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February 2010 Journal Club Article: Regulation of Vitamin D Production Is Independent of Skin Color

Topic article:
Bogh MKB, Schmedes AV, Philipsen PA *et al.* (2010) Vitamin D production after UVB exposure depends on baseline vitamin D and total cholesterol but not on skin pigmentation. *J Invest Dermatol* 130:546–53

Regulation of Vitamin D Production Is Independent of Skin Color
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Vitamin D deficiency has been associated with a variety of negative health outcomes ranging from bone disease (i.e., rickets, osteomalacia, and osteoporosis) to a risk of certain types of cancers, neurologic disease, autoimmune disease, and cardiovascular disease (Stechschulte *et al.*, 2009). Improving vitamin D levels has been advocated and supported by various reports, including a meta-analysis of 18 randomized trials on vitamin D, which found that individuals assigned to receive vitamin D had a 7% reduction in mortality from any cause (Autier and Gandini, 2007). Certain groups, including the elderly and those with darker skin, have lower levels of vitamin D in general, and they appear to be at greater risk for outcomes associated with deficiency (Wolpowitz and Gilchrest, 2006). Some researchers have advocated increasing vitamin D levels through sun exposure. Clearly, a better understanding of the effect of ultraviolet (UV) irradiation on vitamin D levels is needed.

Bogh *et al.* (2010) studied the effect of systematic broadband UVB exposure on individuals with varying vitamin D levels. After an initial screening of vitamin D levels in 182 individuals, 50 were exposed to UVB radiation. From this group, a subset of 28 non–sun worshippers (those who reported behaviors that result in limited sun exposure) were examined in detail to determine the factors that might affect vitamin D levels following UVB irradiation. Individuals with low baseline vitamin D levels produced significantly more vitamin D after UVB irradiation. This increase positively correlated with baseline total cholesterol levels, but it did not correlate with constitutive or facultative pigmentation.

Through the following questions, we examine this paper in greater detail.

1. Describe how vitamin D is produced and how it works.
Vitamin D is a fat-soluble pro-hormone involved in various biologic processes. There are five distinct forms of vitamin D: D_1 (a complex of ergocalciferol with lumisterol), D_2 (ergocalciferol), D_3 (cholecalciferol), D_4 (22-dihydroergocalciferol) and D5 (sitocalciferol). D_2 and D_3 are the most prevalent forms of vitamin D. Vitamin D_2 is made from ergosterol in invertebrates, fungus, and plants in response to UV irradiation. Vitamin D_3 is produced in human skin from 7-dehydrocholesterol (7-DHC), a cholesterol derivative found mainly in the cell membranes of the stratum basale and stratum spinosum. 7-DHC is photolyzed by the UVB photons entering the skin, producing pre–vitamin D_3, which is isomerized at body temperature to vitamin D_3 (Norman, 1998, Tavera-Mendoza and White, 2007; Gilchrest, 2008). 7-DHC absorbs UV radiation at wavelengths between 270 and 300 nm, with an optimal synthesis occurring in a narrow band between 295 and 300 nm. The intensity and wavelengths (spectral irradiance) of UVB irradiation in sunlight strongly influence production of pre–vitamin D_3 in skin; therefore, season, geographic latitude, time of day, cloud cover, skin cover, skin color, smog, and sunscreen—all of which affect UV light absorption—can affect vitamin D synthesis. Under "normal" conditions, sufficient amounts of 7-DHC (about 25–50 μg/cm² of skin) are available for vitamin D_3 production, and adequate vitamin D_3 may be produced after only 10–15 minutes of sun exposure at least two times per week to the face, arms, hands, or back, without sunscreen (Holick, 1995). There is significant debate on whether melanin levels in skin affect the amount of vitamin D produced, and some have reported that individuals with higher skin melanin may require more UVB irradiation to produce the same amount of vitamin D as individuals with lower melanin (Matsuoka *et al*., 1991; Dawson-Hughes, 2004).

