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Evaluation of Effectiveness of Ultraviolet Emitting Lamps on the Cutaneous Production of Vitamin D_3: Relationship of the Lamps Vitamin D_3 Producing Potential to the Production of 8-Hydroxy-2'-Deoxyguanosine and Nitric Oxide

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Abstract. *Background/Aim: To assess the effectiveness of three UV emitting lamps on the cutaneous production of vitamin D_3, a marker of DNA damage and nitric oxide production in human skin. Materials and Methods: Human skin samples (skin types II, III and IV) obtained from surgery were exposed to three different UV emitting lamps for varying times and then extracted and chromatographed to determine the vitamin D_3 content. The skin samples exposed to the 3 UV emitting lamps were also evaluated for 8-hydroxy-2'-deoxyguanosine (a marker of DNA damage) and nitric oxide production. Results: It was observed that the spectral output of the 3 lamps had different effects on the cutaneous production of vitamin D_3, 8-hydroxy-2'-deoxyguanosine and nitric oxide production. One lamp demonstrated optimal production of vitamin D_3 with the least amount of DNA damage and intermediate production of nitric oxide suggesting that it could be developed into a device for treating vitamin D deficiency. Conclusion: The spectral output of the experimental UVB emitting lamps significantly influenced the cutaneous production of vitamin D_3 8-hydroxy-2'-deoxyguanosine and nitric oxide.*

The recognition of the beneficial role of ultraviolet radiation on bone health began in 1919 when Huldschinsky reported that children who were exposed to ultraviolet (UV) radiation from a mercury arc lamp showed significant radiologic improvement of their rickets several months later (1, 2). As a result of this observation several UV emitting devices were produced and sold worldwide for treating and preventing vitamin D deficiency rickets. The Sperti lamp, which contained a mercury arc lamp, was produced in the 1940s in the United States and was available in pharmacies for the treatment and prevention of rickets (1-3). A modern version [Sperti D/UV-Fluorescent lamp (KBD, Inc. Las Vegas, NV, USA)] that contains fluorescent tubes that emit UV radiation was effective in raising blood levels of 25-hydroxyvitamin D [25(OH)D] in healthy adults and in patients with a fat malabsorption syndrome (4, 5).

During exposure to sunlight, ultraviolet B (UVB) radiation between 290-315 nm penetrates into the skin and is absorbed by 7-dehydrocholesterol (7-DHC) resulting in its conversion to previtamin D_3 (1, 3). The activity spectrum for the production of previtamin D_3 revealed that the most efficient wavelengths were 298±2 nm (6). Once formed, previtamin D_3 is thermodynamically unstable, and the triene system rearranges into a more thermodynamically stable form, vitamin D_3 (1).

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 the fat-soluble vitamin D from diet (7, 8). Oral vitamin D supplementation, therefore, has a limited role in many of these patients. It has been reported that irradiation with devices that emit UVB radiation including tanning beds can be used safely and effectively to treat vitamin D deficiency in these patients (1, 8, 9). Thus, patients with malabsorption syndromes are in need of a user-friendly device that can promote the cutaneous production of vitamin D_3 while minimizing the well documented negative consequences including DNA damage and increased oxidative stress due to nitric oxide production. Felton *et al.* have reported that exposure to suberythemal doses of ultraviolet radiation 3 times a week for 6 weeks that was

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Key Words: Previtamin D_3, DNA damage, 8-hydroxy-2'-deoxyguanosine, nitric oxide, vitamin D, nitrite, human skin.

