Metrology of the vitamin-D-synthetic activity of UV lamps

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Abstract. Ultraviolet lamps are widely used in phototherapy, and positive health effect of UV irradiation is associated with the synthesis of vitamin D in human skin. Nevertheless, to avoid harmful effects of UV radiation a variety of commonly used broadband UV detectors have an output in erythema units. Therefore the measurement of the vitamin-D-synthetic capacity is a missing link in the metrology of UV lamps that are used for medical and/or cosmetic purposes. This paper presents original UV measuring device based on the same photoreaction *in vitro* by which vitamin D is synthesized in human skin *via* photo- and thermo- induced conversions of 7-Dehydrocholesterol (provitamin D_3). Thus the targets for UV photons are provitamin D molecules embedded in specially designed UV transparent and stable matrix mimicking biological samples. Three operation modes of varying complexity are developed to follow the photoreaction course in real time.

1 Introduction

It is generally accepted to divide UV radiation into three spectral ranges, i.e., UV-C (100-280 nm), UV-B (280-320 nm), and UV-A (320-400 nm).

Metrology of UV lamps includes measuring a number of physical parameters such as wavelength, intensity, irradiance, etc., and many certified instruments for these measurements are available on the market.

Since the biological effectiveness of UV radiation depends significantly on the radiation wavelength (increasing with increasing energy of UV photons), the measurement of a biological effectiveness of a UV radiation source requires special tool whose spectral sensitivity is correlated with the action spectrum (AS) of a specific biological effect. This goal is served by special biodosimeters which directly measure the integrated biological effect but the result is expressed in specific biological units.

Naturally, most attention is paid to the measurements of erythemic and mutagenic (carcinogenic) biological activity of UV radiation to prevent possible negative effects. The Robertson-Berger (RB) meter is widely used to measure erythemal effectiveness because its spectral sensitivity is similar to the standard erythema action spectrum [1]. And, since carcinogenic biological effectiveness of UV radiation is associated with photoinduced mutations of DNA molecule in living organisms, the measurements of mutagenic (carcinogenic) biological activity are based on the photoinduced DNA damage [2].

However, UV radiation has also a very important beneficial effect, namely, the vitamin D_3 synthesis in human skin which is produced photochemically from 7-Dehydrocholesterol under solar UV-B radiation.

The measurement of the vitamin-D-synthetic ability (or antirachitic activity) of UV lamps used for medical and/or cosmetic purposes is especially important given the essential role of vitamin D in maintaining health [3], as well as taking into account the observed pandemic of vitamin D deficiency among the world's population [4].

1.1. Main features of vitamin D synthesis

As is known, the synthesis of vitamin D occurs in two stages. First, under UV irradiation within the absorption band of provitamin D (**Pro**) it photochemically turns into previtamin D (**Pre**), which is further thermally converted into vitamin D (**D**) (Fig. 1). Thus to measure the vitamin-D-synthetic activity of a UV lamp it is necessary to follow the photoconversion **Pro** → **Pre** and to measure the amount of previtamin D accumulated during an UV exposure. It is this quantity that determines biologically active "antirachitic" UV dose.

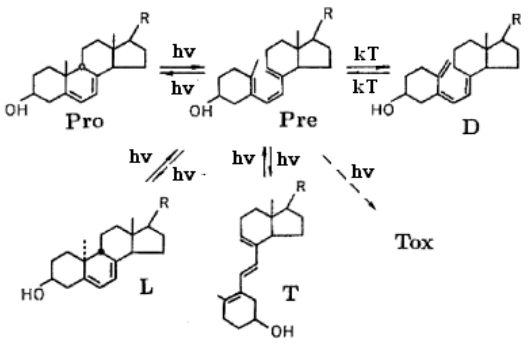

Fig.1. The reaction scheme of vitamin D synthesis. T, L and Tox are by-products tachysterol, lumisterol and toxisterols. Dashed line shows irreversible photoconversions. R=C_9H_{17}, vitamin D_2 series; R=C_8H_{17}, vitamin D_3 series.

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But the task is complicated by the fact that previtamin D itself is not stable to UV radiation and undergoes a number of side photoconversions. As a result, UV irradiation of initial provitamin D gives rise to the formation of a multicomponent photoisomer mixture.

