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The action spectrum for vitamin D_3: initial skin reaction and prolonged exposure

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Vitamin D_3 photosynthesis in the skin is formulated as a set of reaction equations, including side-reactions to lumisterol, tachysterol and toxisterols, and the accompanying reverse reactions, isomerisation of previtamin D_3 to vitamin D_3 and photodegradation of vitamin D_3. The solution of this set is given for the stationary irradiance spectrum. The effective action spectrum for the instantaneous vitamin D_3 production changes shape as a function of exposure, and therefore, no single action spectrum can be used. We assessed the action spectrum for unexposed skin and for skin that has been exposed to 7.5 Standard Erythemal Doses (SED). We constructed two new estimates: (1) the RIVM action spectrum, based on absorption spectra, quantum yields and skin transmission spectra, and (2) the modified QUT action spectrum, which is adjusted for self-absorption and skin transmission. For previously unexposed skin, the modified QUT action spectrum gives a qualitatively similar, but larger estimate than the RIVM action spectrum. We have not been able to solve the lack of quantitative agreement between the vitamin D production estimates from the three action spectrum estimates (RIVM, modified QUT and CIE). All new action spectra have stronger emphasis on the short wavelengths than the CIE action spectrum. We showed that, for wavelengths larger than 300 nm, the bandwidth that was used in the experiment that formed the basis of the CIE action spectrum, gives a red-shift of about 1 nm. Generally, with the formation of previtamin D_3, the return reaction to provitamin D_3 limits the production of vitamin D_3. After some exposure, the new action spectrum has negative values for the longer wavelengths in the UVB. For the RIVM action spectrum, this happens after 7.5 SED, for the modified QUT action spectrum already after 1.25 SED, and after 7.5 SED the net production rate is largely cancelled. Thus prolonged exposure of previously unexposed skin saturates vitamin D_3 formation. For maximum vitamin D production after 1.25 SED, sunscreens should block wavelengths larger than 310 nm. Sunscreens that block only UVB could result in reduction in vitamin D production after prolonged exposure, or even a destruction of vitamin D that has just been formed.

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1 Background

Vitamin D is a fat-soluble prehormone that comes in two forms: ergocalciferol (or vitamin D_2, the vegetable form) and cholecalciferol (or vitamin D_3, produced in animals and humans, but also in some plants). Vitamin D is essential for a healthy bone structure and strong muscles. In addition, more and more health-effects are observed to correlate with vitamin D status, but for most of these associations it is not clear if the relation is causal.[1–3] This research aims at understanding these associations and inferring a vitamin D status maintenance strategy that gives optimal health. It could be that adequate status and oral intake values implemented to day are too low. Apart from this, the kinetics or metabolism of vitamin D status in relation to UV exposure is still not well known. In other words, if people should be persuaded to maintain an increased vitamin D status, how should this be achieved? Overexposure to UV will increase the risk of adverse health effects like skin cancer, cataract and ageing of the skin. Would it be better to take supplements? And how effective or dangerous is that?

For most people, the dominant contribution to their annual vitamin D source is vitamin D_3 that comes from exposure of the skin to UV radiation (in spring and summer mostly from the sun). Some people engage in the use of tanning beds throughout the year. Furthermore, vitamin D can be acquired through nutritional intake (mainly fatty fish and supplements). The assessment of the exposure to UV radiation (how much radiation is commonly received and on what fraction of the total human body?) and its relation to vitamin D_3 production (what is the nature of the chemical reactions in the skin?), requires understanding of the spectral content of the received radiation and radiation driven chemical reactions in the skin. Mostly, cutaneous vitamin D_3 production is estimated with Holick's rule.[4] This rule reads that an exposure of a quarter of the body to a quarter of a "minimal erythemal

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dose" (explained later) is the equivalent of an oral intake of 1000 IU (*i.e.* 25 μg) of vitamin D_3. This in fact only applies to the lamp that has been used in the experiment by Adams *et al.*[5] that was the basis for Holick's rule: an FS40. The effectiveness of UV-radiation to induce erythema or to produce vitamin D_3 has different wavelength dependencies. Therefore, the vitamin D_3 production per erythemal dose varies with the UV-spectrum of the source that is used. An action spectrum is a dimensionless weighting function for the wavelength dependence of the effectiveness of UV-radiation to induce a particular effect. With the CIE action spectrum for previtamin D_3 production (see Bouillon *et al.*[6] this action spectrum has its basis in measurements from MacLaughlin *et al.*[7]), the previtamin D_3 production rate can be calculated. With the CIE action spectrum for erythema (see McKinlay and Diffey[8] and also Webb *et al.*[9]) the rate at which sunburn is caused can be calculated for a known radiation source. The ratio of these two estimates, in combination with the same ratio for the FS40, can be used to apply Holick's rule to estimate vitamin D_3 production for any arbitrary known source of irradiance, *e.g.* for solar radiation under different elevation angles or ozone layer thicknesses.

Nevertheless, there are still several problems in understanding the cutaneous vitamin D_3 synthesis. McKenzie *et al.*[10] pointed out that there is an inconsistency between observation and theory using the CIE spectrum for previtamin D. Webb *et al.*[11] could see no production of vitamin D_3 in winter in Boston (see also Rhodes *et al.*[12] and Webb *et al.*[13]), though production estimates based on the CIE action spectrum suggest an appreciable cutaneous production (see also Webb and Engelsen[14]). Norval *et al.*[15] questioned the applicability of the action spectrum concept to vitamin D photosynthesis: "Is the action spectrum for the UV-induced production of previtamin D_3 in human skin correct? [⋯] In conclusion, the construction of an entirely new computational model to predict previtamin D_3 levels is recommended". Norval *et al.* demonstrated that the photosynthesis of vitamin D_3 is a complex reaction system, that, in principle, cannot be described with a single action spectrum concept. The measurements from MacLaughlin *et al.* considered only the first step: the conversion of provitamin D_3 to previtamin D_3, and disregarded side-reactions. Terenetskaya[16] argued that possibly irreversible conversion of previtamin D_3 to toxisterols under exposure to radiation of long wavelengths played a role in these measurements. Furthermore, she pointed out that the spectral sensitivity of the photoconversion process in the skin may be challenged by the so-called "antenna-effect". Olds *et al.*[17] (see the thesis by Olds[18] for details) have measured the QUT-action spectrum for a different endpoint than previtamin D_3: vitamin D_3 synthesis. These measurements, which have been made in high spectral resolution, pose some challenges: vitamin D_3 production 5-fold less effective than that found on the basis of the CIE action spectrum is reported and Björn *et al.*[19] pointed out that the QUT samples still needed correction for self-absorption. Another problem that one meets when making cutaneous production estimates is the unit of Holick's rule: "oral intake equivalent": what is the efficiency of oral intake? Vitamin D is one of the so-called fat-soluble vitamins and therefore it is more efficiently transported to the blood when taken with some fat, *e.g.* over a meal. To which form of intake does Holick's rule refer?

Often, exposures are measured in minimal erythemal dose (MED), which is the smallest UV exposure that results in a just perceptible redness. This measure varies with the skin type, within one skin type from person to person and within one person, the MED changes with exposure history *via* skin thickening and tanning. The MED is a subjective measure. For this reason, we will use the standard erythemal dose[20] (SED) throughout this study instead. The SED is equivalent to an erythemal effective[8] radiant exposure of 100 J m^{-2}. Our focus will be on the FitzPatrick type II skin (usually burns, tans minimally), for which the minimal erythemal dose has been estimated to be 1 MED $\simeq$ 2.5 SED.

2 Objectives

In this paper, we start with the presentation of a set of reaction equations that describe all photochemical reactions in the skin. It becomes readily apparent that the cutaneous vitamin D_3 production over exposures of the order of a few SED cannot be written in an action spectrum form. Only the instantaneous production rates at any point in time can be formulated as a so-called generalized action spectrum. Some of the chemical products and side-products are photochemically active themselves and thus they alter the equilibrium of the production reactions for previtamin D_3 and vitamin D_3. Consequently, the generalized action spectrum changes in the course of exposure. In Appendix A we show the photochemical reaction equations and solve them analytically under the condition of a stationary spectral distribution of the radiation. For non-stationary irradiance spectra, the generalized action spectrum can only be solved numerically. In this case, the analytical solution for a stationary irradiance spectrum can be used for making time steps.

We use the term "*in vitro*" action spectrum to describe the effectiveness of radiation to drive photochemical reactions in the absence of skin. It can be either inferred from measurements of absorption spectra and quantum yields (taken from the literature) or derived from the QUT action spectrum. In the next step, we combine the two *in vitro* action spectra with spectral transmission estimates for different skin parts from the literature to construct action spectra for the initial reaction of human skin to UV irradiance.

