

The effect of ultraviolet radiation from a novel portable fluorescent lamp on serum 25-hydroxyvitamin D₃ levels in healthy adults with Fitzpatrick skin types II and III

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Summary

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The synthesis of vitamin D begins in the skin with the photoconversion of 7-dehydrocholesterol (7-DHC) to pre-vitamin D₃ as a result of ultraviolet B (UVB) irradiation (1). Pre-vitamin D₃ subsequently undergoes a temperature-dependent process in the skin to form vitamin D₃ that enters the circulation (2). In the liver, vitamin D₃ undergoes hydroxylation by vitamin D-25 hydroxylases (CYP27A1 and CYP2R1) to form 25-hydroxyvitamin D₃ [25(OH)D₃], the major circulating metabolite, which is then converted into its active metabolite 1,25-dihydroxy vitamin D₃ [1,25(OH)₂D₃] by 25-hydroxyvitamin D-1 α hydroxylase (CYP27B1) in the kidney (3).

Prevalence of vitamin D deficiency among individuals with malabsorption syndromes, such as Crohn's disease, ulcerative colitis, cystic fibrosis, short bowel syndrome, or those who have undergone gastric bypass is high due to the reduced ability to absorb vitamin D from diet (4–6). Oral vitamin D supplementation has limited role in many of these patients. It has been reported that irradiation with UVB can be used safely and effectively to treat vitamin D deficiency among these patients (7–10).

Background/Purpose: Ultraviolet (UV) B irradiation may provide a safe and effective method to treat vitamin D deficiency. The objective of this study was to assess the effectiveness of a novel Sperti D/UV-Fluorescent lamp in converting 7-dehydrocholesterol (7-DHC) to pre-vitamin D₃ in vitro and in raising serum 25-hydroxyvitamin D₃ [25(OH)D₃] in healthy adults.

Methods: The lamp was assessed in vitro using a 7-DHC solution and a human skin sample. In a prospective cohort study, five healthy adults with skin types II and III were exposed to a 0.75 minimal erythral dose of UV radiation over ~9% of body surface area three times a week for 4 weeks. The main outcomes were percentage of conversion from 7-DHC to pre-vitamin D₃ in vitro and changes in serum 25(OH)D₃ after irradiation in vivo.

Results: A dose response between UV irradiation time and conversion of 7-DHC to pre-vitamin D₃ was seen in the 7-DHC solution and surgically obtained human skin. The subjects had a significant increase in mean 25(OH)D₃ from 18.4 \pm 8.2 to 27.3 \pm 7.6 ng/ml ($P < 0.001$) after 4 weeks of irradiation. No adverse events occurred.

Conclusion: The Sperti D/UV-Fluorescent lamp is effective in converting 7-DHC to pre-vitamin D₃ in vitro and in raising serum 25(OH)D₃ in healthy adults.

Studies have shown that 1,25(OH)₂D induces activation of the innate immune system of the skin, including expression of the antimicrobial peptide cathelicidin (11, 12). Interestingly, 25(OH)D also induces this process due to the fact that keratinocytes express CYP27B1, allowing local activation of 25(OH)D and autocrine and paracrine effects of 1,25(OH)₂D (11). It is possible that UVB exposure and locally synthesized vitamin D₃ may modulate cutaneous immune function that could have significant implications for both normal individuals and patients, particularly those who are not regularly exposed to sunlight.