It is necessary to emphasize the important features of the photoreaction [5]: (1) the accumulation of previtamin is nonlinearly dependent on a UV dose, (2) the maximum achievable concentration of previtamin D is highly dependent on irradiation wavelength (for monochromatic irradiation) or on the spectral composition of the UV lamp radiation (Fig.2).

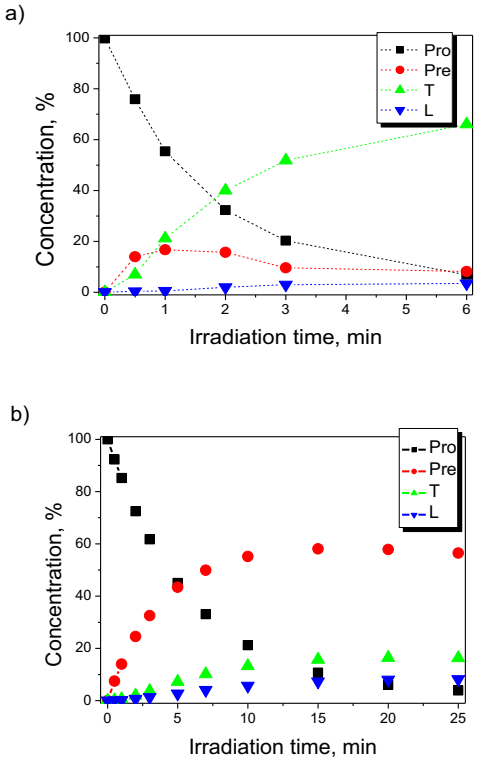

Fig. 2. Kinetics of 7-Dehydrocholesterol (7-DHC, provitamin D_3) photoisomerization under UV irradiation (a) by the low pressure mercury lamp (254 nm) and (b) by the XeCl excimer lamp (308 nm).

It seems unlikely that an optoelectronic device or a photosensitive polymer film could display correctly the above features and adequately measure the vitamin-D-synthetic activity, even if their spectral sensitivity corresponds to the action spectrum of vitamin D synthesis. Obviously, the best tool for this metrology is the *in vitro* model of vitamin D synthesis.

2 Methods

For the first time the *in vitro* model of vitamin D synthesis (cylindrical ampoule with ethanol solution of 7-Dehydrocholesterol) was used to clarify the effect of seasonal and latitudinal changes in solar UVB radiation on vitamin D_3 synthesis [6]. In this study the concentration of accumulated previtamin D was determined using high-performance liquid chromatography (HPLC) which is not suitable for UV measurements *in situ*.

A significant step towards the widespread practical use of the *in vitro* model for on-site measurements was the development of original spectrophotometric analysis of the photoisomer mixture formed under UV irradiation of 7-Dehydrocholesterol [7,8].

Moreover, owing to the detailed studies of the photoreaction using a tunable dye-laser [9] the adequate mathematical model was developed enabling calculation of the photoreaction kinetics for any UV radiation source. The validity of spectral analysis and the mathematical model was confirmed by simultaneous UV measurements using a spectroradiometer within the framework of the EC BIODOS Project [10].

To date, there are three possible measurement methods in which the targets for UV photons are 7-DHC molecules either in solution or embedded in specially designed UV transparent and stable matrix.

2.1. Measurement of Previtamin D concentration using spectrophotometric analysis

Ethanol solution of 7-Dehydrocholesterol is exposed in standard rectangular quartz cuvette. The concentration of 7-DHC (10 ÷ 20 μg/ml) and the cuvette thickness (0.2 ÷ 1.0 cm) are chosen to ensure the conditions of the optically thin layer, i.e. to provide almost uniform illumination of the solution and exclude necessity of its stirring during the UV irradiation.

Before the beginning of an irradiation and after the certain exposures, the absorption spectrum of the solution is registered by a spectrophotometer within a range of 230-330 nm with a 1 nm step. Then for concentration analysis the recorded spectra are processed by computer using original software [8], and the concentration of accumulated previtamin D, which determines 'antirachitic' UV dose, is obtained.

To avoid the inconvenience associated with the use of liquid solution, 7-DHC molecules were incorporated into the hydrogel matrices in the form of 2 mm thick films [11]. It appeared that the absorption spectrum of 7-DHC in the film is almost identical to its spectrum in ethanol that allowed the concentration analysis (Fig. 3) [12].

