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Evaluation of a Ultraviolet B Light Emitting Diode (LED) for Producing Vitamin D_3 in Human Skin

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Abstract. *Aim: A commercially available light emitting diode (LED) that transmitted narrow band ultraviolet B (UVB) radiation was evaluated for its efficacy and efficiency to produce vitamin D_3 in human skin. Materials and Methods: Human skin samples were obtained from surgical procedures. The LED had peak emission wavelength of 295 nm. Skin samples were exposed to the UVB-LED for varying times and then were analyzed by high-pressure liquid chromatography (HPLC) to determine the vitamin D_3 content. Results: There was a statistically significant time- and dose-dependent increase in the percent of 7-dehydrocholesterol that was converted to vitamin D_3 in the skin type II samples; 1.3%±0.5, 2.3%±0.6 and 4.5%±1.67 after exposure to 0.75 (11.7 mJ/cm^2), 1.5 (23.4 mJ/cm^2) and 3 (46.8 mJ/cm^2) minimal erythemal doses (MEDs), respectively. Conclusion: The UVB-LED was effective and efficient in generating vitamin D_3 in human skin, in vitro. The amount of vitamin D_3 production increased in a dose-dependent fashion with increased UVB energy. UVB-LEDs can be developed for devices that can efficiently produce vitamin D_3 in human skin.*

Vitamin D is mainly obtained from sun exposure, as well as from few dietary sources (1, 2). Specifically, during sun exposure, epidermal 7-dehydrocholesterol (7-DHC or provitamin D_3) absorbs solar ultraviolet (UV) B radiation, which results in the thermodynamically unstable molecule, previtamin D_3. Once formed, the triene system in previtamin D_3 rearranges to form the more thermodynamically stable product, vitamin D_3 (1-4). After its formation, vitamin D_3 enters the circulation from the skin and is transported to the liver to be metabolized into 25-hydroxyvitamin D_3 (25(OH)D), and to the kidneys to undergo additional metabolism to 1,25-dihydroxyvitamin D_3 (1, 3, 5-7).

The Sperti lamp, which contained a mercury arc lamp, was produced in the 1940s in the United States, where it was available in pharmacies to treat and prevent the bone disorder known as rickets (1, 6, 7). Since then, there has been an evolution of improved, more user-friendly vitamin D-producing devices for the treatment and prevention of vitamin D deficiency. In particular, the modern version of the Sperti lamp, Sperti D/UV-Fluorescent lamp (KBD, Inc., Las Vegas, NV, USA), was designed with UVB emitting fluorescent bulbs, which have the benefit of a lower heat emission than the previously mercury arc lamps. Additionally, these new UVB-emitting bulbs allow for a larger area of the user's skin to be exposed (8-10). Indeed, the Sperti D/UV-Fluorescent lamp has been shown to be effective in raising blood levels of 25(OH)D in healthy adults, as well as in patients with fat malabsorption syndromes who may not benefit from oral vitamin D supplementation (8, 9).

The modern version of the Sperti lamp includes improved gallium nitride-based UV light-emitting diode (LED), and is commercially available for use in clinical application (11, 12). These LEDs can also be designed to emit specific UV narrow band in order to be utilized therapeutically to convert 7-DHC to previtamin D_3 cutaneously in humans (13). The purpose of this study was to evaluate the capability of human skin to produce vitamin D_3 after exposure to a commercially available LED with a peak emission at 295 nm.