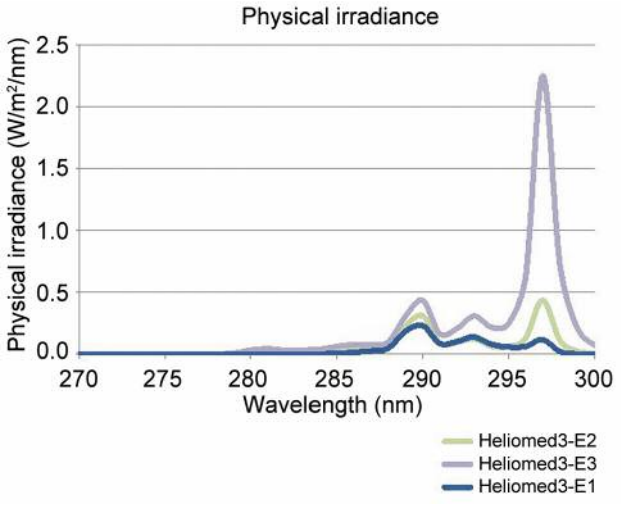

Figure 1. *The emission spectra of the 3 experimental UVB emitting devices. Three Heliomed model (Heliomed3-E1, E2 and E3) lamps (JK-Holding GMBH, Windhagen, Germany) were used in this study. The physical irradiance increased from Heliomed3-E1 to E3. Thus, the MED time decreased from lamps E1 to E3. The physical irradiance and IEC Erythemal Effective Irradiance of the lamp E3 was more focused on the wavelength of 297 nm.*

comparable to summer sunlight exposure in the United Kingdom, resulted in improvement in vitamin D status in adults with skin type 2 (10). They have also reported evidence of DNA damage based on histologic analysis of the skin biopsies and urine analysis for the marker of DNA damage, 8-hydroxy-2'-deoxyguanosine (8-OHdG). After 6 weeks of exposure it was found that there was no significant accumulating increase in DNA damage either in skin biopsies or urine analysis (10).

There continues to be a need to develop devices that emit vitamin D producing UVB radiation while minimizing negative consequences including wrinkling, DNA damage related to an increased risk for nonmelanoma skin cancer and increased oxidative stress (2). There has been an evolution in the development of more user-friendly vitamin D producing devices for the treatment and prevention of vitamin D deficiency including the Sperti D/UV-fluorescent lamp, which was designed with UVB emitting fluorescent bulbs which have lower heat emission than mercury arc lamps and also allow a larger area of skin exposure (4, 11). With the advancement of gallium nitride light emitting diode (LED) technology that emits UV radiation, it is now possible to manufacture LEDs that are efficient and suitable for a wide range of commercial uses (12, 13). These LEDs can be tuned to emit the desired wavelengths including those that can convert 7-DHC to previtamin D_3 in human skin (14, 15).

Table I. *Estimated physical irradiance and MED time calculation for each experimental device.*

Devices	Physical irradiance (W/m^2)	MED time (min)
Heliomed3-E1	1.7	3.3
Heliomed3-E2	2.6	1.9
Heliomed3-E3	6.9	0.6

The purpose of this study was to compare the efficiency and effectiveness of 3 different UV emitting lamps on the cutaneous production of vitamin D_3 and relating them to the production of 8-OHdG and nitric oxide in surgically obtained human skin.

Materials and Methods

UVB emitting lamps and LED spectral characteristics. Three UV emitting lamps with different emission spectra (Figure 1) were provided by JK-Holding GMBH (Windhagen, Germany). The radiation was measured with a Solar meter (Solartech, PA, USA) that had a readout in minimal erythemal dose (MED).

The MED time calculation as well as estimated physical irradiance for each device is shown in Table I. The physical irradiance increased from Heliomed3-E1 to E3. Thus, the MED time decreased from lamp E1 to E3. The physical irradiance of the lamp E3 was more focused on the wavelength of 297 nm (Figure 1).

Study design. Human skin samples of skin types (II, III and IV) obtained from the Plastic Surgery Department at Boston Medical Center and approved by the Institutional Review Board at Boston University Medical Center (BUMC IRB) were evaluated for their efficiency in producing vitamin D_3, 8-OHdG and nitric oxide following irradiance with each of the JK lamps. The skin samples were exposed to an amount of radiation equivalent to 0.5 and 1 MED for the 3 experimental lamps.

Lamp studies. For the lamp study, each piece of skin was processed to remove subcutaneous fat and was punched to provide nine 8 mm diameter skin samples that were used to determine 8-OHdG and nitric oxide concentrations at baseline (0 MED) and immediately after the irradiation. The remaining skin samples after the punches were recovered and were used to determine the effectiveness of the lamps in producing vitamin D_3 as previously described (14). The punched skin samples and the remaining skin samples were exposed in duplicate or triplicate at different times to the 3 lamps that was the equivalent to 0, 0.5 and 1 MED. Three ampules containing 50 mcg of 7-DHC in 1 ml of ethanol were exposed for the same time and used as the positive control for the production of previtamin D_3 (Figure 2).