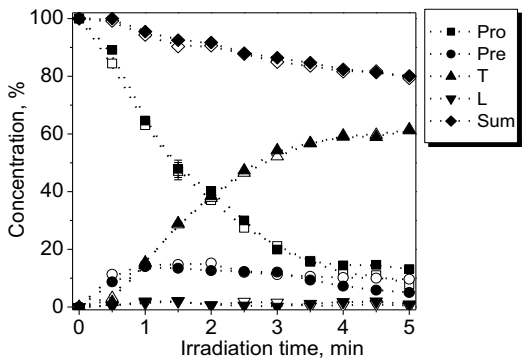

Fig. 3. Comparison of the 7-DHC photoisomerization kinetics in hydrogel matrix (solid symbols) and in ethanol (open symbols) under irradiation with TUV-300 lamp: **Sum = Pro+Pre +T+L.**

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2.2. Measurement of Previtamin D concentration using calibration graph

The spectrophotometric analysis requires a modern spectrophotometer and an experienced professional, that hampers its widespread use. Therefore, for general use, a method can be applied that is similar to measuring erythemic activity with a polysulphone film by the absorbance change at a fixed wavelength [13].

With this aim portable optoelectronic device 'VITA-D' was developed to measure the transparency changes of the film (polymeric or hydrogel) with embedded 7-DHC molecules as a result of UV exposure (Fig. 4). In the device, the film is placed between the photodiode and the LED emitting at the wavelength almost coinciding with the maximum of 7-DHC absorption band [14].

Fig. 4. The external view of the 'Vita-D'. On the laptop screen the LED emission band is shown in relation to the provitamin D absorption spectra transformed due to UV exposure.

It is important to note that for each UV lamp with its unique radiation spectrum, the calibration procedure must be performed, establishing a relationship between the change in the film transmission and the concentration of accumulated previtamin D.

2.3. Visual detection of the vitamin-D-synthetic activity

To provide visual detection, we use the well-known sensitivity of cholesteric mesophase to the smallest changes in molecular structure.

It was found that dissolution of optically active 7-DHC molecules in nematic liquid crystal (LC) induces cholesteric phase with right-handed helix [15]. Under UV irradiation, the alteration of molecular geometry of provitamin D by the photoinduced conversion into previtamin D (with its further *cis-trans* isomerization into tachysterol) significantly affects its helical twisting power, resulting in changes of helical pitch value and allowing visual detection of previtamin D synthesis by color change (like a litmus paper measures pH) (Fig. 5).

Laboratory tests revealed a linear relationship between the shift of the selective reflection band (color change) and the accumulation of previtamin D *in vitro* [16].

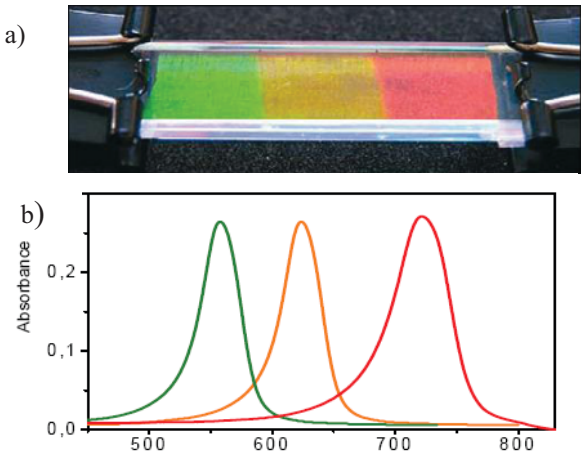

Fig. 5. Photo of personal UV D-biodosimeter with three areas of different UV exposures (a) and corresponding spectra of each section (b)

Recently another method of visual detection of previtamin D photosynthesis has been introduced using the so-called θ-cell in which the rubbing of a front face is parallel to the x-axis and is circular on a rear face. As a result, a topological defect occurs in the form of a disclination line.

When a θ-cell is filled by nematic LC doped with 7-DHC and is irradiated by a UV, the angular deviation of disclination line from its initial position increases (Fig. 6) providing visual detection of antirachitic UV biodose (like measuring the time interval by the deviation of the clockwise arrow) [17].