The new estimates for the cutaneous action spectrum plus the CIE estimate are used to calculate the proportionality coefficient in Holick's rule in terms of "net" intake, *i.e.* nutritional intake corrected for its efficiency. This will be done for different qualities of UV-spectra, parameterized by the solar elevation angle and for the spectrum of the FS40-lamp used in the experiment by Adams *et al.*[5] that forms the basis of Holick's rule. We will then consider prolonged exposure up to

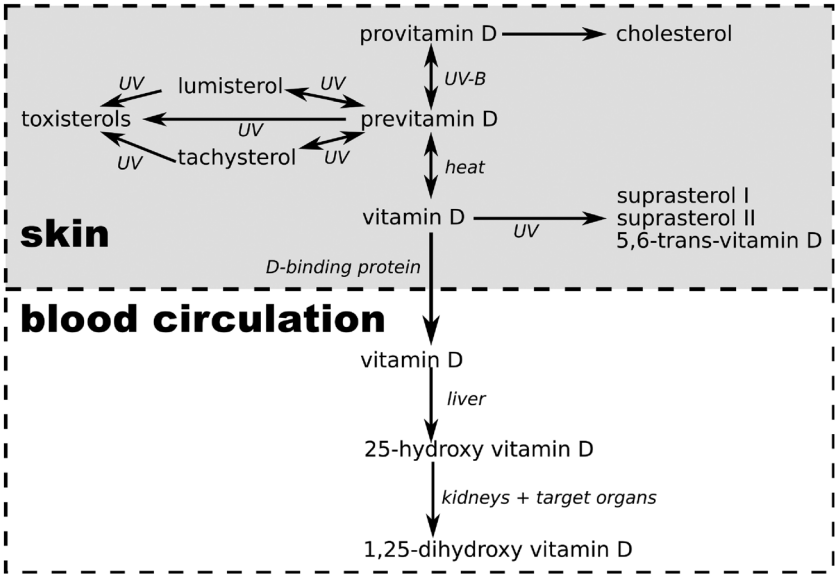

Fig. 1 Photochemistry of vitamin D_3 in skin, transport from skin to the blood stream and subsequent reactions in the human body. Italicized labels next to the arrows give the nature of the mechanisms.

7.5 MED and show the impact of the dose already received on the action spectrum and on the coefficient in Holick's rule. Finally, the new action spectra are compared with the CIE action spectrum. Diurnal and annual cycles of the available vitamin D_3 weighted irradiance in The Netherlands (latitude 52°) are estimated for the different action spectrum estimates. These are used to compare during which parts of the day/year, according to the different estimates, exposure to UV leads to appreciable vitamin D_3 synthesis and when it does not. The impact of the bandwidth used in the measurements by MacLaughlin *et al.*[7] on the CIE action spectrum is analysed by convolution of our action spectra with the slit-function of the monochromator.

3 Chemical reactions involved

Vitamin D_3 is synthesized in the skin. The substrate is provitamin D_3 (7-dehydrocholesterol), the last pre-stage of cholesterol in the cholesterol-synthesis. Under influence of UV-radiation, provitamin D_3 converts to previtamin D_3. Previtamin D_3 slowly isomerizes to the actual vitamin D_3 (cholecalciferol) in an equilibrium process that is temperature-dependent and that does not require radiation. Irradiation of previtamin D_3 can lead to the formation of lumisterol, tachysterol or toxisterols. Irradiation of lumisterol and tachysterol can lead to return reactions to previtamin D_3 or to toxisterols. Toxisterols are photo-insensitive. Webb *et al.*[21] studied the photo-sensitivity of vitamin D_3 in skin. They found that "sunlight regulates the cutaneous production of vitamin D_3 by causing its photodegradation". This degradation was shown to be a transformation of vitamin D_3 to suprasterol I, suprasterol II and 5,6-*trans*-vitamin D_3. In the living skin, the hydrophobic/fat-soluble vitamin D_3 is wrapped in a D-binding protein (DBP) and the wrapped package is transported from the skin to the blood. The set of reactions in the skin is shown in the grey area in Fig. 1, along with the subsequent reactions in the body up to the formation of the active metabolite 1,25-dihydroxy vitamin D_3.

We focus on the cutaneous part of the reactions, *i.e.* all reactions inside the grey box of Fig. 1, hydroxylation in the liver and further reactions fall outside the scope of the present analysis. The time-rate of change of all concentrations $D_i(r)$ due to all reactions in skin as shown in Fig. 1 is given by expression (8), which is derived in Appendix A. Local integration of concentrations $D_i(r)$ over skin depth r gives concentrations $\bar{D}_i$ per unit area of skin. In Appendix A we show that the instantaneous development of $\bar{D}_i$ in a medium (*e.g.* skin) under exposure to an irradiance $I(\lambda)$ can be written in action-spectrum form, *i.e.* a weighted integral of the irradiance, see relation (15). The action spectrum in this relation is the product of three factors: (1) the "*in vitro*" action spectrum, (2) the effective transmission of the medium and (3) the instantaneous concentration profiles of the relevant substances.

4 The *in vitro* action spectrum for pro–pre conversion

Estimating the conversion yields (or rates) in the actual *in vivo* situation, requires (among others) an estimate of the *in vitro* action spectrum F_{ij} from relation (11). Indices (i,j) indicate the generation of substance i out of substance j and therefore, when explicit transitions between chemicals are meant, we use the equivalent notation $F_{j\to i}$. F_{ij} can be estimated on the basis of the spectral molar extinction coefficients from MacLaughlin *et al.*[7] and quantum yields described in Norval *et al.*[15] In some

cases, not all elements of F_{ij} are relevant. When for instance pure provitamin D_3 is irradiated for a short time, then the side-reactions can be neglected and only $F_{\mathrm{pro}\rightarrow\mathrm{pre}}$ matters. This is *e.g.* the case for the measurements by MacLaughlin *et al.*[7] and for the CIE-2006 action spectrum for previtamin D_3 production that has been based largely on these measurements.

Alternatively, we can extract the *in vitro* action spectrum from the measured photochemical production $A_{\mathrm{pre}}(\lambda)$ of previtamin D_3 per dose of monochromatic irradiance with wavelength λ. This is done by inversion of relation (16):

$$F_{\mathrm{pro}\rightarrow\mathrm{pre}}(\lambda) = \frac{A_{\mathrm{pre}}(\lambda)}{T_{\mathrm{eff,\,pro}}(\lambda)\bar{D}_{\mathrm{pro}}} \quad (1)$$

where $T_{\mathrm{eff,pro}}$ is the self-absorption of radiation in the vessel by the provitamin D_3 solution (transmission of quartz is approximately 1). QUT action spectrum A_{QUT}, presented by Olds *et al.*[17,22] provides an estimate for $A_{\mathrm{pre}}(\lambda)$. As put forward by Björn *et al.*[19] and confirmed by Olds and Kimlin,[23] the QUT-action spectrum is not yet corrected for self-absorption. A correction for self-absorption can be made by the use of the Lambert–Beer relation for exponential extinction of irradiance I when it penetrates to depth r in a medium with molar absorptivity spectrum ε and concentration D:

$$T(r) = I(r)/I(0) = 10^{-\varepsilon Dr} \quad (2)$$

Averaging this relation over a vessel with depth R gives the following expression for effective transmission $T_{\mathrm{eff,QUT}}$:

$$T_{\mathrm{eff,QUT}}(\lambda) = (1 - e^{-y})/y \quad \text{with} \quad y = \ln(10)\varepsilon_{\mathrm{pro}}(\lambda)D_{\mathrm{pro}}R \quad (3)$$

The scattered part of the radiation has a longer path through the skin and is therefore more attenuated than follows from Lambert–Beer's law. Bruls[24] measured the angularly resolved transmission of skin and showed that the fraction of scatter radiation at a certain skin depth is small. From this we conclude that our estimate based on Lambert–Beer's law is acceptable. Effective transmission from self-absorption ranges from 0.14 (86% absorption) for 280 nm to 1.0 (no absorption) for 320 nm.