Tissue homogenization procedures. Skin punch biopsy samples (8 mm diameter) were homogenized in 1.5 ml extraction buffer (10 mM Tris pH 7.4, 150 mM NaCl, 1% Triton X-100) per gram of tissue using ultrasonication on ice. The homogenates were transferred to 1.5 ml Eppendorf tubes, centrifuged at 13,000 × *g* for

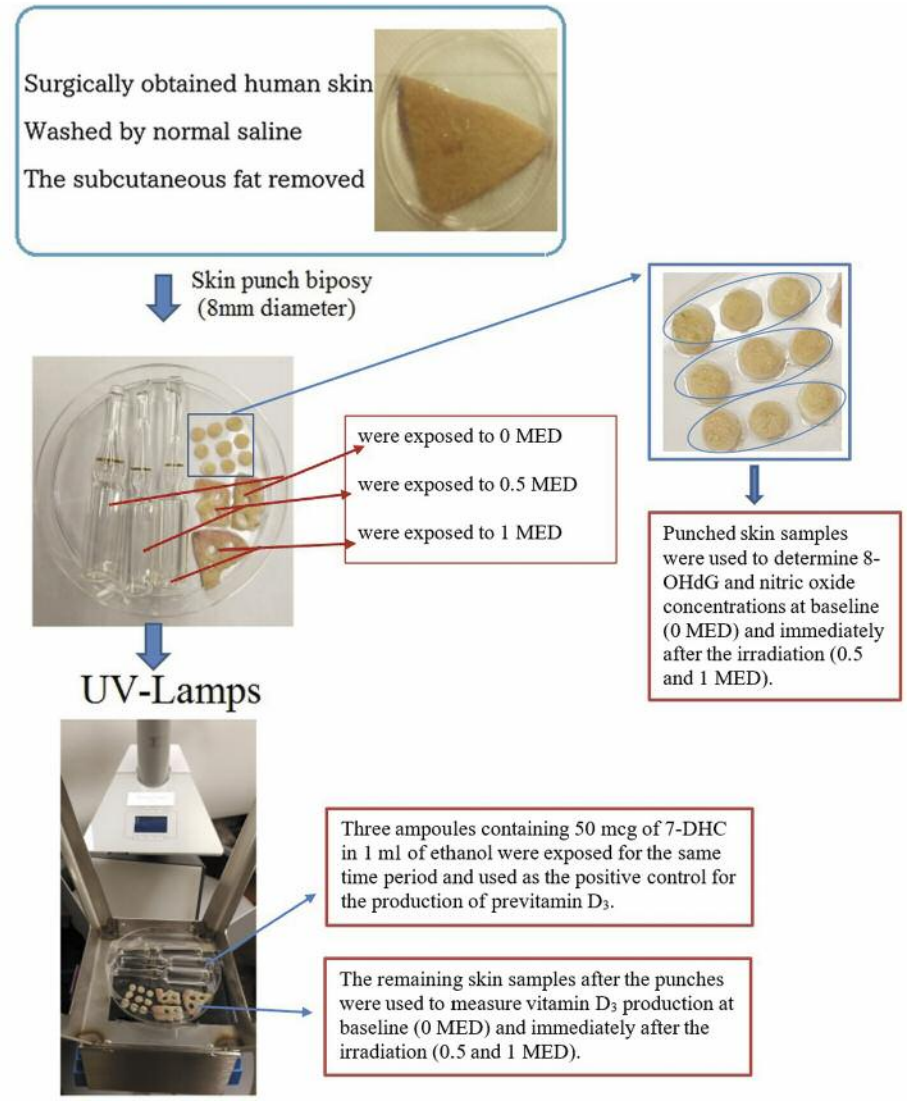

Figure 2. *Photographs of how the skin samples were prepared for the irradiation studies with the 3 JK lamps.*

10 min at 4˚C, and the supernatants was stored at –80˚C until analyzed.

Determination of 8-hydroxy-2'-deoxyguanosine (8-OHdG). We measured the commonly used marker for DNA damage, 8-OHdG in the nonirradiated and irradiated skin samples. Skin homogenates were prepared and 8-OHdG concentrations were determined using an ELISA kit (Enzo Life Sciences, Inc., Farmingdale, NY, USA), according to the manufacturer's instructions. The sensitivity of the kit was 0.59 ng/ml (range=1.875-60 ng/ml).

