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Vitamin D Production after UVB Exposure Depends on Baseline Vitamin D and Total Cholesterol but Not on Skin Pigmentation

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UVB radiation increases serum vitamin D level expressed as 25-hydroxyvitamin-D_3 (25(OH)D), but the influence of skin pigmentation, baseline 25(OH)D level, and total cholesterol has not been well characterized. To determine the importance of skin pigmentation, baseline 25(OH)D level, and total cholesterol on 25(OH)D production after UVB exposure, 182 persons were screened for 25(OH)D level. A total of 50 participants with a wide range in baseline 25(OH)D levels were selected to define the importance of baseline 25(OH)D level. Of these, 28 non-sun worshippers with limited past sun exposure were used to investigate the influence of skin pigmentation and baseline total cholesterol. The participants had 24% of their skin exposed to UVB (3 standard erythema doses) four times every second or third day. Skin pigmentation and 25(OH)D levels were measured before and after the irradiations. Total cholesterol was measured at baseline. The increase in 25(OH)D level after UVB exposure was negatively correlated with baseline 25(OH)D level ($P<0.001$) and positively correlated with baseline total cholesterol level ($P=0.005$), but no significant correlations were found with constitutive or facultative skin pigmentation. In addition, we paired a dark-skinned group with a fair-skinned group according to baseline 25(OH)D levels and found no differences in 25(OH)D increase after identical UVB exposure.

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INTRODUCTION

Vitamin D insufficiency (25-hydroxyvitamin D_3 (25(OH)D) $<50\,nmol\,l^{-1}$) is common and is related to rachitis and osteoporosis, and may increase the risk of some internal malignancies and autoimmune diseases (Chapuy *et al.*, 1992; Ahonen *et al.*, 2000; Munger *et al.*, 2004; Ponsonby *et al.*, 2005; Kemp *et al.*, 2007). UV radiation of the skin provides 90–95% of the total vitamin D requirements for the majority of the population (Holick, 1998, 2004).

Solar UV radiation (290–400 nm) comprises 90–100% UVA (320–400 nm) and 0–10% UVB (280–320 nm). The action spectrum for cutaneous pre-vitamin D_3 synthesis shows that only UVB radiation initiates synthesis (CIE 174 Technical Report, 2006). Conversely, it is also mainly UVB that causes sunburn and carcinogenesis (Diffey, 2002). Despite the risk of skin cancer, sun exposure is often recommended to avoid vitamin D insufficiency (Holick, 2008; Moan *et al.*, 2008); and this has led to scientific debate (Samanek *et al.*, 2006; Gilchrest, 2008).

Studies suggest a faster vitamin D production among individuals with a very low vitamin D level (Viljakainen *et al.*, 2006; Binkley *et al.*, 2007; Brustad *et al.*, 2007; Edvardsen *et al.*, 2007), and skin pigmentation is generally considered to diminish vitamin D production from sun exposure (Holick *et al.*, 1981; Clemens *et al.*, 1982; Chen *et al.*, 2007). However, these theories are not well documented (Young, 2006). Furthermore, cholesterol and vitamin D share the 7-dehydrocholesterol pathway (Slominski *et al.*, 2004; Champe *et al.*, 2005), and may therefore be interdependent. We therefore sought to define the importance of skin pigmentation, baseline 25(OH)D levels, and total cholesterol level on the vitamin D production after UVB exposure.

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Abbreviations: 25(OH)D, 25-hydroxyvitamin-D_3; PPF, pigmentation protection factor; PTH, parathyroid hormone; SD, standard deviation; SED, standard erythema dose

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RESULTS

The initial screening of 182 persons

Of 182 persons screened for serum 25(OH)D level in January (Figure 1), 67% were vitamin D insufficient (25(OH)D $<50\,nmol\,l^{-1}$) and 18% were vitamin D deficient (25(OH)D $\leqslant 25\,nmol\,l^{-1}$). Three groups were selected for further analyses (Figure 2).