The QUT action spectrum has been normalized at its maximum value at 295 nm. At 295 nm, Olds (see Fig. 8.6)[18] gives a converted concentration of 4.89 μg ml^{-1} after exposure of an initial concentration of 100.7 μg ml^{-1} to a dose of 20 J m^{-2} of UV irradiation. However, carefully reading the experimental description reveals that only a third of the volume was actually irradiated. Relation (1) for estimating *in vitro* action spectrum $F_{\mathrm{pro}\rightarrow\mathrm{pre}}$ from the QUT action spectrum thus becomes:

$$F_{\mathrm{pro}\rightarrow\mathrm{pre,\,QUT}} = \frac{3}{T_{\mathrm{eff,QUT}}}\frac{4.89\ \mu\mathrm{g\,ml^{-1}}}{100.7\ \mu\mathrm{g\,ml^{-1}}}\frac{A_{\mathrm{QUT}}}{20\ \mathrm{J\,m^{-2}}} = 7.3\times10^{-3}\frac{A_{\mathrm{QUT}}}{T_{\mathrm{eff,QUT}}}\left[\mathrm{m^2\,J^{-1}}\right] \quad (4)$$

From now on, we will use the term "RIVM action spectrum" for $F_{\mathrm{pro}\rightarrow\mathrm{pre}}$ and A_{pre} if they are estimated *via* relations (11) and (15) and the term "modified QUT action spectrum" if they are estimated *via* relations (4) and (15). We constructed both estimates for the *in vitro* action spectrum. They are shown in Fig. 2, along with their ratio. The two *in vitro* action spectra show good qualitative correspondence. For wavelengths up to 303 nm, the ratio between the two estimates is a flat factor of 20, for longer wavelengths, the ratio changes.

5 The initial vitamin D_3 action spectrum for skin

We have just quantitatively characterized the production of previtamin D_3 in the absence of skin. Now, we include in our analysis the attenuating effect of skin on UV-driven production (-rate). As is pointed out in Appendix A, the instantaneous rates of change of the content in the skin of the individual photochemicals are the only quantities that can be formulated in an action spectrum form. The action spectrum will change in the course of exposure and therefore a single action spectrum only gives a prognostic estimate of the photo-reaction at that particular point in time. It cannot be used to estimate (pre-)vitamin D_3 productions for physiological exposures of the order of a few SED. We use the RIVM- and modified QUT-estimates for *in vitro* action spectrum $F_{\mathrm{pro}\rightarrow\mathrm{pre}}$ from the former

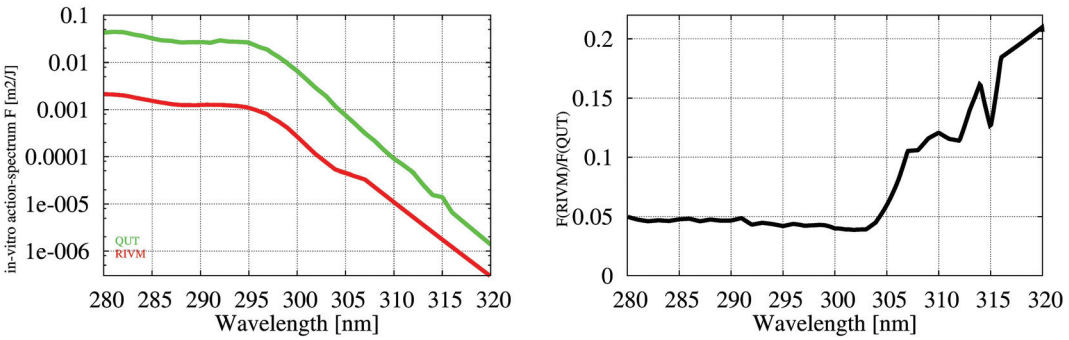

Fig. 2 Left: *in vitro* action spectra for vitamin D_3 from the RIVM model and from (modified) QUT measurements. Right: ratio of estimates from the left panel.

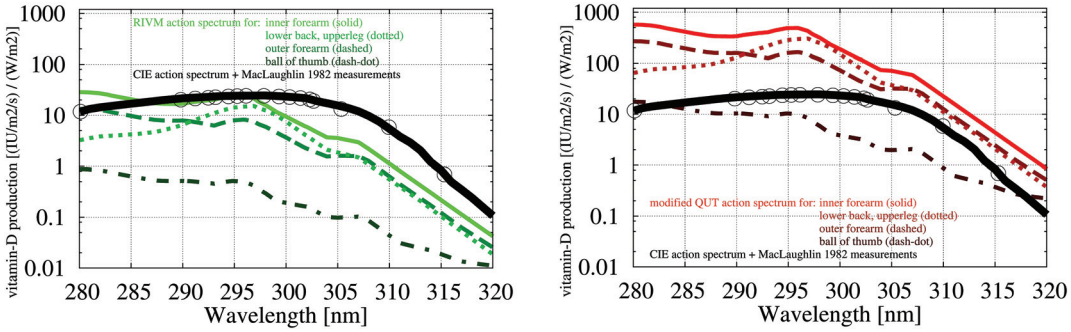

Fig. 3 Action spectrum for initial (pre-)vitamin D_3 production in skin for several transmissions: left: RIVM, right: modified QUT. The CIE action spectrum is shown in black with MacLaughlin's measurements shown as circles.

section to estimate action spectrum A_{pre} from relation (16). We do this for the initial response in previtamin D_3 production from exposure of unexposed skin that contains only provitamin D_3. As a reference for cutaneous previtamin D_3 production, we use CIE action spectrum $A_{\mathrm{pre,CIE}}$. We normalize it such that Holick's rule, when applied to the spectrum of a Westinghouse FS40 lamp (that was used in the study by Adams *et al.*[5] that formed the basis for Holick's rule), gives 1000 IU per quarter of 2.5 SED on a quarter of the total body surface:

$$A_{\mathrm{pre,CIE}}\ [(\mathrm{IU\ m^{-2}\ s^{-1}})\ (\mathrm{W\ m^{-2}})^{-1}] = 23 A_{\mathrm{pre,CIE}}\ [\text{normalized to its maximum}]$$

Our model relation (16) for previtamin D_3 production requires an estimate for the provitamin D_3 profile. We adopt the continuous profile presented by Meinhardt-Wollweber and Krebs[25] (see Appendix B). We use four different estimates for skin transmission $T(r, \lambda)$: from Bruls[24] we use transmission for "lower back and upper leg" and from Meinhardt-Wollweber and Krebs[25] we use "inner fore-arm", "outer fore-arm" and "ball of thumb" (see Appendix B). To facilitate a meaningful comparison, all action spectra are normalized to absolute production per unit of exposed skin surface and per unit of irradiance instead of to their own maximum value. The different estimates for the action spectrum for (pre-)vitamin D_3 production (CIE, RIVM and modified QUT) are shown in Fig. 3. Integration over wavelength of irradiance spectra that have been weighted with one of these action spectra provides the vitamin D_3 production rate per unit area of skin.

6 Holick's rule

The total cutaneous vitamin D_3 production is found by integration of the production rate per unit area of skin (the result of the former section) over the exposed skin surface and over exposure time. We regard the final vitamin D_3 photoproduction to be the sum of productions of previtamin D_3 and vitamin D_3 (see relation (18) and the text above). From now on, we assume that the provitamin D_3 profile does not vary with the body site and can be represented by relation (19), with a concentration per unit area of skin of 1021 ng $\mathrm{cm^{-2}}$, estimated for the human thigh.[26] The production in a short time Δt, in which a fraction f_{body} of the total (homogeneous) body surface S_{body} is exposed, is given by:

$$\text{Vitamin D}_3\ \text{production} = f_{\mathrm{body}} S_{\mathrm{body}} \Delta t \left(\frac{\mathrm{d}}{\mathrm{d}t} \bar{D}_{\mathrm{pre}} + \frac{\mathrm{d}}{\mathrm{d}t} \bar{D}_{\mathrm{vitD}} \right) \quad (5)$$

In our constructions of the action spectrum for previtamin D_3 synthesis, we used skin transmission properties for Fitz-Patrick type II. For this skin-type, Holick's rule applies to the vitamin D_3 production according to relation (5) for $f_{\mathrm{body,Holick}} = 0.25$ and an exposure time $\Delta t_{\mathrm{Holick}}$ such that the erythemal dose sums up to 0.625 SED:

$$\Delta t_{\mathrm{Holick}} \frac{\mathrm{d}}{\mathrm{d}t} D_{\mathrm{ery}} = 0.625\ \mathrm{SED}$$

The erythemal dose-rate can be estimated from the irradiance spectrum by integration against the CIE erythemal action spectrum, see McKinlay and Diffey[8] and Webb *et al.*[9]

$$\frac{\mathrm{d}}{\mathrm{d}t} D_{\mathrm{ery}} = \int A_{\mathrm{ery}}(\lambda) I(\lambda) \mathrm{d}\lambda \quad (6)$$