In the past, mercury arc sunlamps were approved for use in the United States for the production of vitamin D to prevent rickets in children (13). The Sperti D/UV-Fluorescent lamp (KBD, Inc, Crescent Springs, KY, USA), unlike previous mercury lamps, was designed to use UVB emitting fluorescent bulbs that have lower heat emission than mercury arc lamps and also allows a larger area of skin exposure. In addition, the unnecessary UVC has been removed from the output spectrum, and the lamp has been equipped with a timer for improved safety (14). However, the

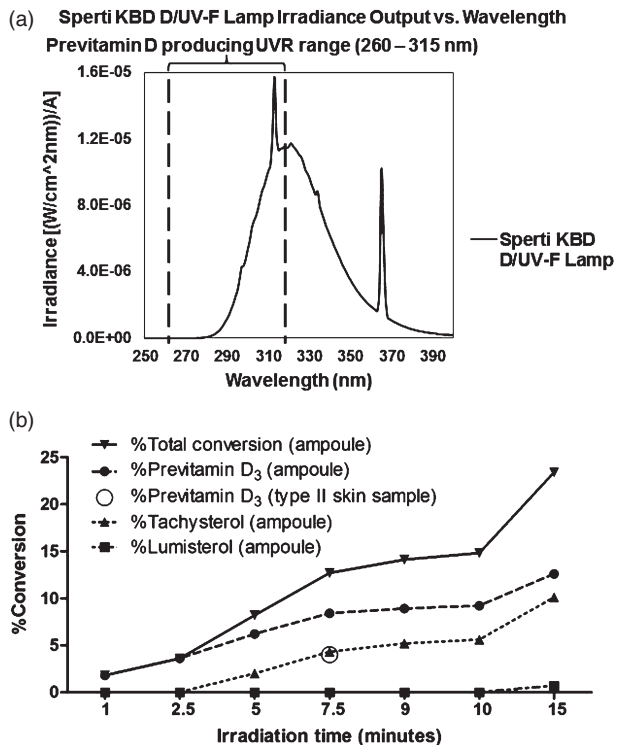


Fig. 1. The Sperti KBD D/UV-F lamp irradiance output and its efficacy in *in vitro* models. (a). The Sperti KBD D/UV-Fluorescent lamp irradiance output overlaps with ultraviolet (UV) wavelengths necessary for cutaneous vitamin D₃ production (260–315 nm) (8). Although the International Commission on Illumination report (15) has suggested that a mathematical model predicted that previtamin D₃ could be made with radiation up to 330 nm, it was concluded by the expert panel that although theoretical, it was not supported by evidence-based data which has clearly demonstrated that previtamin D₃ can be produced in human, rat, and chicken skin only with radiation between 260 and 315 nm. (b). Relationship between irradiation time and total conversion of 7-dehydrocholesterol (DHC) (▼), and between irradiation time and conversion of 7-DHC to previtamin D₃ (●), tachysterol (▲), and lumisterol (■), in borosilicate glass ampoules. Conversion of 7-DHC to previtamin D₃ in a type II human skin sample is represented by the open circle.

efficacy of this device has not been examined. The purpose of this study was to assess the efficacy of this lamp in converting 7-DHC to previtamin D₃ *in vitro* and to assess the clinical efficacy of the lamp in raising serum 25(OH)D₃ levels in healthy adults with Fitzpatrick skin types II and III.

Methods

In vitro studies

Output spectrum of the Sperti D/UV-Fluorescent lamp (~280 to ~400 nm) overlaps with the wavelengths (260–315 nm) effective in producing vitamin D₃ in the skin (1, 14, 15) (Fig. 1a). Borosilicate glass ampoules containing 7-DHC solution in ethanol (50 µg/ml) were exposed to UV radiation (UVR)

from the lamp for 1, 2.5, 5, 7.5, 9, 10, and 15 min at a distance of 15 inches. We determined the percentage conversion of the irradiated 7-DHC solution to previtamin D₃, tachysterol, and lumisterol using high-performance liquid chromatography (HPLC) as previously described (16, 17).

To further evaluate the effectiveness of the lamp, a surgical sample of type II human skin was exposed to UVR from the lamp at 15 inches for 7.5 min. This skin sample was obtained at the time of an elective surgery from a 32-year-old male who was not part of the *in vivo* study. The epidermis was separated from the dermis, then the epidermis and the basal cells were analyzed by HPLC to determine the percentage conversion of 7-DHC to previtamin D₃, tachysterol, and lumisterol as previously described (18).