Whether made in the skin or ingested as a nutritional supplement, vitamin D_3 (cholecalciferol) is then

hydroxylated in the hepatocytes by the mitochondrial enzyme 25-hydroxylase (CYP27A1) to 25-hydroxycholecalciferol [25(OH)D3], or calcidiol, and stored until needed (confusingly, the literature regularly refers to $25(OH)D_3$ concentration in the serum as the vitamin D level). 25-hydroxycholecalciferol is then hydroxylated by the renal mitochondrial enzyme 1α-hydroxylase (CYP27B1) to form 1,25-dihydroxycholecalciferol [1,25(OH)2D3], or calcitriol, the physiologically active form of vitamin D. This conversion is regulated by parathyroid hormone (PTH) and hypophosphatemia; both stimulate renal 1α-hydroxylase. Calcitriol is then released into the circulation, where it binds its carrier protein (vitamin D–binding protein, VDBP) to be transported to target organs (Van den Berg, 1997). Extrarenal activity of 1α-hydroxylase has been reported in various cells, including colon and prostate cancer cells, keratinocytes, and macrophages. Therefore, it has been suggested that local synthesis of calcitriol in tissues from 25(OH)D transferred from the serum to the cells may regulate their growth and differentiation by autocrine and paracrine signaling pathways (Seifert *et al*., 2009).

Calcitriol, the active form of vitamin D_3, mediates its biological effects by binding to the vitamin D receptor (VDR), a member of the nuclear receptor superfamily of steroid/thyroid hormone receptors, which are expressed in most organs, including the brain, breast, gonads, heart, prostate, and skin. VDR acts as a transcription factor to modulate gene expression of transport proteins (including TRPV6 and calbindin), which are involved in calcium absorption in the intestine. VDR activation in the intestine, bone, kidney, and parathyroid gland cells regulates calcium and phosphorus hemostasis in the body (Holick, 2004).

Vitamin D is essential for calcium metabolism, bone mineralization, and bone remodeling by osteoblasts and osteoclasts. Deficiency results in rickets in children and osteomalacia in adults. Vitamin D promotes absorption of calcium and phosphorus in the intestines, and reabsorption of calcium in the kidneys, increasing calcium in the systemic circulation. It also inhibits calcitonin release from the thyroid gland, which acts directly on osteoclasts, inhibiting bone resorption. Vitamin D may also inhibit parathyroid hormone secretion (DeLuca, 2004).

Vitamin D also modulates immunological functions and inflammation. VDR is expressed in innate and adaptive immune cells (monocytes, activated macrophages, dendritic cells, natural killer cells, and activated T and B cells). VDR ligands have been shown to increase the activity of natural killer cells, enhance the phagocytic activity of macrophages, suppress T-cell activation and the induction of regulatory T cells, and affect the maturation, differentiation, and migration of dendritic cells (Hayes *et al*., 2003; Yee *et al*., 2005; Van Etten and Mathieu, 2005). Calcitriol may also enhance macrophage production of the antimicrobial peptide cathelicidin in response to infection (Raloff, 2006). It is not surprising, therefore, that vitamin D deficiency is associated with a higher risk of infection, such as by influenza and tuberculosis (Cannell *et al*., 2006; Nnoaham and Clark, 2008).

Potentiating the activation of VDR with calcitriol may have therapeutic applications in the treatment of inflammatory diseases such as rheumatoid arthritis and psoriatic arthritis, skin disease (psoriasis, actinic keratosis), osteoporosis, cancers (prostate, colon, breast, myelodysplasia, leukemia, head and neck squamous cell carcinoma, and basal cell carcinoma), autoimmune diseases (systemic lupus erythematosus, type 1 diabetes mellitus), and central nervous systems diseases (multiple sclerosis), as well as in preventing organ transplant rejection (Nagpal *et al*.,2005). For a complete review, see Stechschulte *et al*. (2009). Vitamin D deficiency has been associated with the onset of multiple sclerosis and has been implicated in the activation of the histocompatibility gene (HLA-DRB1*1501), which is involved in differentiating between self and foreign proteins in individuals genetically predisposed to multiple sclerosis (Munger *et al*., 2006).

Vitamin D also has important antiproliferative and prodifferentiation effects. Calcitriol regulates cell growth, differentiation, and apoptosis in cancer cells. Cancer cells express VDRs that operate as transcription factors to induce cancer cell death *in vitro* and *in vivo* (Ingraham *et al*., 2007). Evidence suggests a beneficial correlation between vitamin D supplementation of at least 1,000 international units (IU) daily and reduction of colon cancer risk by 50%, pancreatic cancer risk by 43%, and breast and ovarian cancer risks by 30%, at blood levels of 80 nmol/l or higher (Holick, 2003; Gorham *et al*., 2007; Garland *et al*., 2006, Skinner *et al*., 2006; Lappe *et al*., 2007; Lin *et al*., 2007). However, controlled intervention studies have generally failed to show this effect, suggesting that the observed statistical associations may reflect confounding factors (Freedman *et al*., 2007; Reddy and Gilchrest, 2010). Progression in breast cancer and bone metastases and a higher risk of

breast cancer have been correlated with low levels of serum vitamin D and polymorphisms of the VDR gene, respectively (Buyru *et al*., 2003).

