considerations, the computational model for vitamin D production in human skin was set up as follows: To compute the photoinduced production of vitamin D in skin, the

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spatial distribution of the irradiating light within the skin was calculated. The spectral irradiance as attenuated by the optical properties of the skin is calculated for the upper 200 μm of human skin (other depth ranges may be chosen). Only absorption is considered for the attenuation of UV radiation as scattered photons are still available for the photoisomerization reaction. Scattering is likely to change the light distribution within the tissue as backscatter leads to increased subsurface fluence at the cost of available radiation at deeper layers – more intricate approaches to radiation transport will account for this in future versions of the model. If factors outside the skin tissue such as the positioning of the skin towards the radiation source or reflectance from the skin surface are to be taken into account, the spectral irradiance should be corrected for them before use in the computational model. The light distribution within the skin is then weighted with the chosen response function and with the concentration profile of the educt provitamin D to calculate the total amount of previtamin D produced for a given set of parameters.

The computational model for the production of vitamin D in skin takes four input parameters:

- Spectral irradiance $E_0(\lambda)$ [W cm^{-2} nm^{-1}].
- Spectral attenuation coefficients $\mu_a(\lambda)$ [cm^{-1}].
- Response function $R(\lambda)$ [cm^2 J^{-1}].
- Concentration profile $c(z)$ [ng cm^{-2} μm^{-1}] where cm^{-2} is used to indicate flat skin area and μm is used to indicate depth; nm^{-1} refers to the spectral dependency.

The output of the model is a three dimensional production profile for the photoinduced reaction – in other words: How much previtamin D is produced from a given wavelength λ at a given depth z within the skin. This output may be integrated over depth to obtain a previtamin D effective spectral irradiance and/or over wavelength to receive the total or depth dependent amount of previtamin D produced:

$$\text{vitamin D prod.}[\text{ng cm}^{-2}\text{ s}^{-1}] = \int\int dz\, d\lambda E_0(\lambda)e^{-\mu_a(\lambda)z}c(z)R(\lambda)$$

To demonstrate the potential of the model and to show the influence of the input data, a defined set of input parameters were chosen.

Spectral irradiance

The natural source of ultraviolet radiation is sunlight. The spectral irradiance of the sun on earth is strongly dependent on season, time of day, latitude, stratospheric ozone condition, aerosols and weather conditions.[10] Exposure to ultraviolet radiation from artificial sources (UV lamps) is also possible for research, medical or cosmetic reasons. Several types of lamps with distinct spectral characteristics are available and in use for different applications. Some of them have been used for studies on vitamin D production in humans.

As pointed out before, how much irradiance of a given source enters the skin is a field of research on its own. Orientation of a given skin area to the radiation source, reflection from the skin surface and diffuse sources are among the complex parameters defining the available radiation at the skin surface. At this stage, the model does not include an internal module to calculate
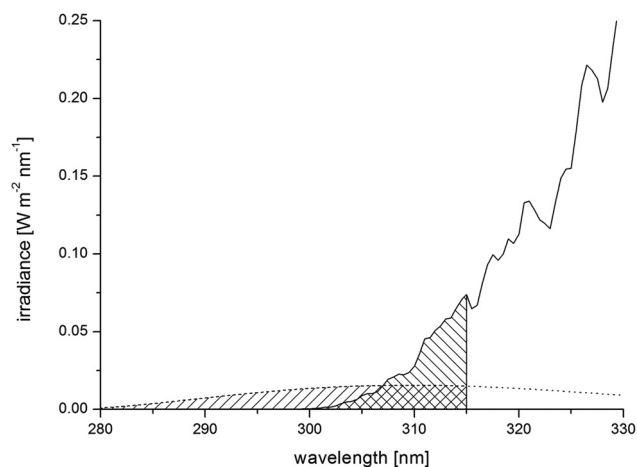

Fig. 1 Solar spectrum [ISO][11] (solid line) and broadband TL-12 lamp spectrum (dotted line) as used for the calculations. TL-12 lamp spectrum was scaled to match the total irradiance of the solar spectrum in the wavelength range 280–315 nm (hatched areas).

alterations of a given source spectrum upon incidence on a topographic skin surface. If desired, the input irradiance should already have been modified to account for these parameters.