Determination of nitric oxide. For the quantitative determination of total nitric oxide (NO) in our experimental model, we used the Total Nitric Oxide Assay Kit (Bender MedSystems GmbH, Vienna, Austria), according to the manufacturer's instructions. The Total Nitric Oxide Assay Kit is a complete kit for the quantitative determination of total nitric oxide (NO) in biological fluids. The kit uses the enzyme nitrate reductase to convert nitrate (NO_3) to nitrite (NO_2). Nitrite is then detected as a colored azo dye product of the Griess reaction that absorbs visible light at 540 nm. The intra- and inter-assay CV were ≤5%.

Power and sample size calculation. In the initial pilot study ampoules containing 7-DHC were exposed to the lamps to determine their effectiveness and the efficiency in producing previtamin D_3. Results from the initial pilot study demonstrated differences in the efficiency and effectiveness of the different lamps in producing previtamin D_3. The differences observed for lamps E1 and E3 in producing previtamin D_3 were about 2% with a standard deviation equal to 1. Assuming that similar differences would be observed in human skin exposed to the 2 lamps, the sample size for the desired power=0.8 and α (type I error)=0.05, it was calculated

Table II. Baseline measurements of nitric oxide production (Nitrite) and DNA damage in human skin samples (all skin types) for the three lamps.

Adjusted by total protein	Lamp	Mean	Std. deviation	Std. error	*p*-Value
Nitrite (μmol/ml of Nitrite per mg/ml of protein)	1	69.3	10.8	2.5	0.9
	2	68.3	7.8	1.8	
	3	67.9	9.7	2.1	
8-OHdG (ng/ml/mg/ml of protein)	1	16.5	3.8	0.90	0.2
	2	15.9	4.1	0.96	
	3	14.5	3.1	0.70	

Table III. *Comparison of the effect of irradiating human skin samples (all skin types) for an equivalent of 0.5 MED with the three lamps on vitamin D_3, nitric oxide (nitrite) and 8-OHdG production.*

	Lamp	Mean	Std. deviation	Std. error	*p*-Value
Nitrite (μmol/ml/mg/ml of protein)	1	104.5	21	7.0	0.2
	2	101.7	8.2	3.3	
	3	91.6	8.1	3.0	
8-OHdG (ng/ml/mg/ml of protein)	1	27.8	13.6	4.5	0.9
	2	25.9	11.1	4.5	
	3	21.2	3	1.1	
Skin production of vitamin D_3 (%)	1	3.6	2.3	1.0	0.8
	2	4.3	2.7	1.2	
	3	3.6	2.7	1.1	
Ampule conversion of 7-DHC previtamin D_3 (%)	1	7.8	2.7	1.2	0.08
	2	5.2	2.5	1.2	
	3	3.8	2.5	1.1	

Table IV. *Comparing the effect of irradiating human skin samples (all skin types) for an equivalent of 1.0 MED with the three lamps on vitamin D_3, nitric oxide (nitrite) and 8-OHdG production.*

	Lamp	Mean	Std. deviation	Std. error	*p*-Value
Nitrite (μmol/ml/mg/ml of protein)	1	157.7	54.5	13.2	0.2
	2	134.9	39.2	10.4	
	3	134.9	37.0	9.8	
8-OHdG (ng/ml/mg/ml of protein)	1	39.3	12.6	3.0	0.04
	2	40.9	9.4	2.5	
	3	31.8	5.5	1.4	
Skin production of vitamin D_3 (%)	1	8.0	2.3	0.7	0.3
	2	8.3	3.5	1.0	
	3	6.8	2.4	0.7	
Ampule conversion of 7-DHC previtamin D_3 (%)	1	15.1	2.7	0.8	0.002
	2	11.9	1.9	0.6	
	3	9.5	3.7	1.3	

to require 5 different human skins per group thereby requiring 15 samples to evaluate the 3 different lamps.

Statistical analysis. The analysis was performed using the SPSS ver 20 for Mac (SPSS, Chicago, IL, USA). For repeated measures, ANOVA was used to compare mean pre-vitamin D_3 conversion, DNA damage and nitric oxide production between baseline and those at subsequent exposure. The criterion for statistical significance was defined as $p<0.05$.

Results

A total of 19 human skin samples were obtained for this study. We evaluated skin types II (n=9), III (n=5) and IV (n=5).