In vitro and *in vivo* studies have shown that calcitriol may suppress proliferation and induce differentiation in human keratinocytes and melanocytes. Keratinocytes have the enzymatic apparatus required to synthesize calcitriol (Seifert *et al*., 2009), and melanoma cells have been shown to be capable of converting vitamin D_3 to its active form [1,25(OH)2D3]. Interestingly, although indoor workers get three to nine times less solar ultraviolet exposure than outdoor workers, only indoor workers have shown an increasing incidence of melanoma, and some have proposed that inadequate cutaneous vitamin D levels may promote the development of malignant melanoma (Godar *et al*., 2009).

Vitamin D has been suggested to play a role in cardiovascular disease. Vitamin D deficiency is associated with an increase in high blood pressure, peripheral artery disease, elevated very-low-density-lipoprotein cholesterol and triglycerides, and impaired insulin metabolism (Wang *et al*., 2008). Studies have shown low levels of vitamin D (<17.8 ng/ml) to be independently associated with an increase in all-cause mortality in the general population (Melamed *et al*., 2008). Higher vitamin D levels have also been associated with longer leukocyte telomere length (Richards *et al*., 2007) and may be an important factor in preventing age-related diseases. Leukocyte telomere length predicts the development of aging-related disease, and the length of these telomeres decreases with each cell division and with increased inflammation (Richards *et al*., 2007).

2. What are considered the "normal" and "abnormal" levels of vitamin D, and how were these determined?

There is controversy over the optimal serum levels of 25(OH)D3 (Reddy and Gilchrest, 2010) as well as their possible causal relationship to the development of vitamin D insufficiency–associated conditions, including autoimmune diseases, cancer, and cardiovascular disease. It is likely that ideal levels vary throughout various life stages, depending on the physiological end point being measured (bone density, bone fractures, concentrations of parathyroid hormone, etc.). A concentration lower than 10–15 ng/ml (25–37.5 nmol/l) is considered deficient, and concentrations between 10–15 ng/ml and 30–50 ng/ml are generally considered insufficient. 25(OH)D3 serum concentrations higher than 30–50 ng/ml (75–125nmol/l) are considered sufficient for overall health and disease prevention, although there are no consistent data to support this suggestion (Holick, 2007;Vieth *et al*., 2007; Reddy and Gilchrest, 2010).

Circulating 1,25(OH)2D3 has a half-life of 15 hours, and it does not decrease until vitamin D deficiency is severe, as 1,25(OH)2D3 serum concentrations are closely regulated by parathyroid hormone, calcium, and phosphate. Therefore, circulating 1,25(OH)2D3 is not a good indicator of vitamin D status. In contrast, 25(OH)D3 has a half-life of 15 days. The serum concentration of 25(OH)D3 is considered the best indicator of vitamin D status, reflecting both vitamin D produced in the skin and vitamin D obtained from food and supplements (Holick, 2007; Jones, 2008; Pilz *et al*., 2009; Seamans and Cashman, 2009). Of note, however, serum 25(OH)D levels do not indicate the amount of vitamin D stored in other body tissues, such as fat.

Under normal physiological conditions, the parathyroid glands secrete PTH in response to low serum calcium levels, to increase the concentration of calcium in the blood by acting on parathyroid hormone receptor in bone, the kidneys, and the intestine. In bone, PTH stimulates resorption by indirectly stimulating the formation of osteoclasts from their precursors (osteoblasts). In the kidneys, PTH increases active reabsorption of calcium and magnesium from distal tubules and the thick ascending tubule. In the intestine, PTH enhances the absorption of calcium by increasing the production of activated vitamin D. PTH upregulates the activity of the renal mitochondrial enzyme 1α-hydroxylase, increasing the hydroxylation of 25-hydroxycholecalciferol to form 1,25-dihydroxycholecalciferol, the physiologically active form of vitamin D. 1,25(OH)2D3 increases the intestinal absorption of calcium via calbindin, a protein in intestinal epithelial cells (enterocytes) that mediates the transport of calcium across the enterocytes from the apical side (regulated by the calcium channel TRPV6) to the basolateral side and then to the systemic circulation via ATPase-dependent calcium pumps (Poole and Reeve, 2005; Barley *et al*., 1999).