Data from ISO 9845-1 describing direct plus circumsolar irradiance was chosen as the reference set of input data for solar spectral irradiance.[11] It assumes an airmass factor of 1.5, with a US Standard Atmosphere, an atmospheric optical depth of 0.084 at 500 nm, and 340 DU ozone. To show the difference to artificial sources of ultraviolet radiation, the base spectrum (without line peaks) of a TL-12 lamp was used. This lamp is widely used in UVB phototherapy. Total irradiance from the TL-12 lamp in the range up to 315 nm was scaled to the solar irradiance in that range (Fig. 1). With this scaling, the biologically weighted irradiation of the TL-12 lamp is *ca.* 2.4 times stronger for erythema and *ca.* three times stronger for vitamin D production than the solar irradiation based on the CIE standard spectra in the UV range >290 nm.

Spectral attenuation

Our own data on optical properties of human skin *in vivo* as partly published previously[12] were used to characterize the attenuation of radiation on its way through the skin (see Fig. 2). Data sets describing average absorption in Caucasian skin of phototypes I–IV ($n = 20$, I ($n = 3$), II ($n = 7$), III ($n = 5$), IV ($n = 5$)) were applied to model the optical characteristics of three different skin sites: inner side of forearm (relatively weak pigmentation), outer side of forearm (higher natural exposure to ultraviolet radiation and potentially stronger pigmentation), and ball of the thumb (weak pigmentation but thick horny layer).

Radiation transport in tissue again is a wide and complex field of research and may be simplified by different models depending on tissue characteristics.

Skin strongly absorbs ultraviolet radiation and scattering is mainly forward-directed.[13] Photons absorbed by other molecules are no longer available for pro- to previtamin D conversion. Scattered photons are still available for previtamin D induction,
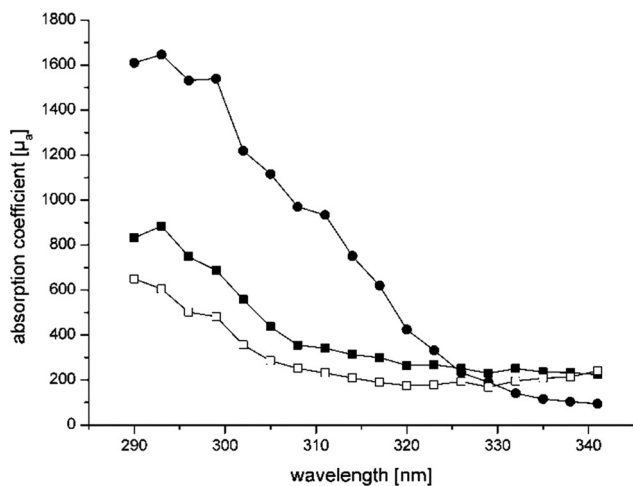
Fig. 2 Absorption coefficients of human skin *in vivo*. Data show average from measurements at the inner forearm (open squares), outer forearm (solid squares) and ball of the thumb (circles) of 20 volunteers.

Fig. 3 Distribution of provitamin D$_3$ (7-DHC) in human skin. Bars show the concentration per skin layer as published in Holick *et al.*[15] The line shows the smooth distribution with equal total concentration per layer.

there probably will however be a displacement in their place of action. Thus absorption may be considered to be more important to the computation of photochemical reactions in the tissue if the location of conversion is not that important. Keeping this in mind and aiming for the simplest approach allowed, we use exponential attenuation (Lambert–Beer law) by absorption only to model the development of the spectral irradiance with increasing depth within the human skin.