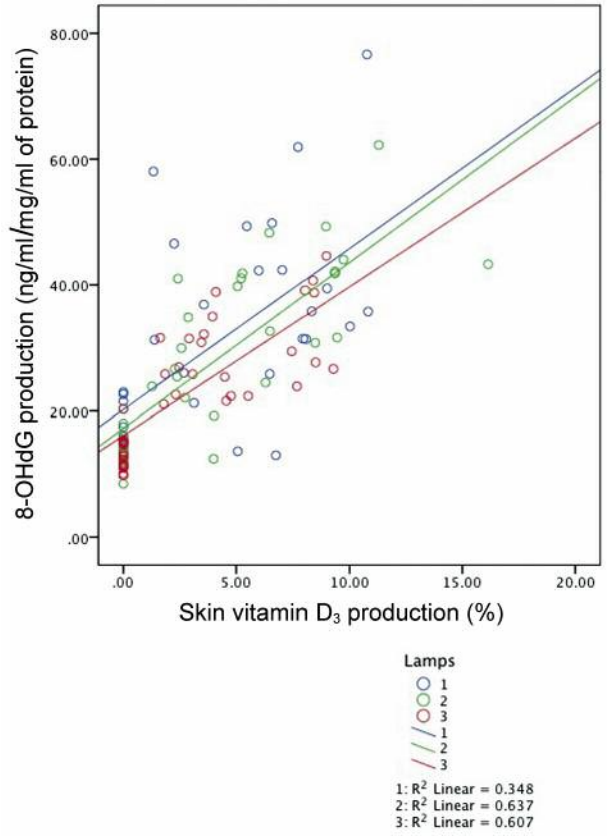

Figure 3. *The comparison of vitamin D_3 and 8-OHdG production following irradiation with the three experimental lamps.*

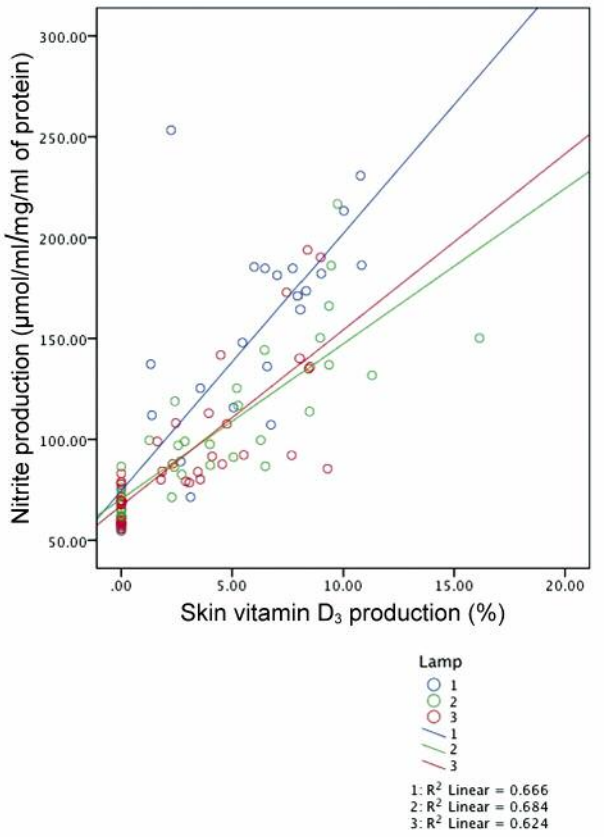

Figure 4. *The comparison of vitamin D_3 and NO (nitrite) production following irradiation with the three experimental lamps.*

The baseline measurement (MED=0) of nitric oxide and 8-OHdG production in the skin samples for the three experimental UVB lamps are shown in Table II. There was no significant difference between these measurements at baseline in the skin samples that exposed to each lamp.

The effect of irradiating human skin samples for an equivalent of 0.5 and 1 MED with the three lamps on vitamin D_3, nitric oxide and 8-OHdG production are compared in Tables III and IV.

The effect of irradiating skin samples on the production of vitamin D_3 and inducing DNA damage is shown in Figure 3. Lamp 3 was more efficient in producing vitamin D_3 with the least amount of 8-OHdG being produced.

The effect of irradiating skin samples on the production of vitamin D_3 and inducing NO production is shown in Figure 4. The lamp 2 was more efficient in producing vitamin D_3 while generating the least amount of NO.