In secondary hyperparathyroidism, which most commonly results from chronic kidney disease, serum calcium levels are decreased, causing the hypersecretion of PTH from the parathyroid glands. PTH in turn increases the consumption of vitamin D, leading to deficiency. In contrast, vitamin D deficiency may cause

secondary hyperparathyroidism, accelerating bone loss, because sufficient vitamin D inhibits PTH secretion from the parathyroid gland. It has been suggested that, at least in part, the beneficial effects of vitamin D supplementation on bone mineral density and reduced bone turnover may be attributed to suppression of secondary hyperparathyroidism by 25-hydroxyvitamin D (Lips, 2001; DeLuca, 2004; Björkman *et al*., 2009).

In cholesterol synthesis, the conversion of lanosterol to cholesterol involves multiple physiological steps, many of which have not yet been elucidated. It has been recognized, however, that 7-dehydrocholesterol (7-DHC) is the final precursor in the cholesterol synthesis pathway, and 7-DHC reductase is the enzyme that converts 7-DHC to cholesterol (Bae, 1999). Vitamin D_3 is produced in the skin from the photoconversion of 7-DHC; therefore, an adequate cholesterol synthesis pathway is necessary for adequate vitamin D synthesis in skin (Slominski *et al*., 2004).

It may be assumed that cholesterol-lowering medications such as hydroxymethylglutaryl CoA reductase inhibitors may inhibit the synthesis of 7-dehydrocholesterol; however, they have been shown to increase vitamin D serum levels. The mechanism of action for this response is not yet understood (Pérez-Castrillón *et al*.,, 2007; Carbone *et al*., 2008).

Vitamin D deficiency may develop from a combination of inadequate oral intake, increased requirement, increased excretion, inadequate sunlight exposure, impaired absorption, or liver or kidney disorders that impair conversion of vitamin D into its active metabolite. Salmon, tuna, and fish liver oils are among the few foods that naturally contain vitamin D. Small amounts of vitamin D are found in beef liver, cheese, egg yolk, and mushrooms. The vitamin D in these foods is found mainly as vitamin D_3 (cholecalciferol) and 25(OH)D3 (Ovesen *et al*., 2003). Vitamin D_3 is said to be three times more effective than vitamin D_2 in raising serum concentrations of 25(OH)D3, and to maintain serum levels longer than does vitamin D_2, although other authorities believe that vitamin D_3 and D_2 are equivalent in this regard (Holick *et al*., 2008). Vitamin D_3 metabolites may also have a higher affinity for their binding proteins in plasma, making vitamin D_3 more available for metabolic processes than other forms (Houghton and Vieth, 2006; Armas *et al.*, 2004). For these reasons, vitamin D_3 is generally preferred for the purposes of vitamin D supplementation.

Current daily intake recommendations for adequate levels of vitamin D are 200 IU for adults under age 50, 400 IU for adults aged 51 to 70, and 600 IU for adults over 71. The American Academy of Pediatrics recommends 400 IU daily for children under 18 (Institute of Medicine, Food, and Nutrition Board, 1997; Wagner and Greer, 2008). Daily supplemental intakes of 400 IU of vitamin D increase 25(OH)D3 concentrations by approximately 3–5 ng/ml; therefore, daily intakes of approximately 1,700 IU may be needed to increase these concentrations from 20 to 32 ng/ml (Vieth *et al*., 2007). Higher daily doses of 1,000 IU of vitamin D are recommended for groups at risk for vitamin D insufficiency, including dark-skinned individuals; elderly and obese persons; individuals with fat malabsorption and photosensitive diseases that demand strict sun protection; and individuals who regularly practice sun-exposure protection or have limited sun exposure owing to geographic location (living in northern latitudes), religious reasons, or limitations that make them homebound (Yetley, 2008; Wolpowitz and Gilchrest, 2006).