Concentration profile

Few data are available on the provitamin D content of skin[14–16] and even less is known about the distribution of provitamin D in skin. Computations presented here are based on the data from Holick *et al.*[15] *ca.* 58 ng cm^{-2} in the stratum corneum and stratum granulosum, 393 ng cm^{-2} in the stratum spinosum, 303 ng cm^{-2} in the stratum basale and 267 ng in the dermis, giving a total concentration of *ca.* 1021 ng cm^{-2}. To obtain a depth profile from this input data, the thickness of these layers were assumed to be 30 μm for the surface layers (stratum corneum and stratum granulosum), 30 μm for stratum spinosum, 15 μm for stratum basale and 125 μm for the superficial dermal layer containing provitamin D. It should be noted however that epidermal layers and overall thickness differs from skin site to skin site and stratum corneum is significantly thicker at the ball of the thumb. Two different approaches were followed concerning the distribution of provitamin D within these layers to obtain an idea of the influence of the distribution on vitamin D production. As a straightforward but oversimplifying approach, a uniform distribution within each layer was assumed leading to a step function for the concentration profile. Secondly, a pharmacological model (sigmoidal dose–response with variable slope) was used to produce a smooth profile with integral provitamin D content per layer as given in the Holick publication. Both distributions are shown for comparison in (Fig. 3).
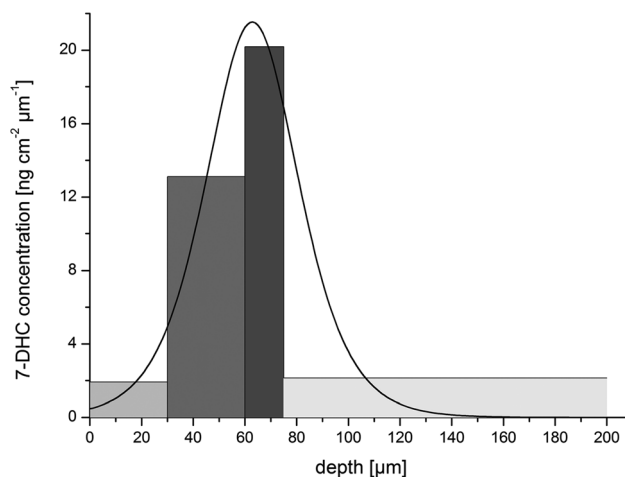
Response function

One branch of computations was done with an *in vitro* action spectrum[17,18] for vitamin D production. Data from this particular action spectrum – which will be referred to as the QUT action spectrum from here on – was chosen, as absolute values for concentrations and doses are available. A solution of 100 μg provitamin D per ml ethanol was used here as *in vitro* model. This concentration is in the range of the average amount of provitamin D per cm³ of (provitamin D containing) skin *in vivo* as used for the concentration profiles (see above). As a 'pure' solution of provitamin D lacks the attenuation properties of the skin tissue matrix, this was combined with input for optical properties of the skin tissue (see above) and concentration profiles (see above).

Comparative calculations were done with a whole skin action spectrum which naturally already includes the influence from provitamin D distribution and optical attenuation. The standard CIE previtamin D action spectrum[19] was taken as the reference spectrum.