In vivo study

The study was reviewed and approved by the Institutional Review Board of Boston University Medical Center, and written informed consent was obtained from each subject. Healthy subjects age 18 years and older, both males and females, with body mass index (BMI) between 18.5 and 30 kg/m² and Fitzpatrick skin types II (beige skin, blue, or gray eyes; blonde or light brown hair and some freckles; with a strong tendency to sunburn outdoors, but sometimes tans) and III (light brown skin, brown eyes and hair; sometimes burns outdoors but always tans) were enrolled into the study. Women were on birth control and not pregnant based on a negative urine pregnancy test at the first study visit. Exclusion criteria included ongoing treatment with pharmacologic doses of vitamin D, treatment with vitamin D metabolites or analogues, history of photosensitivity, chronic hepatic or renal failure, history of skin cancer within 5 years, and use of medications known to cause photosensitivity reactions including hydrochlorothiazide and tetracycline.

The study was performed at Boston University General Clinical Research Unit and consisted of 12 visits; three visits/week. At each visit, subjects were exposed to UVB from the lamp either on the back or abdomen of an area approximately 200 cm² or ~9% of body surface area at a distance of 15 inches while wearing UV eye shield. Exposed areas were rotated at each visit. At each visit, subjects were questioned about their skin and systemic symptoms related to UV irradiation from the prior visit. In accordance with Food and Drug Administration guidelines, subjects received 75% of minimal erythemal dose (MED) of UVR. The exposure time that resulted in 0.75 MED for skin type II at the distance of 15 inches was determined using a radiometer (model 7.0, Solartech, Inc, Harrison Township, MI, USA) to be 4 min. The exposure time for subjects with skin type III was 20% longer than for subjects with skin type II. Blood draws for serum 25(OH)D₃ were performed at baseline and subsequently every week. Serum 25(OH)D₃ levels were determined by liquid chromatography tandem mass spectrometry (19). The intraassay coefficient of variation was 6.0%. The laboratory has been accredited by external quality control agency for serum 25(OH)D (20).

Statistical analysis

The analysis was performed using the data analysis tools package in Microsoft Excel, Office Suite 2007 (Microsoft Corp., Redmond, WA, USA) and Prism 5.0 (GraphPad Software, Inc, La Jolla, CA, USA). Repeated measures analysis of variance (ANOVA) was used to compare mean 25(OH)D₃ levels between baseline and those at subsequent visits.

Results

In vitro studies

The relationship between UV exposure time and conversion of 7-DHC to previtamin D₃, lumisterol, and tachysterol in borosilicate glass ampoules containing 7-DHC in ethanol (50 µg/ml) is demonstrated in Figure 1b. A dose-response relationship between irradiation time and percentage conversion was observed. After the type II skin sample was exposed to UVR, 4% 7-DHC was converted to previtamin D₃, compared with 8.4% of 7-DHC in a borosilicate ampoule (Fig. 1b).

In vivo study

Three adults with skin type II (one male and two females) and two adults with skin type III (both female) were enrolled into the study. The baseline characteristics of these subjects are shown in Table 1. The mean 25(OH)D₃ at baseline was 18.4 ± 8.2 ng/ml (45.9 ± 20.5 nmol/l) and the mean 25(OH)D₃ at the end of the study was 27.1 ± 7.8 ng/ml (67.6 ± 19.5 nmol/l). Changes in serum 25(OH)D₃ compared with baseline in each subject throughout the study is shown in Table 1. Repeated measures ANOVA demonstrated that changes in serum 25(OH)D₃ levels from baseline to subsequent visits reached statistical significance ($P < 0.01$). All subjects tolerated the UV irradiation well, and none reported any skin burn, pain, or other symptoms subsequent to the UV exposures.

Discussion

We demonstrate the efficacy of the Sperti D/UV-Fluorescent lamp in producing previtamin D₃ from 7-DHC *in vitro* and in

raising serum 25(OH)D₃ levels in healthy adults with Fitzpatrick skin types II and III after multiple exposures to a 0.75 MED dose of UVR over a 200 cm² area during a 4-week period.