Combination of relation (5) for the previtamin D_3 production with relations (15) and (18) for the vitamin D_3 action spectrum and relation (6) for the erythemal action spectrum leads to the following expression for the previtamin D_3 production according to Holick's rule:

$$c_{\mathrm{Holick}} \equiv \text{Vitamin D}_3\ \text{production rate}\left(f_{\mathrm{body,Holick}}, \Delta t_{\mathrm{Holick}}\right) = 33.4 \frac{\int \left(A_{\mathrm{pre}}(\lambda) + A_{\mathrm{vitD}}(\lambda)\right) I(\lambda) \mathrm{d}\lambda}{\int A_{\mathrm{ery}}(\lambda) I(\lambda) \mathrm{d}\lambda} \quad (7)$$

where we have taken $S_{\mathrm{body}} = 2.1\ \mathrm{m^2}$. In strict sense,[5] Holick's rule refers to a Westinghouse FS40 lamp, for which it gives a production of $c_{\mathrm{Holick}} = 1000$ IU = 25 μg. In this paper, we will use the term "Holick's rule" in a broader sense as the vitamin D_3 production for any irradiance spectrum according to relation (7). Holick's rule is generally used to linearly scale vitamin D_3 production from other exposures (duration, exposed body fraction). For body fraction, the assumption of linearity is fair, since the photochemical process at one skin site that is exposed does not affect photochemistry at a different skin site. In the next section we will see that linearity

has its limits for prolonged exposure. Relation (7), the extended version of Holick's rule, corrects the original, narrower version for the quality of the spectrum. For the CIE action spectrum for vitamin D_3 production, Dowdy *et al.*[4] have shown that, for noon on March 21 in Boston, under 350 DU total ozone thickness, the sun is 32% more effective (per SED of exposure) in producing previtamin D_3 than an FS lamp. We have repeated this calculation for a more representative total ozone column of 300 DU (and the sun at 48° elevation for noon on March 21 in Boston). For this condition, we estimate the deviation to grow to 44%.

We used the RIVM-, modified QUT and CIE action spectra for cutaneous (pre-) vitamin D_3 synthesis to estimate the coefficient in Holick's rule, eqn (7). We did this for a Westinghouse FS40 lamp and for solar radiation for two elevation angles: 60 degrees (highest sun in Dutch summer) and 30 degrees and for four different skin transmissions. The result is shown in Table 1 in the columns under "initial". The initial conditions are unexposed skin that contains only provitamin D_3. All estimates are "net production", except for the CIE values, which are "oral intake equivalent".

Table 1 gives the vitamin D_3 production rate per erythemal dose, *i.e.* vitamin D_3 production per exposure of 1/4 of the body to 0.625 SED. From the values in the column marked as "initial", we see that vitamin D_3 production rates (per erythemal dose) found with the RIVM action spectra are substantially smaller than what is found with the CIE action spectrum (as could have been expected on the basis of Fig. 3). Similarly, we see that estimates from the modified QUT action spectrum are substantially larger than the estimates based on the CIE action spectrum, except for the ball of the thumb. According to the CIE action spectrum, solar radiation is more potent in producing vitamin D_3 (per erythemal dose) than the Westinghouse FS40 lamp, but all our model estimates, both RIVM and modified QUT, suggest otherwise. Exposure to solar radiation at low solar elevation gives less production (per erythemal dose) than solar radiation at higher elevation, as was expected.

7 Prolonged exposure

All the analyses that we have made in the present study up to this point concerned initial reactions of pure provitamin D_3. For this particular case, the action spectrum of vitamin D_3 could be approximated with the action spectrum for conversion of provitamin D_3 into previtamin D_3. Ingrowth of other components, return reactions and degradation modify the reaction characteristics. As noted earlier: the action spectrum will change in the course of exposure. We now assess the impact of prolonged exposure on the action spectrum. As an example, we work out exposure of a type-II skin to clear sky solar radiation under an elevation angle of 60 degrees (noon in Dutch summer), varying from an infinitesimal dose up to 7.5 SED. As indicated earlier, there is no action spectrum formulation for photoproduction over long exposure periods, but action spectra from relations (16) and (18) can be used to characterize the photochemical reaction process at all stages of exposure. We assume a stationary solar spectrum and therefore we can use the analytical solution from relation (14) to estimate the concentration profiles at relevant stages of exposure. We have 2 estimates for *in vitro* action spectrum $F_{\mathrm{pro}\rightarrow\mathrm{pre}}$, the RIVM one and the modified QUT estimate. For all other components of F_{ij} only the RIVM model gives estimates. To include the QUT action spectrum in the analysis of prolonged exposures, we exploit the similarity between $F_{\mathrm{pro}\rightarrow\mathrm{pre,QUT}}$ and $F_{\mathrm{pro}\rightarrow\mathrm{pre,RIVM}}$ and heuristically approximate $F_{ij,\mathrm{QUT}} \simeq 20F_{ij,\mathrm{RIVM}}$ for all components. We adopt the transmission characteristics for "lower back/upper leg" (representa-

Table 1 Estimates for the coefficient in Holick's rule: vitamin D_3 production rate in IU per 0.625 SED on 1/4 of the body. Results are given for three different action spectra, four different skin types and a set of different irradiance spectra: an FS40 lamp and solar radiation for two elevation angles. Solar spectra are related to ground-level atmospheric conditions, 300 DU total column ozone and clear sky. Columns marked as "initial" refer to initial action spectra for skin profiles with only provitamin D_3, columns marked as "after 7.5 SED" are instantaneous action spectra for skin after exposure to 7.5 SED and columns marked as "convolved initial" refer to convolutions of the initial action spectra with the slit width as most likely used in the experiments by MacLaughlin: ±5 nm full width at half maximum (FWHM)

	Initial			After 7.5 SED			Convolved initial		
		Elevation					5 nm FWHM		
Action spectrum + transmission	FS40	60°	30°	FS40	60°	30°	FS40	60°	30°
CIE (oral intake equivalent):	1000	1492	1180	1000	1492	1180			
RIVM (net production):									
Lower back upper leg	317	201	140	291	136	94	316	230	165
Outer forearm	233	183	146	215	119	92	235	200	164
Inner forearm	585	368	275	510	185	134	589	411	316
Ball of thumb	15	15	14	14	13	11	15	16	15
Modified QUT (net production):									
Lower back upper leg	7045	2960	1565	3322	491	238	7036	3621	2038
Outer forearm	4968	2332	1477	2876	578	325	5011	2695	1787
Inner forearm	12 762	5056	2935	5188	684	340	12 846	6000	3669
Ball of thumb	312	169	124	261	137	106	315	191	143

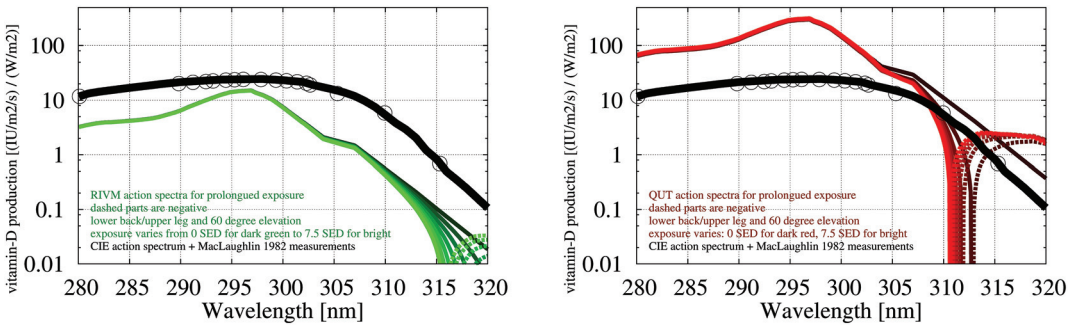

Fig. 4 Action spectrum for previtamin D_3 formation for lower back/upper leg and 60 degree solar elevation angle. Action spectra are presented for different stages of exposure: darkest colour = 0 SED, brightest colour = 7.5 SED. Left: RIVM model, right: modified QUT model, approximated *via* 20 × RIVM model.