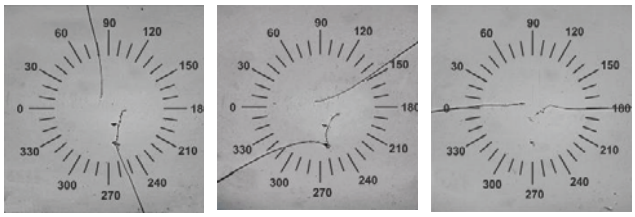

Fig. 6. Polarized optical microscopic image of the θ-cell filled by ZhK-805 doped with 7-DHC (C = 0.2 wt.%) before UV irradiation (a) and after 5 min (b) and 10 min (c) irradiation by the lamp EL-30.

Note that concentration of 7-DHC should be chosen according to a UV lamp intensity for the calibration dependence so that the deviation angle of disclination line during an exposure does not exceed 360 degrees.

3 Results and discussion

A number of laboratory tests were carried out using various UV sources, the radiation of each of them was measured by a spectroradiometer. In addition, the vitamin-D-synthesizing activity of the UV lamp widely used for sanitation was tested. In this section we briefly summarize these results published earlier in various journals, as well as the result of the biomedical research, establishing a link between the measurements *in vitro* and *in vivo*.

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3.1 Testing using the spectroradiometric data

As mentioned above, a significant advantage of the method based on an *in vitro* model of vitamin D synthesis (D-biodosimeter) is the availability of the mathematical model that can provide a link between the biological and physical units. We investigated the validity of this model by measuring and calculating the kinetics of 7-DHC photoisomerization under UV irradiation by UV lamps with available spectro-radiometric data.

3.1.1 Verification of the reciprocity law

The D-biodosimeter was tested with the standard lamp BBH4 (1000W FEL, Osram Sylvania Ltd) which is commonly used for spectroradiometer calibration [18]. Solution of 7-DHC (Sigma) in ethanol (20μg/ml) was irradiated in quartz cuvette (Helma) of 0.5cm thickness at the distance of 50 cm. The absorption spectra were recorded by the Perkin-Elmer Lambda 15 UV/VIS spectrophotometer before and after several exposures, and were further processed by computer to obtain the photoisomer concentrations.

Additionally, the photoreaction kinetics was calculated using the spectral data of the BBH4 lamp at the input of the mathematical model which is the system of differential equations defining time-dependences of the photoisomers concentrations [10]. The same experiment was performed for a 25cm distance between the lamp and the cuvette to increase UV doses without requiring excessively long exposures (Fig. 7).

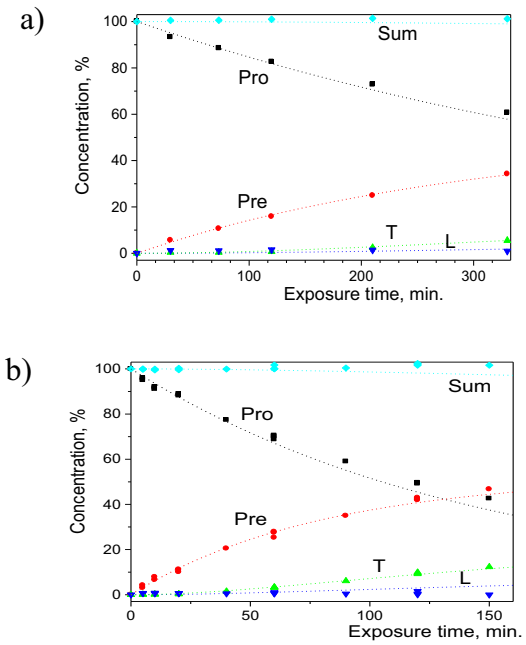

Fig. 7. Experimental (symbols) and calculated (dotted lines) kinetics of previtamin D photosynthesis under BBH4 lamp irradiation: a) 50 cm distance, b) 25 cm distance (the lamp intensity was increased by factor 4).

It is evident from Fig. 7 that there is a close match between the measured and calculated concentrations. Moreover, it was found that doubling the distance between the lamp and cuvette led to a four-fold increase of the exposure time to get the same concentration of previtamin D (Fig.7b). Hence the laboratory test confirmed both the validity of the mathematical model and the implementation of the reciprocity law.