The efficiency of conversion from 7-DHC to previtamin D₃ was higher in borosilicate ampoules containing 7-DHC solution compared with type II human skin samples (8.4% vs. 4% after exposure to UVR from the lamp at 15 inches for 7.5 min). This is consistent with findings from previous studies (16, 18) and likely reflects the effects of UVB-absorbing melanin, DNA, RNA, and proteins in human skin samples.

At the end of the *in vivo* study, all five subjects had a significant increase in their serum 25(OH)D₃ levels of approximately 10 ng/ml regardless of their baseline levels, and their 25(OH)D₃ levels reached a plateau by week 3 of the study. This is equivalent to what was observed when healthy adults ingested vitamin D₃ 1000 IU/day or 7000 IU/week for 11 weeks (21). Because the subjects were irradiated three times a week, each UVB exposure provided an equivalent of ~2300 IU of vitamin D₃. Koutkia et al. (9) exposed a patient wearing a one-piece bathing suit for 10 min, three times a week to UVR from a tanning bed (~54% of the body surface area), and in 4 weeks, her 25(OH)D₃ levels increased by 357% from 7 to 32 ng/ml. Our subjects experienced an average 47.5% increase in 25(OH)D₃ levels with only ~9% of the total body surface area being exposed to UVR.

There appeared to be some variation in the subjects' response to UV irradiation (Table 1). The likely explanation is the varying amount of 7-DHC and melanin in the skin of each individual. 7-DHC is the essential substrate that is converted to previtamin D₃ by UVB. Melanin, which determines skin pigmentation, absorbs UVR from 290 to 700 nm (22) and competes with 7-DHC for UVB photons (16). Clemens et al. (23) exposed two white and three black individuals to one MED of UVR. There was a 30% to 50% increase in the serum 25(OH)D₃ levels in the white adults, and no significant increase in the black adults. In order to achieve similar serum 25(OH)D₃ levels as the white adults, one black subject had to be exposed to a dosage of UVR six times the original amount. Adiposity is one of the determinants of an individual's response to UVR. Wortsman et al. (24) demonstrated that peak serum vitamin D concentration after UV irradiation was inversely correlated with weight and BMI. All subjects who participated in our study were lean.

Table 1. Characteristics of the subjects in the *in vivo* study ($n = 5$), baseline serum 25(OH)D₃ levels, and changes in serum 25(OH)D₃ levels from baseline throughout the study period which reached statistical significance ($P < 0.01$, repeated measures ANOVA)

Subject	Age (years)	BMI (kg/m ²)	Sex	Ethnicity	Skin type	Baseline serum 25(OH)D ₃ (ng/ml)	Change in serum 25(OH)D ₃ from baseline (ng/ml)			
							Week 1	Week 2	Week 3	Week 4
1	29	20.4	F	White	II	14.0	+5.0	+8.0	+15.0	+13.0
2	25	21.4	F	White	II	10.5	+3.9	+7.3	+9.1	+9.7
3	29	21.9	M	White	II	22.6	+1.2	+12.7	+9.0	+8.3
4	26	21.1	F	White	III	33.0	+6.0	+4.0	+13.0	+6.0
5	24	21.9	F	Hispanic	III	14.0	+7.0	+9.0	+14.0	+14.0
Mean ± SD	26.6 ± 2.3	21.4 ± 0.7	—	—	—	18.8 ± 9.1	+4.6 ± 2.2	+8.2 ± 3.1	+12.0 ± 2.8	+10.2 ± 3.3

25(OH)D₃, 25-hydroxyvitamin D₃; ANOVA, analysis of variance; BMI, body mass index; SD, standard deviation.