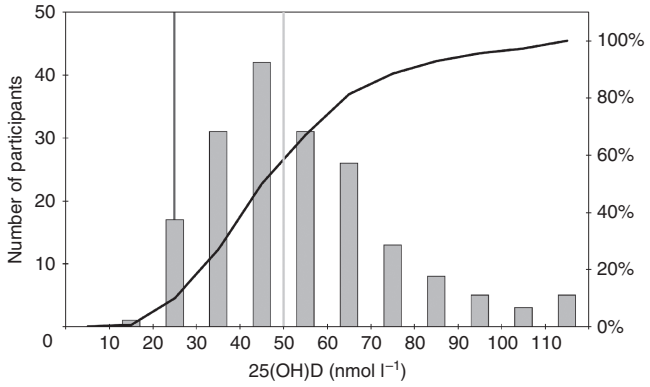

Figure 1. Distribution of 25(OH)D levels for the 182 participants at the initial screening. 67% Of participants were vitamin D insufficient (25(OH)D < 50 nmol/l), and 18% were vitamin D deficient (25(OH)D ⩽ 25 nmol/l). The dark and light grey lines show cutoffs at 25 nmol/l and 50 nmol/l, respectively.

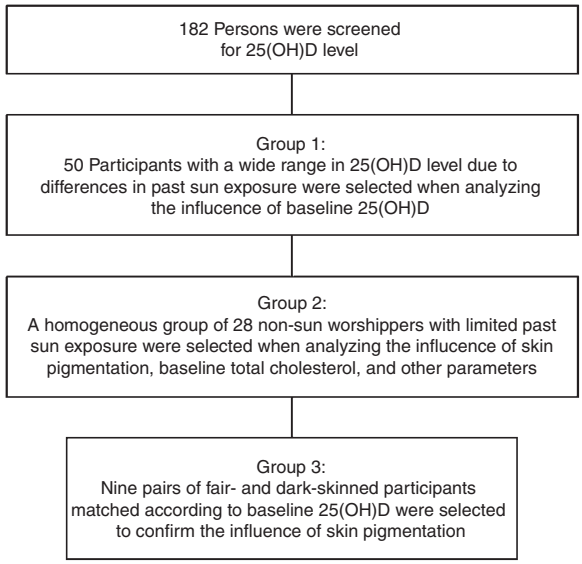

Figure 2. Flowchart describing the selection and purpose of selecting the 3 groups.

Group 1: 50 participants with a wide range in baseline 25(OH)D levels

When the influence of baseline 25(OH)D level on vitamin D production after exposure to UVB radiation was examined, 50 participants (Table 1) with all levels of 25(OH)D due to previous different sun exposures were included: 25 with an insufficient 25(OH)D level (evenly distributed 25–50 nmol l^{-1}), 15 with a deficient 25(OH)D level (⩽25 nmol l^{-1}), and 10 with a highly sufficient 25(OH)D level (>70 nmo l^{-1}). As the level of baseline 25(OH)D is expected to be of importance to the baseline parathyroid hormone (PTH) level, this was also examined in this group.

The 25(OH)D (mean (standard deviation, SD)) level increased by 23.3 nmol l^{-1} (10.6) in response to the UVB treatments, with a strong negative correlation between the increase in 25(OH)D (Δ25(OH)D) and baseline 25(OH)D levels ($P<0.001$; $R^2=0.313$, Figure 3).

We found a significant negative correlation between baseline 25(OH)D and baseline PTH levels ($P=0.025$; $R^2=0.104$, Figure 4), but we found no significant correlations between baseline 25(OH)D level and body mass index.

However, we found a positive linear relation between baseline 25(OH)D levels and the number of fish meals per week ($P=0.009$; $R^2=0.132$), with the baseline 25(OH)D level being 12 nmol l^{-1} higher in the group eating fish more than once a week.

As expected, the facultative skin pigmentation (pigment protection factor (PPF) upper body) increased from (mean (SD)) 6.5 (2.9) to 7.9 (3.0) ($P<0.001$), and the skin redness percent increased from (mean (SD)) 22.8 (6.6) to 25.5 (7.4) ($P=0.004$) after the UVB treatments.

Group 2: a homogeneous population of 28 non-sun worshippers

A homogeneous population of 28 non-sun worshippers (group 2) (Table 1) with limited past sun exposure were selected from the 50 participants (group 1) and 22 sun worshippers with excessive and very different sun exposure were excluded (Figure 2). We found a significant difference in baseline 25(OH)D level ($P=0.018$) between the 28 non-sun worshippers and the 22 sun worshippers due to different amounts of previous sun exposure.

