The Effect of Different UV Absorbing Substances on the Production of Vitamin D₃ in Human Skin Exposed to UVB LED Irradiation

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Abstract
Recent studies have identified ultraviolet radiation emitting LEDs as a potential treatment option for vitamin D deficient patients who are unable to obtain sufficient amounts of vitamin D through their diet. Vitamin D deficiency can be caused by fat malabsorption syndromes. 293nm wavelength emitting LEDs were previously found to be 2.4 times more efficient than sunlight in vitamin D₂ production. In this study, 3.5mm thick layers of different UV absorbing substances were used to determine the most efficient arrangement to maximize vitamin D₂ production in a clinical setting. Radiation from a 293nm emitting LED passed through a quartz glass containing distilled water, 0.14% saline solution, or Crisco brand canola oil before irradiating an 8mm diameter punch biopsy of Fitzpatrick type II surgically obtained skin. Three punch biopsies were used in each sample. The amount of vitamin D₂ in each sample was then quantified using HPLC. It was found that water and saline solution had little effect on the conversion of 7-dehydrocholesterol to previtamin D₂ and vitamin D₂, but there was no conversion for the skin exposed over the oil layer. A follow up study examined the effect of layers of different types of oil on vitamin D conversion. Generic brand safflower oil and peanut oil both prevent conversion of vitamin D. This information could be crucial in a clinical setting for maximizing the amount of vitamin D production in the shortest amount of time, thus minimizing potential damage in the skin from UV radiation during treatment.

Methods

1. Take three 8mm diameter punch biopsies of type II skin for each substance.
2. Fill apparatus with at least 35ml of one substance. Ensure no air bubbles are directly underneath the sample. (Height of ring limits to 3.5mm Thickness)
3. Expose each biopsy to 2 MEQ [0.0132 (cm²) of 293nm LED radiation. Repeat steps 2-3.

Canola Oil, Safflower Oil, Peanut Oil
1. Take three 8mm diameter punch biopsies of type II skin for each substance.
2. Fill apparatus with at least 35ml of one substance. Ensure no air bubbles are directly underneath the sample.
3. Expose each biopsy to 3 MEQ [0.0178 (cm²) of 295nm LED radiation. Repeat steps 2-3.

Vitamin D Analysis
1. Submerge punch biopsies in 60°C water bath for 1 minute. Collect epidermis by scraping.
2. Add 2.5ml of 8% acetic acid in hexane to each test tube. Sonicate for 10 seconds.
3. Incubate overnight at 50°C.
4. Transfer supernatant to a test tube and dry with nitrogen gas.
5. Add 140µl of 9% IPA in hexane. Analyze on straight phase HPLC at a flow rate of 1.5ml/min.

Water, Saline, and Canola Oil
1. Take three 8mm diameter punch biopsies of type II skin for each substance.
2. Fill apparatus with at least 35ml of one substance. Ensure no air bubbles are directly underneath the sample.
3. Expose each biopsy to 3 MEQ [0.0178 (cm²) of 295nm LED radiation. Repeat steps 2-3.

Results

Introduction
Vitamin D is an important nutrient with many important benefits regarding bone, cardiovascular, and metabolic health. It can be obtained in two ways: ingestion of foods rich in vitamin D, such as mushrooms and vitamin D fortified milk, or cutaneous production through exposure to ultraviolet radiation, typically via sunlight exposure. In the skin, 7-dehydrocholesterol is converted into previtamin D₃ upon exposure to UV radiation, and is then thermodynamically converted into vitamin D₃.

Previous studies have found that UV LEDs can be used to more efficiently produce vitamin D. LED therapy is a potential clinical treatment option for those suffering from vitamin D deficiency who are unable to absorb vitamin D through their diet. One study demonstrated that 293nm emitting UV LEDs are 2.4x more efficient than sunlight in cutaneous vitamin D production.

Although several studies have looked into the absorbance of different substances commonly found on human skin or used in by some people to prevent UV burns, few have acknowledged their effects on the efficiency of cutaneous vitamin D₃ production. It is possible that the different mediums could diffract the UV radiation waves emitted by the LED, since ultraviolet radiation is among the shortest wavelengths and is affected more by scattering. Another possibility is that the different mediums could interfere with the amount of UV radiation allowed to reach the skin by absorbing certain amounts of radiation.

This study examines the effect of layers of various types of potential UV absorbing substances (water, salt, canola oil) on vitamin D₃ production in Fitzpatrick type II skin. The effect of layers of different types of oil (canola, safflower, peanut) on type IV skin was also examined. This data can be used to determine more ideal conditions for UV LED irradiation in clinical treatments.

Discussion
Studies have been conducted to determine the efficiency of UV LEDs in cutaneous vitamin D₃ production, but there are still numerous studies that remain untested. Human skin is rarely dry; there is usually a thin layer of sweat or oil. In this study, it was determined that oil effectively prevented 7-dehydrocholesterol conversion to previtamin D₂, the precursor to vitamin D₂. In a clinical setting, this means that patients with oily skin will be less able to produce vitamin D₂. There are ways to account for the observed reduced efficiency, such as removing the oil before exposure, in practical clinical.

It was also found that water and saline solution had little effect on the conversion of vitamin D₂ produced. As human sweat consists mainly of water, sodium, and urea, the clinical implication is that sweat interference with UV irradiation from LEDs is improbable. This also implies that vitamin D₂ production in skin exposed to sunlight will be mostly unaffected by perspiration. Thus, vitamin D₂ production in humans exposed to sunlight who are exercising or swimming in water with few other dissolved substances should experience similar vitamin D₂ production to when their skin is dry. Future studies would need to look further into the effect of other constituents of perspiration and other minerals found in water at varying concentrations.

Future studies could also look into the effect of varying layer thicknesses on vitamin D₂ conversion, as the amount of UV radiation absorbed by each substance would likely increase with layer height. Each layer was limited to 3.5mm in this study due to the apparatus, but future studies could examine heights which more accurately represent oil in human skin. New methods could be developed to irradiate the samples so that some of the oil is absorbed by the skin, again to more accurately represent oil in human skin. Even though different types of oil were tested, oils more similar to the oil in human skin, such as those found in cosmetics or animals, should be tested as well.

Conclusions
Water and 0.14% saline solution both had little impact on vitamin D₂ conversion in type II skin. Canola oil, safflower oil, and peanut oil effectively prevented any vitamin D₂ conversion in the samples which were irradiated.

References
2. Kalajian, T. A. Ultraviolet B Light Emitting Diodes (LEDs) Are More Efficient and Effective in Producing Vitamin D₃ in Human Skin Compared to Natural Sunlight. Ultraviolet B Light Emitting Diodes (LEDs) Are More Efficient and Effective in Producing Vitamin D₃ in Human Skin Compared to Natural Sunlight, 2017.

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