3.1.2 The calibration using narrow band filters

Radiometric characterization of the method has been performed using UV irradiation from the xenon arc lamp with a number of narrow band filters ($\Delta\lambda$ = 2 nm). Special calibration facility was used to establish the relation between physical, erythemal and antirachitic UV doses for quasi-monochromatic UV irradiation [19].

As mentioned above, the time-dependence of previtamin D accumulation is linear only at the beginning of UV exposure, and this linear part of the dependence was used to relate physical, erythemal dose and previtamin D concentration, i.e. for all the wavelengths the doses needed for 5% formation of previtamin D were calculated using the spectroradio-metric data (Table 1).

Table 1. Relationship between antirachitic, physical and erythemal doses depending on the radiation wavelength

Wavelength (nm)	Antirachitic dose (% Pre)	Physical dose (J/m^2)	Erythemal dose (MED)
260	5	55	0.275
270	5	42.7	0.213
280	5	40	0.2
290	5	95	0.475
300	5	205	0.703
310	5	10400	3.874

More clearly dramatic change in the ratio between antirachitic and erythemal doses in the longwave region is shown in Fig.8.

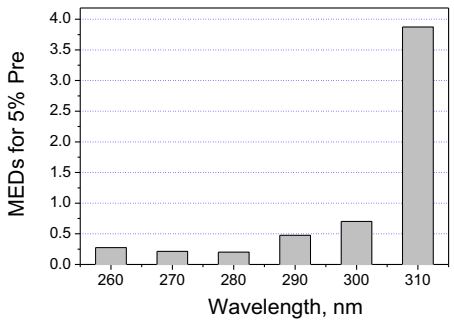

Fig. 8. The wavelength dependence of the MEDs quantity required to yield 5% of previtamin D

Since the ethanol solution of 7-DHC (C = 20 μg/ml) was irradiated in the cuvette of 0.5 cm thickness, the amount of 7-DHC 10 μg/cm^2 was 10 times more than the amount of 7-DHC in the skin (1μg/cm^2) of a 20-year-old person [20]. Since the rate of photochemical processes

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does not depend on the concentration of the initial substance and, in accordance with the law of photochemical equivalence, is determined only by the intensity of the absorbed light, from these data it is possible to obtain the relations MED/Pre *in vivo*, taking into account the transmission of the skin for every UV wavelength.

3.1.3 Comparison of two UV lamps emitting UV-B and UV-A radiation

Exceptional sensitivity of previtamin D formation to the spectral composition of UV radiation was demonstrated during the laboratory test using the UV lamps FS20 and TL01 whose radiation spectrum were measured by a spectroradiometer (Fig. 9).

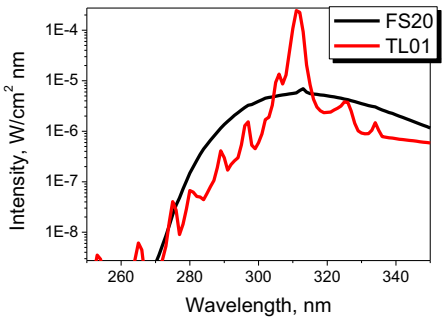

Fig. 9. Radiation spectra of the two UV lamps

At first glance, both lamps have approximately equal radiation intensity in the UV-B region of the spectrum, but in fact intensity of TL01 is 6 times higher due to the intensive line at 313nm. But it turned out that the rates of previtamin D accumulation are very different, and not in favor of the TL01 lamp. (Fig. 10). In addition, the photoreaction kinetics was calculated using the spectro-radiometer data at the imput of the photoreaction model [10]. These calculated kinetics of previtamin D accumulation are shown in Fig. 10 together with the experimental data. A good agreement between the calculated and experimental data proves the adequacy of the model and, furthermore, shows that this method can have practical significance in UV-B-actinometry.

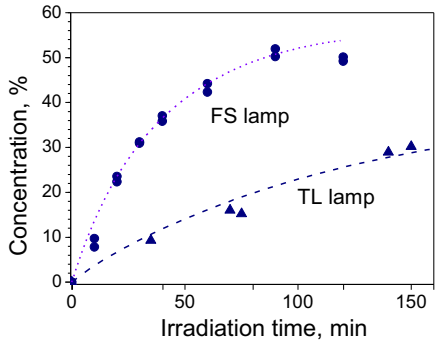

Fig. 10. Experimental *(*symbols*)* and calculated *(*lines*)* kinetics of previtamin D formation under irradiation by the UV lamps.