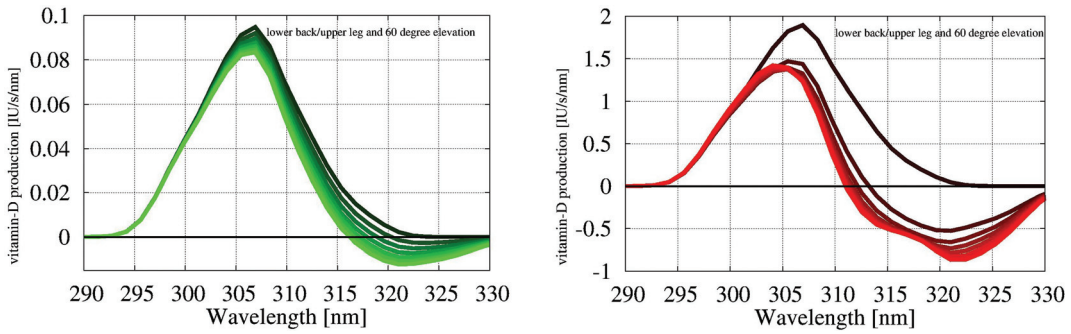

Fig. 5 Weighted irradiance for previtamin D_3 formation associated with the action spectra shown in Fig. 4 for lower back/upper leg and 60 degree solar elevation angle. Action spectra are presented for different stages of exposure: darkest colour = 0 SED, brightest colour = 7.5 SED. For absorption and quantum yields at wavelengths larger than 320 nm the value at 320 nm was used.

tive for a large area of body) and estimate the action spectrum for vitamin D_3 as defined by relations (16) and (18) at exposure stages varying from 0 SED to 7.5 SED with step 1.25 SED. The result is shown in Fig. 4 (negative parts are plotted as dashed lines). The brighter the colour in Fig. 4, the longer the exposure. Associated weighted irradiance spectra are shown in Fig. 5.

The impact of prolonged exposure on the efficiency of cutaneous previtamin D_3 production rate as expressed by relation (7), the extended version of Holick's rule, is shown in Table 1 in the columns under "after 7.5 SED". The CIE-based production rates are insensitive to exposure duration by definition. According to the RIVM action spectra, previous exposure to 7.5 SED substantially reduces production for solar radiation, but not for the FS40 lamp. According to the modified QUT action spectrum, production after 7.5 SED is reduced to half of the initial rate for the FS40, and to a small fraction of initial production for solar radiation.

8 Comparison of new action spectra with CIE-2006

The measurements performed by MacLaughlin *et al.*[7] (see black line plus circles in Fig. 3) form the fundament of the CIE action spectrum. A qualitative difference is observed between the new (RIVM and modified QUT) action spectra and the CIE action spectrum: all new estimates have more emphasis on the shorter wavelengths. This affects the diurnal and annual cycles of vitamin D_3 weighted solar irradiance. For a large part of year at mid-high latitudes, the solar elevation remains low and lack of UV radiation arriving at the Earth, and then the skin surface leads to a lack of vitamin D synthesis (observed *in vivo*, see Webb *et al.*[13]). With our new action spectrum estimates, vitamin D_3 production will be estimated to be significant during a smaller portion of the day around local noon and during a shorter season of year around the summer solstice. This is demonstrated in Fig. 6, where we show normalized dose-rates within one day and total available daily dose for a whole year for The Netherlands. Inlays show zoomed-in parts for the periods in time where the weighted irradiances and daily doses are 10 percent of their maximum value (other threshold choices give similar results). The curves in red represent the CIE-2006 vitamin D_3 action spectrum. All new action spectra require a higher solar elevation to reach the 10 percent level. The largest deviation is found for the modified QUT action spectrum in combination with transmission spectra for "lower back, upper leg": for this part of the skin and focussed on vitamin D_3 production, a summer's day is

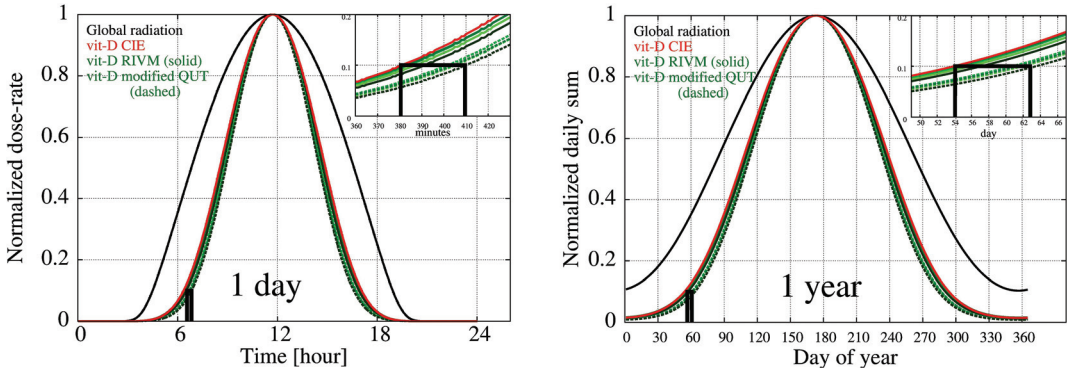

Fig. 6 Diurnal cycle of vitamin D_3 weighted irradiance for 21 June and annual cycle of total available daily vitamin D_3 weighted dose in The Netherlands. Comparison of estimates for different action spectra plus curve for global radiation.

1 hour shorter and the year is 18 days shorter than estimated on the basis of CIE-2006. For other skin sites, the effect is slightly less and for the RIVM action spectrum, the effect is about one-third of that for the modified QUT action spectrum.

Part of the stronger emphasis of the new action spectra on short wavelengths in comparison with the CIE action spectrum may come from the finite bandwidth of the radiation source used by MacLaughlin *et al.*[7] Most likely, the Full Width at Half Maximum (FWHM) of the monochromator has been 5 nm, although possibly this width may refer to Half Width at Half Maximum (HWHM). For this reason, we estimated the impact of the finite band width for both interpretations, but focus our analysis on 5 nm FWHM. As the spectral distribution of the 5 kW Xe arc lamp plus monochromator was not reported, we adopt an ideal white light source plus an ideal triangular slit function as a monochromator. With this triangle, we convolved both the RIVM action spectra and the modified QUT action spectra. The results for the RIVM action spectrum for lower back/upper leg after convolution with triangles with 5 nm HWHM and with 5 nm FWHM respectively are shown in Fig. 7, along with the non-convolved version from Fig. 3 and the CIE action spectrum. The effect of the convolutions is the strongest for wavelengths larger than 300 nm: a 5 nm FWHM slit function gives a red-shift of 1 nm and a 5 nm HWHM slit function gives a red-shift of 3 nm. The impact of convolution on the other estimates for the action spectrum (modified QUT and other skin sites) was similar.

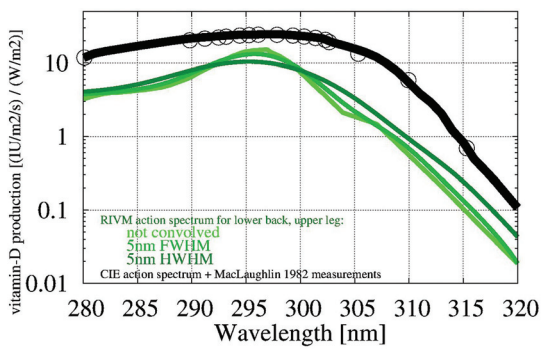

Fig. 7 Impact from non-ideal bandwidth during the experiment on the RIVM action spectrum for vitamin D_3 for lower back/upper leg. Three band-widths are shown in green: (1): ideal monochromatic radiation (same as the corresponding curve in the left panel in Fig. 3), (2): triangular slit function with 5 nm full width at half maximum and (3): 5 nm half width half maximum. The CIE action spectrum is shown in black.

The impact of 5 nm FWHM band-width on the coefficient in Holick's rule is demonstrated in Table 1 in the columns under "convolved initial". For the FS40 lamp, there is no significant effect. For solar radiation, the convolved action spectra give an up to 20 percent larger production than before convolution. When 5 nm HWHM was used (not shown in Table 1), then, according to the RIVM action spectra, the FS40 lamp and solar radiation under 60 degrees were equally potent in producing vitamin D per erythemal dose. For the modified QUT action spectra, the FS40 was still stronger than the sun, but the difference was much reduced.

9 Discussion and conclusion

We have constructed a set of equations describing the photochemical reactions involved in the production of vitamin D_3 in the skin. This set is solved analytically for stationary irradiance spectra (relation (14)). This solution cannot be written in action spectrum form, but the instantaneous production rate of any of the substances involved can (relation (16)). We constructed two sets of estimates for the action spectrum for the formation of vitamin D_3 in different skin sites. One set, the RIVM action spectrum, is based on a theoretical derivation from first principles and the other set on the basis of the QUT action spectrum. The two sets show qualitative agreement for wavelengths shorter than 303 nm. In comparison with the CIE action spectrum, the modified QUT action spectrum gives a 7× larger estimate and the RIVM action spectrum a 3× lower estimate. For larger wavelengths the ratio between the RIVM action spectra and the modified QUT estimates reduces to a factor of 5. The normalization of the action spectra in terms of absolute production should be addressed in new studies. The lack of coherence in the new action spectrum estimates means

that we cannot propose to replace the CIE action spectrum with either of the new ones. Until there is convergence of new insights, the CIE standard action spectrum for previtamin D_3 synthesis should be used.