Materials and Methods

Equipment and sample exposure to UVB radiation. The UVB-LED was obtained from RayVio Corp. (Hayward, CA, USA) and spectral characteristics of the LED are shown in Figure 1. The peak

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Table I. *Percent production of previtamin D_3 in ampoules and vitamin D_3 in skin type II samples, after exposure to various doses of UVB radiation.*

	Ampoules	*p*-Value*	Skin samples	*p*-Value*
Energy exposed	Production of previtamin D_3 (%), mean±SD	0.02	Production of vitamin D_3 (%), mean±SD	0.04
11.7 mJ/cm^2	1.8±0.3		1.3±0.5	
23.4 mJ/cm^2	3.2±0.5		2.3±0.6	
46.8 mJ/cm^2	5.5±0.6		4.5±1.7	

*Analysis of variance for the three exposures.

wavelength of the LED was 295 nm (Figure 1). A digital UV Solarmeter (Solar Light Company Inc., Glenside, PA, USA) was used to measure radiation in minimal erythemal doses (MEDs) in which 1 MED is equivalent to 15.6 mJ/cm^2. Human skin tissue samples of Fitzpatrick skin type II were collected from five healthy individuals during plastic surgeries at the Department of Surgery of Boston Medical Center, and the tissue sample retrievals were approved by the Institutional Review Board (IRB) at the Boston University Medical Center (BUMC). The skin samples were cut into 1 cm^2 pieces. Duplicate skin samples were exposed to UVB-LED radiation for different times and the percent production of vitamin D_3 from 7-DHC was evaluated as previously described (14) (Figure 2). Borosilicate ampoules (Wheaton. Millville, NJ, USA) containing 50 μg of 7-DHC dissolved in 1 ml of ethanol were exposed to the same amount of UVB radiation as the human skin samples and served as the positive controls, as previously described (14).

Three ampoules containing 7-DHC and the duplicate human skin type II samples were placed in a quartz dish. The quartz dish was placed on top of a plastic apparatus containing a 1-cm^2 opening at its center at a distance focused 10.0 mm±1.0 mm from the top of the LED. The 1-cm^2 pieces of skin were placed over the 1-cm^2 opening, and were exposed to UVB-LED irradiation for varying times equivalent to 0.75 (4 min 12 s or 11.7 mJ/cm^2), 1.5 (8 min 30 s or 23.4 mJ/cm^2), and 3 MEDs (9 min or 46.8 mJ/cm^2). The ampoules were exposed for the same time as were the skin samples as previously described (14). Ampoules in triplicate and a skin sample that were not exposed to the UVB-LED served as negative controls.

Vitamin D_3 and previtamin D_3 content analyses. After the exposure to UV-LED, each skin sample was placed in water at 60˚C for 1 min to separate the epidermal from the dermal layer. This epidermal layer was then completely removed using a clean scalpel. The dermis was discarded and the epidermis was kept for further analysis. Each epidermal sample was then submerged in a test tube containing 4 ml of methanol, sonicated for 20 s, and immediately incubated overnight at a temperature of 50˚C to facilitate the conversion of previtamin D_3 to vitamin D_3. After the incubation period, the supernatant, which contained the lipid extract, was dried down under nitrogen gas, resuspended in 1ml of 0.8% isopropyl alcohol (IPA) in hexane and centrifuged. The lipid extract was dried under nitrogen gas and resuspended in 130 μl of 0.8% IPA in hexane. The mixture was then transferred to vials for analysis by straight-phase high-performance liquid chromatography (HPLC) with a flow rate of 1.5 ml/min to determine the amount of previtamin D_3 and vitamin D_3 that was produced as previously described (14). The same procedure was followed for the skin sample that was not exposed to UVB-LED radiation.

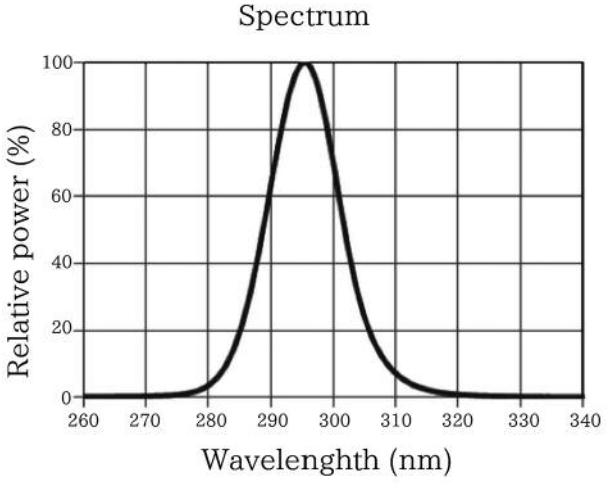

Figure 1. *The spectral output of the light emitting diode (LED) with a peak at 295 nm.*