3.2 Biomedical testing of UV lamps

For the first time the method described in section 2.1 was applied for the measurement of the vitamin-D-synthetic activity of the UV lamps recommended for health exposure at home and also used in a solarium with the cosmetic purposes (tanning).

3.2.1 Testing of high pressure mercury lamp

The UV arc discharge mercury lamp of high pressure DRT-125-1 is used in many household appliances of UV irradiation in the FSU countries. Taking into account that in the lamp spectrum there is UV radiation of three ranges (UV-A, UV-B and UV-C) (Table 2), it can be assumed that at certain doses UV irradiation can provoke bactericidal, erythemal and/or antirachitic effects.

Table 2. The intensity distribution in the UV radiation spectrum of the lamp DRT125-1

Intensity distribution in spectral regions (W/m^2)		
UV-A	UV-B	UV-C
1,57	1,85	1,45

In the manual to this lamp, in addition to the recommendations for its use for sanitary purposes, it is also mentioned that UV irradiation contributes to the production of vitamin D in the skin. We measured the accumulation of previtamin D *in vitro* at two distances (90 and 180 cm) recommended. The corresponding concentration dependences are shown in Fig. 11 a) and 11 b) [21].

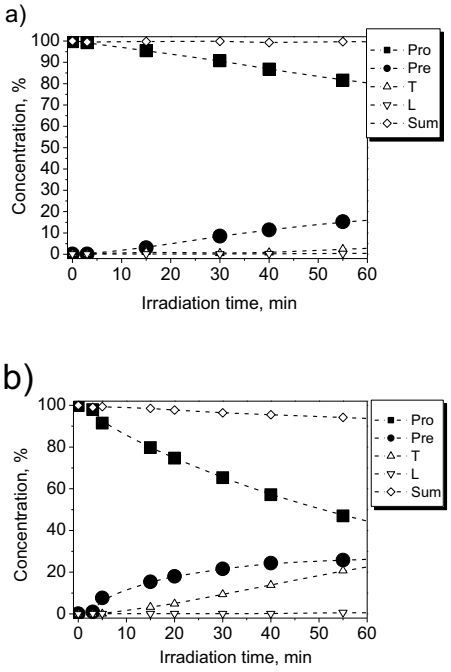

Fig.11. Change in the concentration of the initial 7-DHC (squares) and formed previtamin D (circles) under irradiation by the lamp DRT-125-1 at the distances of 180 (a) and 90 cm (b).

As can be seen from Fig. 10b), the previtamin D accumulation can be approximated by a linear dependence only with short exposures (up to 10 min). With further irradiation, the curve goes to the saturation, mainly due to the side photoconversion of previtamin D into tachysterol.

If we assume that only 10% of the UV-B radiation penetrates the epidermis [22] and all previtamin D formed during UV irradiation completely turns into Vitamin D then a simple calculation shows that the irradiation of 1 m^2 surface of a human body with the DRT-125 lamp for 10 minutes can ensure the formation of the required amount of vitamin D.

However, measurements of the spatial distribution of light intensity showed that, due to the small size of the DRT-125 lamp, it is impossible to obtain uniform illumination over an area of 1 m^2. Therefore, irradiation at the distance of 180 cm cannot provide even a minimum daily dose of vitamin D. But with the decrease in distance, the presence of a bactericidal component in the lamp emission spectrum can have a negative (mutagenic) effect.

Therefore, in addition we measured the vitamin-D synthesizing power of the Philips TL 20W / 01 RS SLV and Philips TL 20W / 12 RS SLV lamps recommended for the treatment of psoriasis. As can be concluded from Fig. 12, where formation of previtamin D is shown with respect to converted provitamin D, these lamps are more favorable for the synthesis of previtamin D. The lamp Philips TL 20W / 12 RS SLV is especially good, since 60% of the previtamin is produced at 90% conversion of initial provitamin D.