The vitamin D_3 production rates per SED are eight times smaller when the RIVM action spectrum is applied than when the CIE action spectrum is applied. Similarly, applying the modified QUT action spectrum, the rates are twice as high. Both sets of new action spectra have more emphasis on the shorter wavelengths than the classical CIE action spectrum.

The RIVM action spectrum was constructed on the basis of an estimate for the absorption spectrum of provitamin D_3, which we copied from Fig. 1c in the paper by MacLaughlin *et al.*[7] The values shown for wavelengths greater than 303 nm are so small that the relative error in the derived action spectrum is large for these wavelengths. In Fig. 5 we can see that this implies that the weighted irradiance is dominated by a spectral region where the absorption spectrum of provitamin D_3 is not that accurate. A better estimate is needed here. The QUT action spectrum has been estimated independently from previous measurements of the provitamin D_3 absorption spectrum and therefore this action spectrum estimate does not suffer from the reported inaccuracy. However, the modification that we made to the QUT action spectrum, to compensate for self-absorption, still required the absorption spectrum. For wavelengths larger than 303 nm this is a small correction into which inaccuracy of the absorption spectrum does not propagate significantly. Therefore, in this respect (*i.e.* concerning inaccuracy of the available provitamin D_3 absorption spectrum for wavelengths larger than 303 nm), the modified QUT action spectrum has an advantage over the RIVM estimate.

We have shown that for wavelengths larger than 300 nm the CIE action spectrum is red-shifted by about 1 nm because of the bandwidth used in the underlying experiments by MacLaughlin *et al.*[7] and hence the true action spectrum is blue shifted relative to the CIE spectrum. It is uncertain as to which exact monochromator settings have been used in the different parts of the spectrum, so a mathematical deconvolution, to compensate for the CIE action spectrum for bandwidth, is not feasible. Furthermore, we expect that inter skin-site and inter-person variability of the parameters that are relevant for vitamin D production makes any vitamin D action spectrum only applicable for making rough estimates of the production. We therefore suggest leaving the CIE action spectrum as it is.

The new action spectra suggest that the sun is less effective in vitamin D_3 production per erythemal dose than the FS40 lamp, which has been used to calibrate Holick's rule. For the CIE action spectrum, Dowdy *et al.*[4] found the opposite: that (for high elevation angle) solar irradiance is up to 32% more efficient. Thus, estimates from Holick's rule for exposure time required for a given vitamin D production will be under-estimates and not over-estimates (as suggested by Dowdy *et al.*[4]). Webb *et al.*[11] saw no production of vitamin D_3 in winter in Boston (see also Webb *et al.*;[13] Rhodes *et al.*[12]), but model estimates based on the CIE action spectrum (see *e.g.*[10,14]) suggest an appreciable cutaneous production (see Webb[27] for a review of all relevant parameters in cutaneous vitamin D synthesis). From this, McKenzie *et al.*[10] concluded an "inconsistency" between model and measurement. The emphasis on shorter wavelengths of the new estimates for the action spectrum helps explain a tiny bit of this inconsistency. For all skin sites for which we constructed vitamin D action spectra, we calculated during which fraction of the day and during which part of the year, significant vitamin D synthesis should be possible. When the modified QUT action spectra for vitamin D synthesis are used, then a summer's day in The Netherlands is up to 1 hour shorter and the year is up to 18 days shorter than estimates of the productive periods on the basis of the CIE-2006 action spectrum. For the RIVM action spectra the time reductions are about one-third of those for the modified QUT action spectra.

Prolonged exposure of previously unexposed skin saturates vitamin D_3 formation. Generally, with the formation of previtamin D_3, the return reaction to provitamin D_3 limits the production of vitamin D_3 more and more. A different strong limiting effect on the production of vitamin D_3 is caused by photo-degradation of vitamin D_3 in skin by UV-radiation with longer wavelengths. When the solar elevation angle is small, then this effect is large and prolonged UV-exposure is ineffective. Disagreement between the absolute calibrations of the RIVM action spectrum and the modified QUT action spectrum leaves the dose at which this will become relevant undetermined. For the RIVM action spectrum, vitamin D_3 production is substantially reduced after 7.5 SED, a dose that induces a severe sun-burn. For a 20× more potent action spectrum (our heuristic approximation of the full modified QUT action spectrum) production is already substantially suppressed after 1.25 SED, a normal physiological dose that many people may receive several times in a year. Rhodes *et al.*[12] gave 1.3 SED of simulated sunlight per session, 3 sessions per week during 6 weeks and they reported a mean status change of 25 nmol l^{-1}. Whether or not this is "substantially reduced" depends on which status change would have been expected without saturation of the photochemical reaction, which is unknown. A sunscreen that eliminates radiation for wavelengths larger than 310 nm would prevent photo-decay of vitamin D. Sunscreens that preferentially block UVB may be counter-productive for vitamin D production. It is unclear if, as conjectured by Webb *et al.*,[21] in winter orally taken vitamin D_3 is destroyed in the skin. Caution should be taken in the assignation of consequences to the negative action spectra in our model for wavelengths larger than 310 nm. As indicated earlier, the RIVM action spectrum suffers from inaccuracy of the relevant absorption spectra for wavelengths larger than 303 nm. In studies on cutaneous vitamin D production by both solar and artificial UV-radiation, McKenzie *et al.*[28–30] observed significant vitamin D production even when the sun had a low elevation angle or when lamps were used with primarily only UVA.

Further research into cross-reactions with lumisterol and tachysterol and all return reactions will also have to include

the behaviour of these substances. How long does it take for the provitamin D_3 profile to refill after exposure? How long do side products lumisterol and tachysterol remain in the skin? What happens to their profiles when new cell layers are formed? And how fast is vitamin D_3 wrapped in D-binding protein and taken away from the skin into the blood stream?

Olds *et al.*[17] report a five-fold lower vitamin D_3 production under the QUT action spectrum than found *via* the CIE action spectrum. We have two considerations that place this finding in a different perspective: (1) as is customary with action spectra, Olds *et al.*[17] have normalized their QUT action spectrum by dividing out its maximum value. This operation has masked the characteristic of the *in vitro* experiment that the absolute yield was much larger than that in the experiments in the skin that formed the basis for the CIE action spectrum. Adjustment of the QUT action spectrum for the absolute yield reverses productions: now QUT no longer gives 5 times less than CIE, as suggested by Olds *et al.*,[17] but 27× more for irradiance from the sun in the zenith and even 247× more for the Westinghouse FS40 lamp. (2) The QUT action spectrum cannot be directly used to estimate cutaneous vitamin D_3 production, since it does not account for transmission through skin.

The CIE action spectrum is based on measurements in the skin and the QUT measurements have been done in solvent (and probably also the absorptivity and quantum yield measurements that we used to construct the RIVM action spectra). This leads to a different conformer of previtamin D_3: *cis–cis* in skin and *cis–trans* in solvent, which has a small effect on the photochemical reactions.[7,31] Furthermore, Tang *et al.*[32] stated: "the solvent environment plays a significant role in the conformational equilibrium of previtamin D_3 and on the various photochemical pathways" (see also Dmitrenko *et al.*;[33] Dmitrenko and Reischl[34]). This aspect of skin *versus* solution is possibly one of the causes of the differences between the new action spectrum estimates and the CIE action spectrum.

Our action spectra and production rates are constructed for the FitzPatrick Type-II skin only. Darker skin-types have a more effective shielding against UV radiation and different transmission spectra. Mixed populations will thus have mixed vitamin D_3 photosynthesis characteristics and vitamin D status optimization of such a population will therefore require a mixed approach. In this context, it would be a valuable addition to see the results of our model for all skin types.

Our estimates for the coefficient in Holick's rule are sensitive to the shape of the provitamin D_3 profile in skin as a function of skin depth. More accurate estimates of cutaneous vitamin D_3 production require better understanding of the provitamin D_3 profile for different skin sites, for different skin types, and for both genders separately. Datta *et al.*[35] recently showed that the change in vitamin D status that results from UV-exposure has a large inter-personal variation. It would be interesting to see if these variations can be linked to variations in biological parameters in our model, *e.g.* variations in the provitamin D_3 content or -profile and therefore to inter-individual variations in action spectrum. For chicken, Tian *et al.*[31] have shown that the provitamin D_3 content varies with the skin-site by a factor of 30: from 120 ng cm^{-2} for skin on the back to 3500 ng cm^{-2} for skin on the legs. The provitamin D profile should also be studied for different exposure histories (related to tanning and skin-thickening). Bruls[24] has for example shown that skin with an exposure history transmits about 3 times less than unexposed skin. Abundance of the D-binding protein is not yet well understood and thus little is known yet about possible saturation of the DBP-mediated transport of vitamin D_3 from the skin to the circulation.