The percent conversion of 7-DHC to previtamin D_3 in the control ampule and the amount of vitamin D_3 that was produced in the different skin types for all lamps are compared in Figure 5.

The ratio of 8-OHdG and NO production to vitamin D_3 in skin type IV was lower than that in skin type 2 (Figure 6).

Discussion

Felton *et al*. (10) have reported the results of their elegantly designed study on vitamin D status and skin DNA damage in healthy adults with skin types 2 and 5 exposed to low-level summer sunlight simulated exposure as would be experienced at a latitude in the UK for 6 weeks.

As expected, they observed a significant, 49%, increase in the circulating levels of 25(OH)D as a result of UVB radiation absorbance (290–315 nm) by 7-DHC in the epidermis and its conversion to previtamin D_3 (10). However, as UVB was penetrating into the epidermis to form previtamin D_3, it was also absorbed by DNA resulting in the formation of cyclobutane pyrimidine dimers (CPD) and other pyrimidine photoproducts that have been associated with increased risk for non-melanoma skin cancer (15). Felton *et al*. concluded that after 6 weeks of sub-erythemal exposure to solar simulated UV radiation there was no evidence for

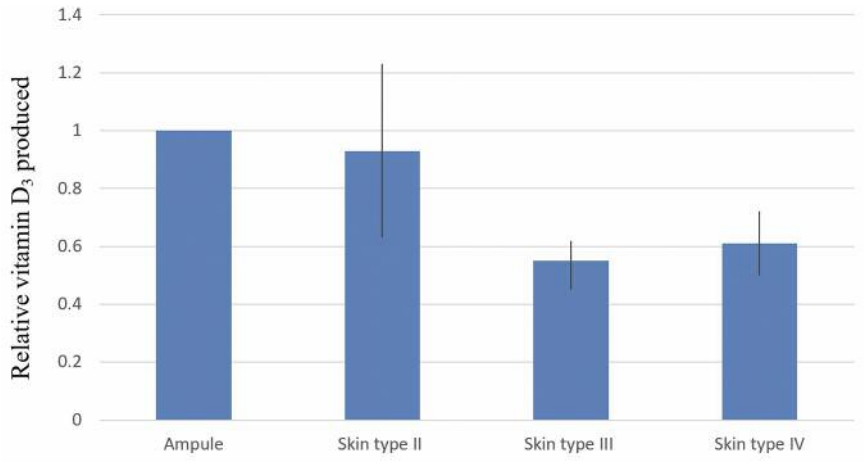

Figure 5. *The relative conversion of 7-DHC to Vitamin D_3 in different skin types compared to the conversion of 7-DHC to previtamin D_3 in the positive control ampule for all lamps. The figure shows that the conversion of 7-DHC to vitamin D_3 in skin type II was about 90% compared to the ampule.*

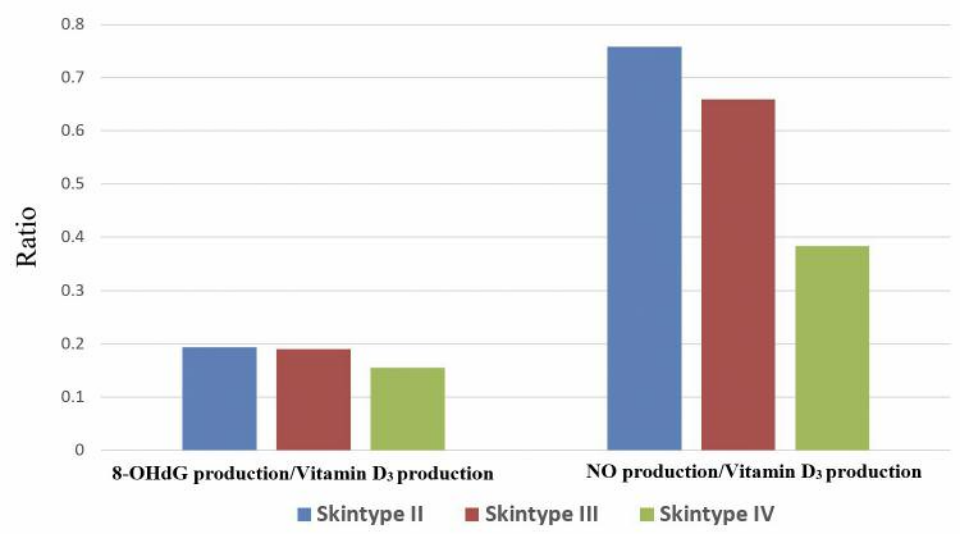

Figure 6. *The comparison of the biological response of skin types to UV irradiation for all lamps. The ratio of 8-OHdG and NO production to vitamin D_3 in skin type IV was lower than that in skin type 2.*

accumulation of DNA damage suggesting that the skin adapted to the exposure by inducing DNA repair enzymes (10).