Comparison of results obtained with the CIE action spectrum with results based on other input is not straightforward, for several reasons: the optical properties of the skin samples are not available, the doses of ultraviolet radiation applied to the skin to induce previtamin D are unpublished and the target effect was not and probably could not be quantified – that is, we do not get to know how many nanograms previtamin D were produced from how many ng provitamin D at the given doses. Strictly speaking, only relative statements can be made upon this response function and no absolute comparison to, *e.g.*, *in vitro* data is possible. A useful hint given in the CIE publication may still allow to estimate absolute production in order to allow for discussion. "They carefully evaluated the action spectrum for previtamin D$_3$ production in human skin by making certain that no more than 5% of the 7-dehydrocholesterol (7-DHC) in human skin was converted to previtamin D$_3$".[19] The QUT data was scaled on this basis. For reasonable scaling, we looked for the value of maximal depletion (%) of 7-DHC (provitamin D) in the
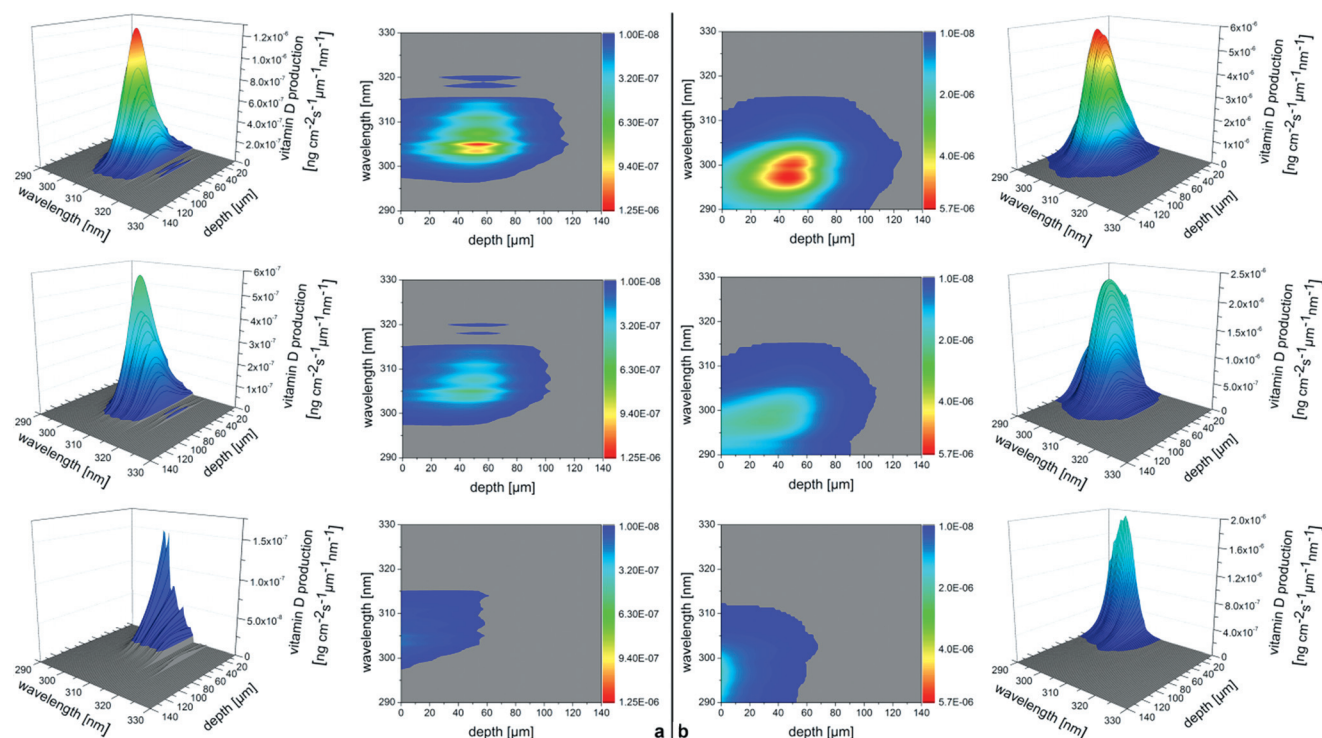
Fig. 4 Spectral depth profiles for previtamin D induction. Production from solar irradiation is shown in 3D and as contour plots at the left side (a). Production from a TL12 lamp is shown in the 3D and contour plots at the right side (b). Different skin sites are shown: inner side of forearm (top row), outer side of forearm (middle), ball of the thumb (bottom row). Note that colour coding scale is different for TL-12 and solar irradiation but remains the same within each group.

original QUT action spectrum data. A maximal depletion of 6.8% is found for a dose of 20 J m^{-2} at a wavelength of 296 nm. If the irradiation parameters for the QUT action spectrum were to be chosen in order to limit maximal depletion to the value of 5% as described for the CIE action spectrum, only 14.7 J m^{-2} (~73.5% of the original dose) should have been applied here, assuming a linear dose–response-relationship (which might not be true). The whole QUT action spectrum was weighted accordingly to reduce potential previtamin D depletion to the 5% criterion of the reference spectrum. In this way, some degree of comparability is allowed between results from the computational model applying *in vitro* spectrum, skin optics, and provitamin D concentration profile on the one hand *vs.* results obtained using the CIE action spectrum, no additional influence from skin optics, unknown distribution but an assumed equal total concentration of provitamin D on the other hand.