The transmission measurement data that we used may not be representative for *in vivo* skin. Our estimate is that this may have led to a bias where the actual photon flux at any skin depth is underestimated. Improvement of this detail may thus lead to higher photoproduction rates from our model.

In the derivation of the RIVM action spectrum and of the effective skin-transmission that we have used to modify the QUT action spectrum, we have assumed all irradiance impinges perpendicularly to the skin. In practical situations, the irradiance will come from many different directions. Thus, the irradiance will penetrate less deeply into the skin (orthogonally seen), leading to some red-shift in the action spectrum and less vitamin D_3 production. The red-shift of the action spectrum from less deep penetration will be similar to the red-shift from an inward-shifted provitamin D_3 profile.

A. Photochemical production and generalized action spectrum

In this appendix, we present a mathematical model for all photo-chemical and isomerization reactions shown in Fig. 1 that occur in the skin, *i.e.* up to and including translocation away from the skin to the circulation, but hydroxylation by the liver and further reactions are not included. We show how the set of equations can be solved in practice and for constant irradiance spectra an analytical solution is presented. We use the mathematical representation of the photochemical reaction set to derive a (time-dependent) generalized action spectrum, which gives the instantaneous weighting function for irradiance to estimate the production rates of substances involved in the reactions.

Substances are numbered as follows: 1: provitamin D_3, 2: previtamin D_3, 3: lumisterol, 4: tachysterol, 5: vitamin D_3, 6: any toxisterol, suprasterol or other decay product. The reaction set is quantified spectrally in five steps, tracing the photoconversion processes. (1) We start with irradiance spectrum $I(\lambda)$. (2) From this irradiance spectrum, we estimate the photon flux density. (3) The flux is then attenuated on its way to depth r in the skin by a transmission factor $T(r, \lambda)$ to account for absorption and scatter. (4) The reduced photon flux at a given skin depth is (in part) absorbed by locally available substances with molar absorptivity spectrum ε_i for substance i. (5) The quantum yield $\phi_{i\rightarrow j}$ specifies which fraction of the absorbed photons leads to the actual conversion of substance i into substance j. The time-rate of change of the first five concentrations $D_i(r)$ at depth r in the (epi-)dermis, resulting from exposure to

irradiance spectrum $I(\lambda)$, is thus given by relation (8). For completeness, we added two terms to relation (9): the first term on the right hand side gives the heat-driven isomerisation reaction of previtamin D_3 to vitamin D_3 (defined in relation (12)) and the second term gives the translocation process of vitamin D_3 from the skin to the circulation (defined in relation (13)).

$$\frac{\partial}{\partial t}D_i(r) = \sum_j \tilde{B}_{ij}(r)D_j(r) + \text{refill of provitamin D}_3 \qquad (8)$$

In this relation, relative conversion rate $\tilde{B}_{ij}$ (*i.e.* the time rate of change of concentration D_i of substance i relative to instantaneous concentration D_j of substance j) is defined as:

$$\tilde{B}_{ij}(r) \equiv \tilde{B}_{ij,\text{iso}} + \tilde{B}_{ij,\text{trans}} + \int_\lambda B_{ij}(r,\lambda)\mathrm{d}\lambda \qquad (9)$$

where spectral relative conversion rate B_{ij} is defined as:

$$B_{ij}(r,\lambda) \equiv I(\lambda)T(r,\lambda)F_{ij}(\lambda) \qquad (10)$$

and the *in vitro* action spectrum F_{ij} as:

$$F_{ij} \equiv \frac{10^{-10}\ln(10)\lambda}{hcN_\text{A}} \times \begin{pmatrix} -\varepsilon_1\phi_{1\to2} & \varepsilon_2\phi_{2\to1} & 0 & 0 & 0 \\ \varepsilon_1\phi_{1\to2} & -\varepsilon_2(\phi_{2\to1}+\phi_{2\to3}+\phi_{2\to4}+\phi_{2\to6}) & \varepsilon_3\phi_{3\to2} & \varepsilon_4\phi_{4\to2} & 0 \\ 0 & \varepsilon_2\phi_{2\to3} & -\varepsilon_3(\phi_{3\to2}+\phi_{3\to6}) & 0 & 0 \\ 0 & \varepsilon_2\phi_{2\to4} & 0 & -\varepsilon_4(\phi_{4\to2}+\phi_{4\to6}) & 0 \\ 0 & 0 & 0 & 0 & -\varepsilon_5\phi_{5\to6} \end{pmatrix} \qquad (11)$$

In this relation,† λ is expressed in nm, I in W m^{-2} nm^{-1} and molar absorptivity spectra ε_i refer to extinction according to Lambert–Beer's law with base 10 (see relation (2)), N_A = 6.022 × 10^{23} mol^{-1} is Avogadro's number, h = 6.626 × 10^{-34} [J s] is Planck's constant and c = 3 × 10^8 [m s^{-1}] is the speed of light.

We adopt the spectral molar absorptivities for provitamin D_3, previtamin D_3, lumisterol and tachysterol from MacLaughlin and the quantum yield estimates for transitions between these substances collected by Norval *et al.*,[15] see Fig. 8. For the molar absorptivity spectrum of vitamin D_3, we use the reference provided by Sigma-Aldrich.[36] This spectrum includes a factor R, the optical path length, which is prescribed in the reference procedure[38] to be R = 1 cm. We estimated the quantum yield for conversion of vitamin D_3 into decay products (suprasterol I, suprasterol II and 5,6-*trans*-vitamin D_3, see Fig. 1) in a numerical simulation of the degradation experiment of vitamin D_3 in its solvent performed by Webb *et al.*[21] Least squares optimization of the quantum yield gave a provisional estimate of 0.405 ± 0.005, which we consider to be sufficiently accurate for the present study. We plan to publish a more robust estimate in the near future. Concerning the photo-decay of previtamin D_3: Abillon and Mermet-Bouvier[37] estimated the quantum yield for conversion of previtamin D_3 to toxisterols to be 0.039. We include this characteristic in our model and leave all other conversions to toxisterol out of the analysis from now on.

Isomerisation of previtamin D_3 to vitamin D_3 is temperature dependent. In skin, the forward reaction takes place on a characteristic time-scale $\tau_{\text{iso,forward}}$, which has been estimated to be 2.5 hours.[31] The return reaction of vitamin D_3 to previtamin D_3 has a characteristic time-scale $\tau_{\text{iso,backward}}$ of 24 hours.[31] Isomerisation is represented by the following (non photo-chemical) production relations:

$$\begin{aligned} \tilde{B}_{52,\text{iso}} &= -\tilde{B}_{22,\text{iso}} = \ln(2)/\tau_{\text{iso,foward}} \\ \tilde{B}_{25,\text{iso}} &= -\tilde{B}_{55,\text{iso}} = \ln(2)/\tau_{\text{iso,backward}} \\ &\text{(all other elements of } \tilde{B}_{\text{iso}} \text{ are 0)} \end{aligned} \qquad (12)$$

Due to lack of information, we assume that in skin provitamin D_3 is refilled instantaneously by its precursors involved in the cholesterol synthesis. In all our model calculations, the fraction of provitamin D_3 that was converted was so small that the assumption of instantaneous refill had no impact on the results. Translocation of vitamin D_3 from the skin to the circulation takes place on a characteristic time-scale τ_{trans}, which has been estimated to be 9 hours.[31] This transport is represented by the following loss term in relation (9):

$$\tilde{B}_{55,\text{trans}} = -\ln(2)/\tau_{\text{trans}} \quad \text{(all other elements of } \tilde{B}_{\text{trans}} \text{ are 0)} \qquad (13)$$

Relation (8) can be used to simulate the photo-conversion of provitamin D_3, previtamin D_3, lumisterol, tachysterol and vitamin D_3 at any depth r in the medium. There is no general analytical solution for equation (8), concentrations have to be found numerically. For the limited set of situations when the shape of the irradiance spectrum is stationary (intensity may vary) and transmission is dominated by stationary characteristics of the medium (*e.g.* skin pigmentation and thickness) and not by dynamical quantities (like the concentrations of the substances involved in the photobiological process), then matrix $\tilde{B}_{ij}(r)$ can be calculated once in advance of the simulation and used throughout. The solution of differential equation (8) for concentrations at time t is then found from the set of initial concentrations *via* matrix exponential, for which we adopt the implementation in FORTRAN-90 by Alan Miller as provided by Blevins:[39]

$$D_i(r,t) = \sum_j \left[e^{\int_0^t \tilde{B}(r,\tau)\mathrm{d}\tau} \right]_{ij} D_j(r,t=0) \qquad (14)$$

† The factor 10^{-10} in definition (11) has the following origin: I gives irradiance in [J s^{-1} m^{-2} nm^{-1}]. 10$^{-9}I\lambda/(hcN_\text{A})$ gives the photon flux in [mol s^{-1} m^{-2} nm^{-1}]. T is a dimensionless correction for skin transmission. 10$^{-10}I\lambda T\varepsilon \ln 10/(hcN_\text{A})$ gives the absorption concentration rate of photons per unit concentration of target material in [(mol L^{-1}) (mol L^{-1})$^{-1}$)s^{-1} nm^{-1}]. Multiplication with the dimensionless quantum yield gives the conversion rate in [s^{-1} nm^{-1}].