Serum 25(OH)D3 concentrations higher than 200 ng/ml are considered toxic and are usually caused by a high intake of supplements (such as large amounts of cod liver oil). Vitamin D toxicity may present with nonspecific symptoms such as nausea, vomiting, anorexia, constipation, and weakness. It may also increase calcium serum levels, causing changes in mental status, cardiac conduction abnormalities, and renal stones (Jones, 2008). Excessive sun exposure alone does not result in vitamin D toxicity, because excess previtamin D_3 is converted to inactive photoproducts as it is formed (Holick, 2007).

Vitamin D levels differ by gender, age, physical complexion, ethnic group, season, and geographic location. Vitamin D serum levels tend to be lower in people living in the northern latitudes during the winter season and higher in the fall, after a summer of sun exposure. Males usually have higher vitamin D 25(OH)D3 concentrations than females. Children tend to have higher vitamin D serum levels than adults. The increasing use of multivitamin and -mineral dietary supplements in adults is not associated with a corresponding increase in serum 25(OH)D3 concentrations. Leaner individuals have higher 25(OH)D3 serum concentrations than heavier individuals, and non-Hispanic white women of reproductive age tend to have higher vitamin D levels than African-American women and Mexican Americans (Nesby-O'Dell *et al*., 2002; Yetley, 2008; Huotari and Herzig, 2008).

3. How did the design of the study affect its results?

Previous studies have suggested that individuals with low vitamin D levels are able to produce vitamin D faster than individuals with normal levels of vitamin D (Viljakainen *et al*., 2006). It had been proposed that skin pigmentation may affect vitamin D production from sun exposure (Chen *et al*., 2007). Therefore, the investigators proposed to determine the influence of baseline 25(OH)D3 levels, constitutive and facultative skin pigmentation, and total cholesterol levels on baseline 25(OH)D3 serum levels after UVB irradiation.

In January, when the levels of UV radiation and serum concentrations of vitamin D are considered the lowest in Denmark, 182 persons were screened for 25(OH)D3 baseline levels. From this group, 50 participants with a wide range in baseline 25(OH)D3 levels were selected, 25 with insufficient 25(OH)D3 levels (25–50 nmol/l or 10–20 ng/ml), 15 with deficient 25(OH)D3 levels (<25 nmol/l or 10 ng/ml), and 10 with sufficient levels of 25(OH)D3 (>70 nmol/l or 28 ng/ml). A major problem with the study design is that participants were exposed to four doses of UVB irradiation every second or third day, at an intensity of three standard erythema doses (SEDs), to 24% of their body surface area (chest and back). SED is not to be confused with minimal erythema dose (MED), the dose of UV required to produce minimally detectable but sharply demarcated erythema 24 hours later in a specific individual. The SED, a term coined by the senior author and colleagues in the 1990s, is a dose of 10 J/cm^2 using a specific light source (TL 12 bulbs) that is said to represent 1 MED for very fair-skinned individuals. Each subject received four exposures of three SEDs, and the dark-skinned subjects were said to have a photoprotection factor of approximately 10 SEDs on average. Because maximal vitamin D photosynthesis occurs after approximately one-third MED, most subjects probably achieved maximal vitamin D photosynthesis regardless of their epidermal melanin content. A more logical study design to detect an effect of skin pigmentation on vitamin D photosynthesis would be to use a modest, constant UV dose for all subjects, a dose likely to maximize vitamin D photosynthesis in fair-skinned but not dark-skinned subjects, a scenario more relevant to everyday life for most individuals. The reader can only presume the study was not originally designed to address the influence of pigmentation on UV-induced vitamin D levels.. Several variables were measured: skin pigmentation (facultative skin pigmentation measured as pigment protection factor in the upper body), skin redness, and 25(OH)D3 levels were measured before and after the irradiations, and age, gender, body mass index , alkaline phosphatase levels, ionized calcium levels, total cholesterol levels, PTH levels, and number of fish meals per day were measured at baseline.

The 50 individuals were divided into two groups according to their sun-exposure behaviors, resulting in 22 sun worshipers and 28 non–sun worshippers.

Among the 28 non–sun worshippers, the investigators paired a group of dark-skinned individuals with a group of fair-skinned individuals, based on their baseline 25(OH)D3 levels, to determine whether constitutive or facultative skin pigmentation affects 25(OH)D3 levels after identical UVB irradiation, normalized for individual UV exposure by the SED determination.