Once formed, previtamin D$_3$ either thermally converts to vitamin D$_3$ or photoisomerizes to other photoproducts. Most of these photoreactions are reversible. If no further (UVB) radiation is available for photoisomerizations, only the thermal conversion remains. The thermal conversion from previtamin D to vitamin D is also reversible. A net conversion is maintained until a conversion rate equilibrium is reached. In the skin, vitamin D$_3$ is transported out of the skin.[20] So the thermal conversion of previtamin D to vitamin D is perpetuated. For thermodynamic reasons, vitamin D will never be fully depleted from the skin but a low base concentration will remain. Nonetheless, we can assume that all newly photoinduced previtamin D will be converted to vitamin D eventually and thus use any previtamin D

action spectrum in the place of a vitamin D action spectrum and *vice versa*.

Calculated vitamin D production (for different sets of parameters)

Human skin

The computational model predicts that vitamin D production is different for skin regions with different optical properties. Production profiles for the inner (volar) and the outer (dorsal) side of the forearm as well as for skin from the ball of the thumb are shown in (Fig. 4a/left) based on solar irradiation and a smooth provitamin D distribution within the skin.

For forearm skin, production from solar irradiation is most efficient in the viable layers of the epidermis and in the spectral range from 300–315 nm. More than 90% of the production in volar forearm skin is produced in the range from 301–313 nm with a maximum at 305 nm. The depth range at which 90% of previtamin D is produced is between 15 and 85 µm with a most effective production at 54 µm. Only about 11.5% of the total production is from the dermis *i.e.* at depth >75 µm in this model, the stratum spinosum contributes most (50%) to the overall production, followed by the stratum basale (22%) and the superficial layers (stratum corneum and stratum granulosum, 16.5%). As the attenuation of vitamin D-efficient radiation is weaker in the lighter skin of the inner side of the forearm, overall vitamin D production is higher here than on the outer side of the forearm. The stronger pigmentation at the outer side of the arm also

causes a slight shift (~0.5 nm) of efficient production towards higher wavelengths and towards the surface as more than 90% are produced at depths between 3 and 78 μm with a maximum around 49 μm. The strong attenuation of ultraviolet radiation in the skin is the major limiting factor for the depth at which effective provitamin D conversion is still possible. At a depth of 100 μm, less than 1% (15%) of the incident radiation in the range below 300 nm (315 nm) is still available even in the weakly pigmented skin of the inner side of the forearm.

The optical properties of the thick horny layer at the ball of the thumb lead to a very different vitamin D production profile. The majority of the overall weak production takes place at the skin surface and production rapidly decreases with depth in the skin. At the depth of the basal layer, vitamin D production already becomes negligible, even though the substrate, *i.e.* provitamin D, concentration is highest here.

Spectral quality – sunshine *vs.* artificial irradiation

As expected, the spectral quality of the radiation that is available for provitamin D conversion has a strong impact on the production profiles. While the solar spectral irradiance generally increases with wavelength in the UV, the TL-12 lamp spectrum shows maximal intensity at around 308 nm. Thus, for the normalization shown in Fig. 1, much more radiation is available from the TL-12 lamp in the wavelength range where provitamin D conversion is particularly efficient.

Fig. 4b shows the production profiles for TL-12 lamp irradiation. Due to the broad spectral distribution of TL-12 lamp irradiance in the short range UVB, which matches much better the wavelength range of effective provitamin D conversion than the solar spectral irradiance, effective conversion is achieved on a much broader spectral range.

The spectral range where most of the photoconversion takes place is blue-shifted for the TL12 lamp by more than 8 nm compared to solar production with a maximum between 297 nm and 298 nm. Besides, the depth at which 90% of the production is achieved at the inner side of the forearm is in the range up to 78 μm with a maximum at 48 μm, *i.e.* most efficient vitamin D production is located further towards the surface for TL-12 lamp irradiation than for solar irradiation.