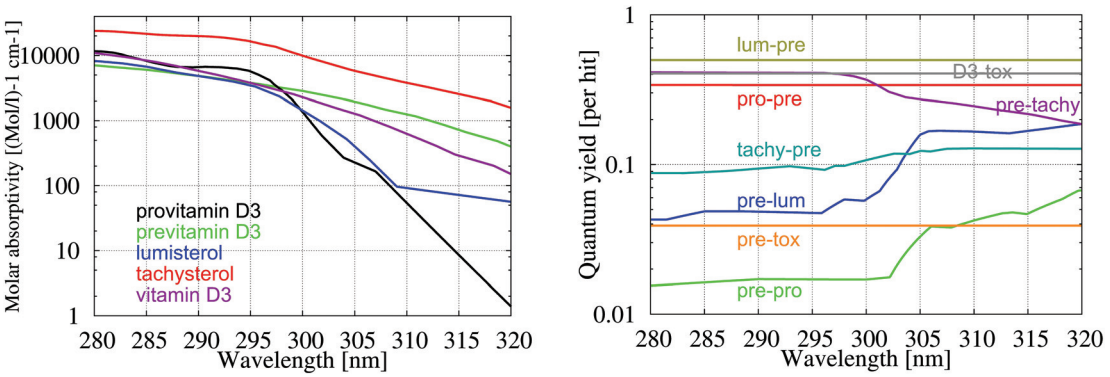

Fig. 8 Left: molar absorptivity spectra for provitamin D_3, previtamin D_3, lumisterol and tachysterol from MacLaughlin *et al.*[7] and for cholecalciferol from Sigma-Aldrich.[36] Right: quantum yield spectra from Norval *et al.*[15] plus our own estimate for the quantum yield for conversion of vitamin D_3 into decay products and the estimate from Abillon and Mermet-Bouvier[37] for conversion of previtamin D_3 to toxisterols.

In the case of non-stationary irradiance spectra, relation (14), the analytical solution for a stationary irradiance spectrum, can be used for integration *via* quasi-stationary time steps. The instantaneous total photo-chemical conversion rate at any point in time in the skin is found *via* integration of relation (8) over the skin-depth. $\bar{D}_i$ giving the skin-depth integrated concentration of substance i (excluding the stratum corneum, which is isolated from the rest of the epidermis) per unit area of skin:

$$\bar{D}_i \equiv \int_r D_i(r)\mathrm{d}r$$

The photo-conversion rate of the different surface-concentrations $\bar{D}_i$ can be written as the integral of irradiance spectrum I, weighted with an action-spectrum A_i, as follows:

$$\frac{\partial}{\partial t}\bar{D}_i = \int_\lambda I(\lambda)A_i(\lambda)\mathrm{d}\lambda \qquad (15)$$

Quantity $A_i(\lambda)$ is called the action spectrum and has the following definition:

$$A_i(\lambda) \equiv \sum_j F_{ij}(\lambda)T_{\mathrm{eff},j}(\lambda)\bar{D}_j \qquad (16)$$

Effective transmission $T_{\mathrm{eff},j}(\lambda)$ represents skin transmission weighted with the profile of substance j and is defined as:

$$T_{\mathrm{eff},j}(\lambda) \equiv \int_r \frac{D_j(r)}{\bar{D}_j}T(r,\lambda)\mathrm{d}r \qquad (17)$$

Production in macroscopic time-steps, as given by relation (14), cannot be formulated *via* an action-spectrum. The action spectrum formulation can therefore only be used prognostically, to monitor and characterize instantaneous conversion rates at different stages of exposure. In this sense, the now extended action spectrum for previtamin D_3 synthesis can still be a valuable tool. In this paper, we seek the action spectrum for vitamin D_3, but this substance is not directly produced in photosynthesis, its only direct relation with UV radiation is its degradation. If the exposure stops at a certain moment, photochemistry halts and all previtamin D_3 that is formed will eventually become vitamin D_3. This makes it clear that at any point in time, the sum of the previtamin D_3 content and vitamin D_3 content is a measure of the final vitamin D_3 content. Therefore, we define the true action spectrum of vitamin D_3 as the sum of the separate action spectra for pure previtamin D_3 and the degrading action spectrum for actual vitamin D_3:

$$\text{“true”}A_{\text{vitamin D}_3}(\lambda) \equiv A_{\text{previtamin D}_3}(\lambda) + A_{\text{vitamin D}_3}(\lambda) \qquad (18)$$

B Skin characteristics

Profile $D_{\mathrm{pro}}(r)$ for provitamin D_3 in the skin is taken from Meinhardt-Wollweber and Krebs:[25]

$$D_{\mathrm{pro}}(r) = 4.1 \times 10^5 \times \mathrm{Gau}\beta\ (r, r_0 = 62\ \mu\mathrm{m}, \sigma = 20\ \mu\mathrm{m}) \quad [\mathrm{IU\ m^{-2}\ \mu m^{-1}}] \qquad (19)$$

Meinhardt-Wollweber and Krebs[25] adopted the concentration per unit area of skin from Holick *et al.*[26] who took their samples from hypopigmented Caucasian human leg skin (thigh). They reported 4.1×10^5 [IU $\mathrm{m^{-2}}$]. We follow Bruls[24] and adopt a stratum corneum depth of 20 μm and locate the basal layer at 70 μm. Photoproducts in the stratum corneum are assumed not to leave this skin layer and are therefore irrelevant for vitamin D_3 synthesis. Photochemical conversion rates at depth r in the (epi-)dermis depend on actinic photon flux $\Phi(r)$, which is the integral of the photon flux over all directions. We now relate the actinic flux $\Phi(r)$ at a given depth r in the skin to the incident irradiance I_0 on the skin that we assume to be perpendicular. The medium through which the incoming radiation has to travel attenuates the radiation. The attenuation varies with skin thickness and, to a much smaller degree, with pigmentation. Apart from the radiation or photons that still have a direction perpendicular to the skin, there is a scattered or diffuse photon density. Fig. 2 on page 61 of the thesis by Bruls[24] shows the angular distribution of radiation transmitted through the epidermis for different angles of

incidence. Scatter does not seem to be dominant, and therefore focussing on the irradiance is a good approximation for the actinic photon flux in the epidermis. We model skin as a homogeneous material with transmission $T(\lambda, r)$ according to Lambert–Beer's law:

$$\Phi(r,\lambda) \simeq T(\lambda,r)I_0 \quad \text{with} \quad T(\lambda,r) = e^{-\mu(\lambda)r}$$

where $\mu = \mu_a + \mu_s$ is the extinction coefficient, which consists of an absorption part and a scattering part.

We use observations from the literature of irradiance extinction for different thicknesses of skin samples as an estimate for μ to get the photon flux at any given depth in the epidermis. Jacques[40] has compiled a review on optical properties of biological tissues. In Fig. 12 of ref. 40, spectra for absorption coefficient μ_a (both measured and modelled) are shown for (components of) cutaneous melanosomes. The overall correspondence between the proposed parameterizations and the experimental data over the wavelength range from 200 nm to 1200 nm is good, but the match in our range of interest, *i.e.* from 280 nm to 320 nm, is much weaker. This is the reason why we don't use the absorption coefficient spectra from Jacques,[40] but instead use experimentally estimated spectra from experiments that were dedicated to the UV range. Bruls[24] has reported spectral measurements on transmission of human skin (non-exposed lower back and upper leg of young Caucasians). Meinhardt-Wollweber and Krebs[25] reported absorption-spectra for three sites of (white Caucasian) skin: inner fore-arm, outer fore-arm and ball of the thumb. When we compare the transmission estimates based on Bruls with estimates based on Meinhardt-Wollweber and Krebs, we see that the lower back and upper leg results from the former have the best match with the outer fore-arm results from the latter. Bruls and Meinhardt-Wollweber and Krebs collected their transmission spectra *in vitro*, where the skin samples were attached to a diffusor. In this way, the photon fluence component from back-scatter, which will exist in real living skin, is excluded. As a result, the estimates of transmission from experiments will underestimate *in vivo* transmission.

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