Website advertising for the recruitment of subjects may have led to selection bias. Relevant variables such as age, diet, state of health, and lifestyle may not have been well represented in the selected individuals; for instance, older people were not included in the study, and they are known to produce less vitamin D in response to sun exposure (Holick, 2007). Although the use of dietary supplements and consumption of fish meals per day were measured, a more meticulous dietary log including the consumption of beef liver, cheese, eggs and mushrooms—all important sources of 25(OH)D3 that might have influenced participants' vitamin D levels—was not employed.

Because few dark-skinned individuals were included in this study, the authors were unable to randomize the subjects or control for confounding factors when comparing dark-skinned and fair-skinned groups. The investigators also commented on a high variance in the liquid chromatography–tandem mass spectrometry analysis when measuring 25(OH)D3 levels, despite including two serum samples from each individual and performing the analysis twice on each sample. This variance may have confounded the results.

4. What are the results of the study?

From the initial group of patients screened for serum 25(OH)D3 levels (n = 182), 67% were found to be vitamin D–insufficient (25(OH)D3 levels <50 nmol/l) and 18% vitamin D–deficient (25(OH)D3 <25 nmol/l). Similar data on the prevalence of vitamin D deficiency and vitamin D status in northern latitudes have been reported (Prentice, 2008).

From the selected group of 50 individuals exposed to UVB irradiation (with a wide range of baseline 25(OH)D3 levels), the investigators found that the increase in 25(OH)D3 levels negatively correlated with baseline 25(OH)D3 levels ($P < 0.001$). Individuals with low baseline 25(OH)D3 levels showed a significantly higher production of 25(OH)D3 after UVB irradiation, consistent with previous studies reporting that individuals with low vitamin D levels produce vitamin D faster than individuals with normal levels of vitamin D. The exact physiological mechanism for this response is unclear (Viljakainen, 2006).

The investigators also found that the increase in 25(OH)D3 levels positively correlated with baseline total cholesterol levels ($P = 0.005$), suggesting that normal cholesterol levels are important for adequate vitamin D production. PTH levels negatively correlated with baseline 25(OH)D3 levels ($P = 0.025$), and no significant correlation was found between baseline 25(OH)D3 levels and body mass index, in contrast to earlier studies (Reddy and Gilchrest, 2010). A positive correlation was found between baseline 25(OH)D3 levels and the number of fish meals per day ($P = 0.009$). Facultative skin pigmentation and skin redness increased with UVB irradiation in a statistically significant manner.

The authors found a significant difference in baseline 25(OH)D3 levels between non–sun worshippers ($n = 28$) and sun worshippers ($n = 22$) ($P = 0.018$). The differences in sun habits between the groups may explain previously reported lower levels of vitamin D in dark-skinned persons (Nesby-O'Dell *et al*., 2002).

In the 28 non–sun worshippers exposed to UVB radiation, the authors found no significant correlation between the increase in 25(OH)D3 levels and their constitutive or facultative pigmentation. However, a significant positive correlation was found in this group between the increase in 25(OH)D3 levels and baseline total cholesterol ($P = 0.005$) The investigators also found a significant positive correlation between the increase in 25(OH)D3 levels and male gender ($P = 0.01$). No significant correlation was found between the increase in 25(OH)D3 levels and age, body mass index, PTH levels, alkaline phosphatase levels, ionized calcium levels, number of fish meals per day, or skin redness after UV irradiation.

When comparing the increase in 25(OH)D3 levels of the paired dark-skinned and fair-skinned groups, the investigators found no significant differences despite their significant difference in skin pigmentation ($P = 0.008$). No significant difference in 25(OH)D3 increase was found between the dark-skinned individuals and the 25(OH)D3 baseline-matched fair-skinned individuals, a result that does not resolve the question of the effect of constitutive and facultative skin pigmentation in increasing 25(OH)D3 levels after sun exposure (Chen *et al*., 2007; Brazerol *et al*., 1988; Rockell *et al*., 2008).

5. What are the limitations of the study?

The small sample size used for the assessment of changes in vitamin D level after sun exposure and the small sample size used to compare the effect of skin pigmentation between dark-skinned and light-skinned individuals may have affected the results.