It is now recognized that there are several other photochemical processes that occur in the skin during exposure to sunlight (16). These include the production of nitric oxide, which is considered to be an oxidant that can cause DNA damage. However, it is also known that nitric oxide can help lower blood pressure and reduce risk for heart disease. Exposure to UV radiation has been shown to increase the expression of the proopiomelanocortin gene resulting in the production of beta endorphin and adrenocorticotropic hormone (ACTH) in the epidermis. Beta endorphin has been associated with improvement in the feeling of well-being and reduction of the risk for depression and ACTH increases cortisol thereby also influencing a variety of physiologic processes including immunomodulation (2, 16).

In the 1960s, Furchgott and associates accidentally made the observation that daylight irradiation of blood vessels induces dilation, a phenomenon called photorelaxation (16). This effect was markedly potentiated by solutions containing nitrite (17, 18), indicating that under certain circumstances nitrite may exhibit relaxation activities comparable to NO. Studies in environmental chemistry have revealed that both

the nitrite anion and nitrous acid in aqueous solutions undergo photodecomposition when irradiated with UV radiation at 200-400 nm, resulting in the formation of NO (19, 20). In human skin another source of NO is the nitric oxide synthetase (21).

Several lines of evidence indicate that NO is involved in the control of wound healing processes, allergic skin manifestations, microbicidal activity, antigen presentation, hair growth, proliferation and differentiation of epidermal cells, and the regulation of innate immune reactions and inflammatory responses (22). In addition, UV-induced processes such as erythema and edema formation, as well as melanogenesis, are also mediated by NO (21). Furthermore, NO is an effective inhibitor of lipid peroxidation (23), and the coordinated action of NO on gene expression and preservation of membrane function may play a pivotal role in protecting against either UVA- or reactive oxygen species (ROS)-induced cell death (24). Since NO has been found to play important roles in regulating skin pigmentation, keratinocyte cell growth and differentiation, it appears likely that photodecomposition of intradermal nitrite and RSNOs, as well as of extra dermal nitrite, results in NO formation. This may affect processes in human skin such as melanogenesis (25) or may confer protection against UV-induced cell damage (23, 24).

Our current study focused on comparing the differential effects of exposing the same surgically obtained live skin sample to 3 different sources of UVB radiation on the production of vitamin D_3, nitric oxide and 8-OHdG in skin type II and IV.

There was a trend from skin type II to IV to have a better protection against any UV induced damage (Figure 6). These findings are consistent with previous observations. Albrecht *et al*. (26) have reported that after 4 min of solar-simulated exposure (UV-NIR), the radical formation in skin types IV-V was 60% of that in skin type II. Felton *et al*. (10) have observed that adults with skin type V who were exposed to repeated low-level simulated summer sun light exposure were unable to significantly raise their blood level of 25(OH)D and had little or no DNA damage when compared to adults with skin type II.

Conclusion

Our results suggest that Lamp 1 created the most DNA damage and had the least production of vitamin D_3 making it the least desirable to consider for further development. Lamp 2 showed less production of nitric oxide compared to lamp3. There was no significant difference between Lamps 2 and 3 in vitamin D_3 production. Overall Lamp 3 seems to be the best choice for optimal production of vitamin D_3 with the least amount of DNA damage and intermediate production of nitric oxide.

Conflicts of Interest

M.H. received an unrestricted research grant from JK-Holding GMBH (Windhagen, Germany), and is a consultant for Quest Diagnostics Inc. and Ontometrics Inc, and on the speaker's Bureau for Abbott Inc. The remaining Authors declare no conflicts of interest regarding this study.

Authors' Contributions

Study design: A.S. and M.H. Data acquisition: A.S., K.P. and M.H. Analysis: A.S. and M.H. All Authors reviewed and edited the manuscript. M.H. is the guarantor of this work, and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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