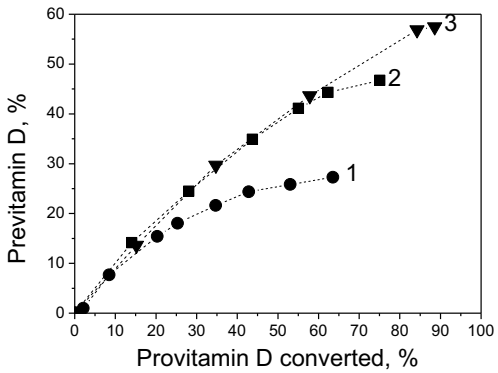

Fig.12. Comparative dependencies of previtamin D photosynthesis relative to converted provitamin D for three lamps: 1 - DRT-125-1, 2 - Philips TL 20W / 01 RS SLV, 3 - Philips TL 20W / 12 RS SLV.

3.2.2 Testing of a sunbed

Direct measurements of the vitamin D level in blood of healthy volunteers exposed to artificial UV source (sunbed) in parallel with measurements of vitamin D generation *in vitro* were performed to provide a missing link between *in vivo* and *in vitro* measurements [23].

As is known, after its formation in skin vitamin D_3 is metabolized to 25-hydroxyvitamin D (25(OH)D) in liver and in several other tissues [24]. The *in vivo* level of 25(OH)D measured in the blood serum of 22 healthy volunteers exposed to UV radiation in the sunbed was compared with *in vitro* measurements of previtamin D formation.

The UV source was a commercially available and approved sunbed Wolff Suveren 53IG (Wolff System, Basel, Switzerland).

It was found that the increase in 25(OH)D concentration depended both on the initial 25(OH)D level and on the cumulative sunbed exposure time.

It is interesting to note that the non-linear character of 25(OH)D formation observed *in vivo* is similar to non-linear previtamin D accumulation *in vitro,* and, as a result, a linear correlation with high correlation coefficients R has been revealed between the *in vivo* and *in vitro* data for the three groups of volunteers with different initial 25(OH)D levels (Fig.13).

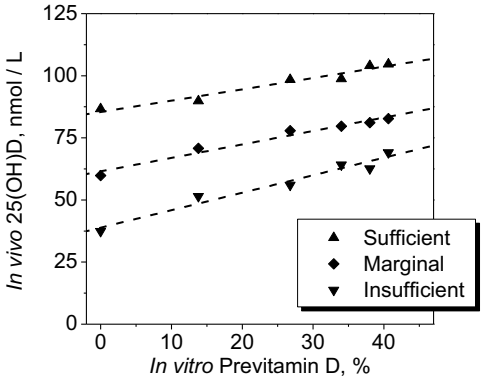

Fig. 13. Linear correlations between the *in vivo* changes in the median of 25(OH)D distribution for three volunteer groups and *in vitro* photosynthesis of previtamin D.

As seen, the overall growth of 25(OH)D level under sunbed exposures is more noticeable for people with initially lower vitamin-D status.

It is important to note that observed linear correlations between *in vivo* and *in vitro* data allow predicting the changes of vitamin D status after UV exposures using only one pre-exposure blood sample analysis combined with further measurements of previtamin D accumulation *in vitro*. Certainly, correlations obtained in this study should be re-measured for each sunbed equipped with other fluorescent lamps.

4 Conclusions

Wide use of UV radiation in medicine requires reliable control because high energy UV photons initiate a variety of photochemical reactions that have both negative and positive consequences.

Little attention is still given to the determination of the lowest healthy UV doses that are extremely necessary to overcome the vitamin D deficiency by maintaining optimal levels of 25-hydroxyvitamin D in the human blood. This is especially important in view of its role against many types of cancers, in regulating cell growth and modulating the immune system [25].

In view of strong wavelength dependence of vitamin D synthesis, the specification of artificial UV radiation in radiometric units is of limited value if the spectral content is not taken into account. As illustrated in Fig. 8, most of broad-band UV detectors measuring erythemal activity of UV lamps are unsuitable for determining the specific antirachitic UV exposures.

It is hoped that the results given in this article confirm the ability of the presented methods to provide a reliable measure of the antirachitic radiation of UV sources and will form a basis for the future mandatory metrology of the vitamin D synthetic activity of UV lamps used both for medical and cosmetic purposes.

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