The investigators failed to mention how the group of 50 individuals with a wide range in baseline 25(OH)D3 levels was selected from the initial group of 182; selection bias and other confounding factors may have negatively affected the accuracy of the results. It is unclear whether this group was randomly selected, but it was implied that "a wide range" of 25(OH)D3 levels were included. There is no mention of evaluating participants for liver and renal disease, but proper function of these organs is key in the hydroxylation process that forms the active form of vitamin D.

When describing the dark-skinned and light-skinned individuals selected for the study, there was no mention of ethnic background. This factor may be important, as genetic differences in baseline levels of vitamin D and genetic polymorphisms of the vitamin D receptors may be observed among diverse ethnicities. In addition, when selecting the nine matched pairs of dark- and light-skinned individuals, the investigators did not control for biophysical variables (age, ethnicity, body mass index, etc.), cholesterol levels, and PTH levels.

6. What are the conclusions and clinical implications of the study?

Bogh *et al*. performed this experiment in winter, when melanin is located mostly in the basal layer, as opposed to summer, when sun exposure stimulates melanin to move higher up in the epidermis; they suggested that the UV irradiation dose was sufficient to reach a "state of saturation" in the production of vitamin D. The exact mechanism of this saturation process is unclear. The investigators suggest that sufficient vitamin D may be produced in both dark-skinned and light-skinned individuals with few, low doses of ultraviolet B exposure.

Still, they recommend oral supplementation of vitamin D to establish appropriate serum levels while avoiding the potential carcinogenic effects of UV irradiation to the skin.

Based on the study's results and limitations, additional experiments could be performed. The investigators recorded participants' vitamin D levels and retrospectively searched for possible modifying variables implicated in the synthesis of vitamin D following UV irradiation, such as cholesterol levels and skin pigmentation. A different design could be used in which selected subject cohorts are matched and randomized to adjust for variables that may influence vitamin D levels and responses to sun exposure in the production of vitamin D.

A more comprehensive study may include a larger sample, broader age groups, established ethnic backgrounds, different latitudes, or studies of the same group of subjects during different seasons. In addition, other variables affecting vitamin D production, such as diet and behaviors among certain ethnic groups, could be included.

Several studies have reported vitamin D levels after sun exposure increase to a greater extent in individuals with low baseline vitamin D levels, but the physiological mechanisms implicated in this response have not been elucidated (Viljakainen *et al*., 2006). Conversely, individuals with apparently adequate UV exposure have been reported to have low serum 25(OH)D3 levels. A variable responsiveness to UVB irradiation among individuals, genetic differences in the amount of vitamin D necessary to maintain optimal physiological functions, genetic differences in the cytochrome P450 enzymes activating and degrading vitamin D, and regulation of vitamin D transport from the skin to the circulation may play a role in these findings. (Binkley *et al*., 2007; Edvardsen *et al*., 2007). It may be important to investigate why individuals with low baseline levels of vitamin D have a higher production of 25(OH)D3 when exposed to UV radiation. This would require a control for all possible factors, including those considered in this study (age, gender, body mass index, skin pigmentation alkaline phosphatase levels, ionized calcium levels, total cholesterol levels, PTH levels, and number of fish meals per day), as well as hepatic and renal status and dietary habits. Appropriate controls would also entail assessing the genetic and constitutive differences in the metabolic processes involved in the synthesis, activation, transport and degradation of vitamin D and the variety of vitamin D levels required to maintain adequate physiological homeostasis. In addition, a large sample population and subjects from multiple centers (to ensure an appropriate representation of various geographic areas, ethnic groups, and related lifestyles) should be included.

Vitamin D deficiency produces significant morbidity through effects on bone, and vitamin D insufficiency has been suggested to play an important role in various physiologic functions, including cardiovascular, immunological, and anticarcinogenic homeostasis as well as cognitive function and overall mortality (Stechschulte *et al*., 2009). More than 50% of the world's population is at risk for vitamin D deficiency or insufficiency, owing to the inadequate fortification of foods with vitamin D, poor supplementation, and other factors, including diet and lifestyle habits (Holick, 2008). UV irradiation is an important factor contributing to skin aging and skin cancer. Despite the risk of carcinogenesis, sun exposure has been recommended to avoid vitamin D deficiency (Reichrath, 2007). However, vitamin D is readily available from oral supplements, providing a safe and sufficient source of vitamin D required for all known and hypothesized functions, and this is recommended as the first line of vitamin D supplementation (Gilchrest, 2008; Reddy and Gilchrest, 2010).

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