The relation between volar and dorsal forearm vitamin D production is weakly dependent on the spectral quality of the radiation source. For the solar spectrum dorsal production amounts to 51% of the volar production – for the TL-12 lamp, it is 44%. The absolute values must be taken with care as the wavelength range <290 nm is cut from the calculations due to missing data on skin optical properties, even though the TL-12 profiles indicate that there is still some production in this range. However, the figures indicate that generally similar results would be found for the full wavelength range.

Response functions for vitamin D production

Fig. 5 shows a comparison of the computational results for the spectral production found by either applying weighting of the spectral irradiances with the CIE action spectrum or calculations
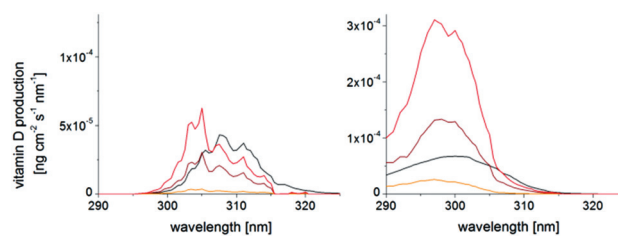
Fig. 5 Spectral production profiles as predicted by the computational model or the CIE reference spectrum (black lines). Different UVB sources (sun (left) or TL-12 lamp (right)) were used and calculations for several skin sites (inner–volar side of forearm: red lines, outer–dorsal side of forearm: brown lines, ball of the thumb–thenar: orange lines) are shown for the model.
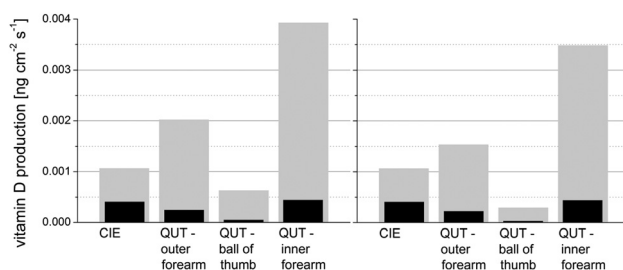
Fig. 6 Predicted vitamin D production for different input parameters: smooth provitamin D concentration profile (right panel) *vs.* step function profile (left panel), solar (black) *vs.* artificial (grey) irradiation, and three different skin sites. Calculations with the CIE action spectrum where skin properties are implicit are given as reference. Note that production from the TL12 lamp is underestimated by the model (QUT) calculations.

based on provitamin D distribution, spectral attenuation and the QUT *in vitro* action spectrum.

Introducing only the assumption of no more than 5% photoconversion for QUT and CIE response functions, the vitamin D effectiveness calculated with the two different approaches notably is comparable within each irradiation scenario. Looking at the spectral distribution of vitamin D effectiveness, a shift of 2–3 nm towards longer wavelengths is seen for the calculations based on the CIE spectrum (90% of production in 303–317 nm range with the CIE data and solar radiation *vs.* 90% in 301–313 nm range calculated with the model for inner arm skin and solar irradiation). This effect appears for both irradiation scenarios applied in this study. If the TL-12 lamp spectrum had been normalized to equal solar erythemal effectiveness in the range 290–315 nm, the vitamin D production scale from the left panel would be valid for both graphs.

The right panel of Fig. 6 shows the respective total production. If the process is driven by solar radiation and a smooth concentration profile for provitamin D is assumed, total vitamin D production predicted by use of optical properties of forearm skin sites and the *in vitro* model are close to that calculated with the CIE action spectrum: In the weakly pigmented skin at the inner side of the forearm, vitamin D production as predicted by the model is 8% higher than the CIE value. On the outer side of the forearm, predicted production is 45% below the CIE value. For TL-12 lamp irradiation, model and reference calculations

differ more strongly: On the inner (outer) side of the arm, 3.25 (1.44) times more production is predicted by the model. If the TL-12 lamp spectrum was scaled to give the same erythemal effectiveness as the solar spectrum in the range 280–400 nm, the grey TL-12 bars would be reduced by a factor of *ca.* 2.4.