In 2010, the US Institute of Medicine (IOM) published its Report on Dietary Reference Intakes for Calcium and Vitamin D (25, 26), which concluded that serum 25(OH)D of ≥ 20 ng/ml covers the requirements of 97.5% of the healthy population. The US Endocrine Society also issued its Clinical Practice Guideline on evaluation, treatment, and prevention of vitamin D deficiency in 2011 (27), which defined vitamin D deficiency as serum 25(OH)D < 20 ng/ml as vitamin D insufficiency as serum 25(OH)D of 21–29 ng/ml. The difference in these recommendations reflects different goals and views on current evidence. The mean serum 25(OH)D among the subjects at the start of the *in vivo* study (18.4 ± 8.2 ng/ml) would be considered insufficient by both the recommendations from the IOM and the Endocrine Society, while the mean serum 25(OH)D at the end of the study (27.1 ± 7.8 ng/ml) would be considered sufficient according to the IOM recommendations but not the Endocrine Society guideline. The amount of 7-DHC in the skin (which is at least partly determined by age), skin pigmentation, and adiposity must be taken into account when evaluating the serum 25(OH)D response to UVR.

Most patients will be able to achieve these recommended serum 25(OH)D levels by taking oral vitamin D supplements or vitamin D-fortified foods. However, this is not the case among patients with malabsorption syndromes who have limited ability to absorb orally administered vitamin D. High-dose oral vitamin D, up to 200 000 IU/day, have been used successfully in some, but not all patients with malabsorption (28). Successful use of parenteral, particularly intramuscular, vitamin D has been reported among these patients (29–31). Intramuscular preparations of vitamin D were available in the United States in the past (32). However, at present no parenteral form of vitamin D is available aside from 200 IU of vitamin D in the commercially available intravenous multivitamins (28, 33). Occasionally, the only alternative for patients with severe malabsorption to replete their vitamin D status is through cutaneous exposure to UVR. Concerns have been raised regarding the fact that UV irradiation has been associated with skin cancer and photoaging (34, 35). These concerns must be weighed against the morbidity associated with osteomalacia, osteoporosis, and decreased muscle function as a result of severe vitamin D deficiency (3) on a case-by-case basis. In some patients with symptoms of severe vitamin D deficiency among whom oral vitamin D supplementation is not effective or not tolerated, the benefit of UV irradiation under close medical supervision outweighs the risk and becomes a reasonable treatment option.

This is the first study that evaluates the efficacy of the Sperti D/UV-Fluorescent lamp both in *in vitro* systems and *in vivo*. The strengths of this study include the use of validated *in vitro* models as the basis for evaluation of the clinical efficacy of this device. The use of a homogeneous group of young, healthy, and lean subjects allow assessment of the effects of the lamp on serum 25(OH)D₃ with limited variation from age, body size, or medical comorbidities. The limitations of this study include a small sample size in the *in vivo* study and a relatively short treatment period that does not allow assessment of the efficacy

of the device in maintaining stable 25(OH)D₃ levels over a longer period of time. Future studies are warranted to determine the optimal dose of UVR irradiation in patients who may require intensification of UVR therapy in order to reach their 25(OH)D goal (either by increasing irradiation time or shortening of the distance from the lamp) such as the elderly (among whom the amount of 7-DHC is decreased), the obese (who have greater volume of distribution of vitamin D), and those with greater amount of skin pigmentation (Fitzpatrick types IV, V, and VI). Subjects with Fitzpatrick skin type I were excluded from this study due to their tendency to develop skin burns from UVB. Because the application of this device would potentially be most valuable in patients suffering from malabsorption syndromes, assessment of the efficacy of this fluorescent lamp in this population is warranted. All subjects in this study underwent UV irradiation in a supervised setting. Because the device would be most useful as a device that could be used by patients in their homes after receiving detailed instructions, the effectiveness of the device in this setting remains to be evaluated.

Conclusions

In summary, the Sperti D/UV-Fluorescent lamp is efficacious in increasing 25(OH)D₃ levels in healthy adults with Fitzpatrick skin types II and III after multiple exposures over a 4-week period, and provides an effective and relatively inexpensive method to improve vitamin D status particularly in patients with malabsorption syndromes.

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