Statistical analysis. The SPSS version 25 software for Mac (SPSS, Chicago, IL, USA) was used to perform statistical analysis. Analysis of variance (ANOVA) test was used to compare the mean vitamin D_3 production between skin samples exposed to three different energy levels, 11.7 mJ/cm^2, 23.4 mJ/cm^2, and 46.8 mJ/cm^2, to determine if there were statistical differences. A $p<0.05$ was considered to be statistically significant.

Results

The HPLC analysis of the content of 7-DHC, previtamin D_3 and vitamin D_3 is shown in Figure 2. Ampoules that were not exposed to the UVB-LED did not demonstrate any production of previtamin D_3 (Figure 2B). In the ampoules exposed to varying doses of UVB radiation previtamin D_3 was produced (Figure 2C). More specifically, a significant time- and dose-dependent increase in the percent conversion of 7-DHC to previtamin D_3 was observed ($p=0.02$) (Table I). The HPLC analysis of the lipid extract of the negative control skin sample did not show any vitamin D_3 content (Figure 2D). On the other hand, in the 5 duplicate skin type II samples (10 lipid extract samples) that were exposed to varying doses of UVB radiation vitamin D_3 content was observed (Figure 2E). In these samples,

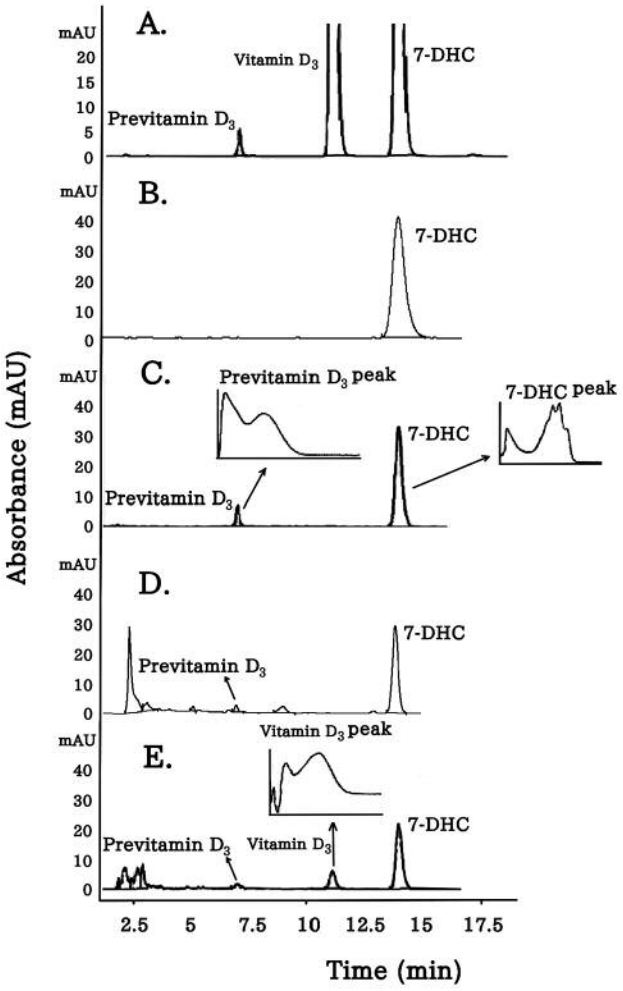

Figure 2. *High-pressure liquid chromatography (HPLC) chromatograms display the previtamin D_3, vitamin D_3 and 7-DHC content in an ampoules and skin tissue samples. A standard chromatogram for previtamin D_3, vitamin D_3 and 7-DHC is presented (A). Representative chromatograms of an ampoule not exposed to (B), or exposed to the ultraviolet B (UVB)-light emitting diode (LED) for 3 minimal erythemal doses (MEDs) (C) show previtamin D_3 production after the exposure to UVB-radiation. Representative HPLC chromatograms of a lipid extract of 1 cm^2 human skin type II sample not exposed to (D) or exposed to 3 MEDs of UVB-LED (E) are also presented, confirming the production of vitamin D_3 after UVB radiation.*

the percent of vitamin D_3 produced from the epidermal 7-DHC was significantly increased (p=0.04) in as time- and dose-dependent manner, after exposure to UVB radiation (Table I).