Vitamin D distribution

If a step function is assumed for the distribution profile of provitamin D, the predicted vitamin D production is always higher than that predicted under the conditions of a smooth profile (compare Fig. 6).

This is due to the fact that more educt is available towards the surface where more radiation is available and less in the depth of each layer in a step function distribution compared to the smooth distribution profile. The more conditions favour superficial previtamin D production (*i.e.* extraordinary strong effective attenuation within the skin, short wavelength irradiation spectra), the stronger the effect. Thus, the effect is almost negligible for the combination solar irradiation and inner side of forearm (less than 1% more production predicted from the step function) and rises to 10% at the outer side of the arm. For the blue shifted TL-12 lamp irradiation, 13% (inner side) resp. 32% (outer side) more production are predicted with the step function compared to the smooth distribution. At the ball of the thumb, where strong scattering and absorption confine efficient previtamin D production to a few superficial micrometers, this effect becomes particularly prominent: For TL-12 lamp induced previtamin D production, the model predicts more than twice as much previtamin D production for the step function than for the smooth function – for solar irradiation, this factor is as high as 17.

Results for the TL-12 lamp must be considered with care however, as the model cuts spectral data to the range where data from each parameter is available. As a consequence, production at wavelength shorter than 290 nm are not taken into account. The general trend of the effect should not be affected.

Conclusions

The difference in optical properties between average inner and outer forearm skin of Caucasians, which does not seem to be particularly large at first sight, resulted in twice as much total vitamin D production in inner forearm skin than in outer forearm skin. These findings support the hypothesis that skin phototype or pigmentation have a considerable effect on individual vitamin D production as discussed since the 1960s.[21–26] Calculations based on the optical properties of different Fitzpatrick skin phototypes (same skin site) could show how strong an influence is to be expected.

Even though total provitamin D concentration and its rough distribution is important, the exact distribution profile, *e.g.* step-function *vs.* smooth distribution, is not. The calculated contributions of the skin layers to overall previtamin D production qualitatively agrees with published data,[15] *i.e.* most previtamin D is found in the stratum spinosum and stratum basale, least in the dermis.

The spectrum of the irradiation is very important. The model calculations give a strong impression of how sensitive previtamin

D production is to the spectral quality of the UV source. Within the most effective wavelength range, even small shifts may have significant impact – narrow line peaks from artificial sources definitely will.

The strong attenuation of short range ultraviolet radiation in the skin is the major limiting factor for the depth at which effective provitamin D conversion is still possible. Together with an *in vitro* – 'pure' – action spectrum, it yields an effective vitamin D production spectrum which is shifted by 2–3 nm towards shorter wavelengths compared to calculations based on the CIE action spectrum. Total vitamin D production as predicted by the computational model is comparable to results calculated with the CIE reference spectrum for previtamin D production if lightly pigmented skin and solar irradiation are assumed in the model. This may be read as an indicator of the model's validity.

Compared to the widely used calculations based on the CIE action spectrum, the benefit of the computational model is that variation of the parameters allow deeper insight into the dependencies of cutaneous production of vitamin D. It shows where in the skin how much (pre-)vitamin D can be produced for a given spectral irradiance and allows to estimate the impact of the parameters on total vitamin D production. This is supported by the fact that absolute quantitative results can directly be obtained. Even though qualitative results are easily obtained from the model at this stage, a decent choice of input data with a well matched focus on variable parameters is prerequisite to obtain reliable quantitative results from the model. If the model is used to balance skin damage against vitamin D production for example, response functions and irradiation spectra should be carefully chosen and scaled. Predictions about how much vitamin D can be produced rely on realistic irradiation spectra and amount and quality of exposed skin area. Further development of the model will improve its capability to study cause and effect.

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† Vitamin D denotes vitamin D_3 unless otherwise indicated.

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Photochem. Photobiol. Sci., 2012, **11**, 731–737 | 737