Discussion

Patients suffering from fat malabsorption syndromes, including patients with cystic fibrosis, inflammatory bowel disease, as well as those who have undergone a gastric

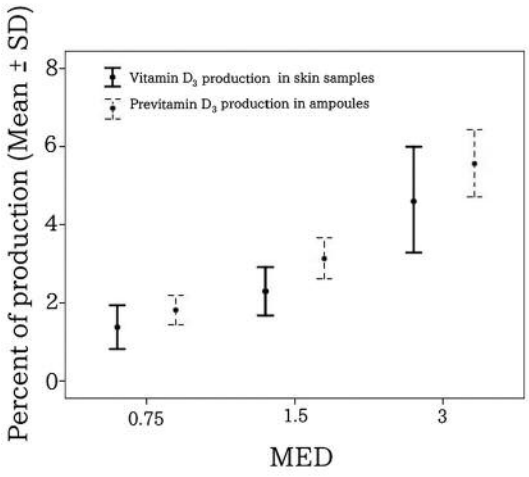

Figure 3. *Percent conversion (mean±SD) of 7 DHC to previtamin D_3 in ampoules and vitamin D_3 in skin samples for each minimal erythemal dose (MED). Analysis of variance for the three exposures showed that there was a significant difference of 0.02 and 0.04 for previtamin D_3 and vitamin D_3 production respectively.*

bypass surgery, are at a high risk for developing vitamin D_3 deficiency (9). These patients would greatly benefit from a simple and convenient device that could enhance the cutaneous production of vitamin D_3. Gallium nitride LEDs, which are capable of emitting the specific narrow band UVB radiation that converts 7-DHC to previtamin D_3, are commercially available. However, the capability of commercially available UVB-LEDs to produce vitamin D_3 in human skin has not been studied. The current study aimed to determine the production of vitamin D_3 in surgically obtained human skin samples after exposure to the UVB-LED from RayVio Corp., which emitted a peak wavelength of 295 nm.

Previous *in vitro* studies (3, 14) and the Comite International de l'Eclairage (CIE) (15) as well have reported that the narrow band of UV light is able to convert 7-DHC into previtamin D_3 in human skin. Specifically, UV narrow band between 290 and 300 nm was reported to be the most efficient for production of vitamin D_3 in human skin samples (3). In addition, Morita *et al*. (2) compared different wavelengths of radiation and reported that although 316 nm was less effective in producing vitamin D in mice than wavelengths between 268-305 nm, the serum levels increased in the wavelengths in this range as compared to a control group.

The major advantage of the present study was that the specific UV narrow band of 295 nm was shown to efficiently increase the production of previtamin D_3 in ampoules and vitamin D_3 in human skin in a dose-dependent manner (Figure 3). Therefore, the commercially available UVB-LED

that was tested may be useful for the treatment and prevention of chronic vitamin D deficiency in patients who suffer from fat malabsorption syndromes, since these patients are not able to easily absorb orally ingested vitamin D.

Conflicts of Interest

All Authors have no conflicts of interest.

Authors' Contributions

A.J. Veronikis, M.B. Cevik, and M.F. Holick designed the experiments. R. Allen helped design the LED device. A.J. Veronikis, M.B. Cevik, A. Sun and K. Persons conducted the studies. A.J. Veronikis, M.B. Cevik, and M.F. Holick analyzed the data. A.J. Veronikis, M.B. Cevik, A. Shirvani, and M.F. Holick drafted the manuscript. All authors contributed to and reviewed the manuscript. M.F. Holick provided final approval.

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