In the selected 28 non-sun worshippers (group 2), 25(OH)D levels increased by (mean (SD)) 25.3 nmol l^{-1} (10.5) in response to the UVB treatments. No significant correlations were found between Δ25(OH)D and constitutive (PPF buttock) ($P=0.5$) or facultative ($P=0.4$) skin pigmentation. However, we found a significant positive correlation between Δ25(OH)D and baseline total cholesterol, the precursor of vitamin D ($P=0.005$; $R^2=0.265$, Figure 5). Furthermore, we found a significant relation between Δ25(OH)D and sex, as 25(OH)D (mean (SD)) increased by 20.6 nmol l^{-1} (13.3) among female participants compared with 28.8 nmol l^{-1} (6.0) among male participants ($P=0.01$). There were no significant relations between Δ25(OH)D and age, height, weight, numbers of fish meals per week, body mass index, PTH, alkaline phosphatase, ionized calcium, or skin redness percent after exposure. The facultative skin pigmentation increased from (mean (SD)) 7.1 (3.5) to 8.8 (3.5) ($P<0.001$), and the skin redness percent increased from (mean (SD)) 21.8 (7.2) to 24.2 (6.8) ($P=0.027$) during the course of UVB treatments.

Group 3: nine matched pairs

A total of 18 participants (group 3) (Table 1) consisting of 9 pairs of dark- (skin types V–VI (Fitzpatrick, 1988)) and fair-skinned participants (skin types I–IV (Fitzpatrick, 1988)) were matched according to ''identical'' baseline 25(OH)D levels (Table 2). They were used to confirm whether constitutive or facultative skin pigmentation has a role for Δ25(OH)D after UVB exposure. We found no significant differences in Δ25(OH)D ($P=0.7$) between the dark- and the fair-skinned group despite their significant difference in constitutive and facultative skin pigmentation ($P=0.008$).

Table 1. Participants baseline characteristics[1]

	Group 1: 50 participants with a wide range in baseline 25(OH)D	Group 2: a homogeneous population of 28 non-sun worshippers	Group 3: nine matched pairs
Number of participants	50	28	18
Number of males	21	16	11
Number of females	29	12	7
Number of skin type I[2]	4	4	2
Number of skin type II	15	5	2
Number of skin type III	9	4	2
Number of skin type IV	12	5	3
Number of skin type V	7	7	7
Number of skin type VI	3	3	2
PPF—buttock[3]	5.9 ± 3.5 (1.5–18.7)	6.5 ± 4.4 (1.5–18.7)	7.1 ± 4.1 (1.5–17.4)
PPF—upper body[4]	6.5 ± 2.9 (1.7–17.7)	7.1 ± 3.5 (1.7–17.7)	7.8 ± 3.8 (1.7–17.7)
Skin redness, percent	22.8 ± 6.6 (2.2–34.7)	21.8 ± 7.2 (2.2–33.9)	20.0 ± 8.4 (2.2–33.9)
Age	31.0 ± 8.1 (19–48)	31.0 ± 7.5 (19–48)	41.3 ± 8.3 (19–48)
Weight, kg	76.6 ± 16.5 (55–120)	78.9 ± 17.5 (55–120)	78.9 ± 16.0 (55–120)
Height, m	1.74 ± 0.1 (1.56–1.96)	1.75 ± 0.1 (1.56–1.89)	1.74 ± 0.1 (1.56–1.89)
Body mass index, $kg\,m^{-2}$	25.1 ± 4.7 (19.2–40.8)	25.8 ± 4.8 (19.2–37.0)	26.0 ± 4.4 (20.0–37.0)
Serum 25(OH)D, $nmol\,l^{-1}$	36.5 ± 26.5 (5–116)	27.8 ± 18.0 (5–88)	20.3 ± 8.9 (9–41)
Serum PTH, $\rho mol\,l^{-1}$	4.4 ± 2.1 (0.7–12.0)	4.8 ± 2.5 (0.7–12.0)	5.5 ± 2.5 (2.8–12.0)
Serum ionized calcium, $mmol\,l^{-1}$	1.22 ± 0.03 (1.16–1.30)	1.22 ± 0.03 (1.16–1.30)	1.21 ± 0.03 (1.16–1.26)
Serum alkaline phosphatase, $U\,l^{-1}$	68 ± 15.5 (32–106)	72.4 ± 13.4 (49–106)	70.2 ± 13.5 (49–106)
Serum total cholesterol, $mmol\,l^{-1}$	5.0 ± 0.9 (3.6–8.0)	4.7 ± 0.7 (3.6–6.3)	4.8 ± 0.5 (3.7–5.5)

25(OH)D, 25-hydroxyvitamin-D_3; PPF, pigmentation protection factor.
[1]Mean ± SD (range).
[2]Fitzpatrick, 1988.
[3]Constitutive skin pigmentation.
[4]Facultative skin pigmentation.

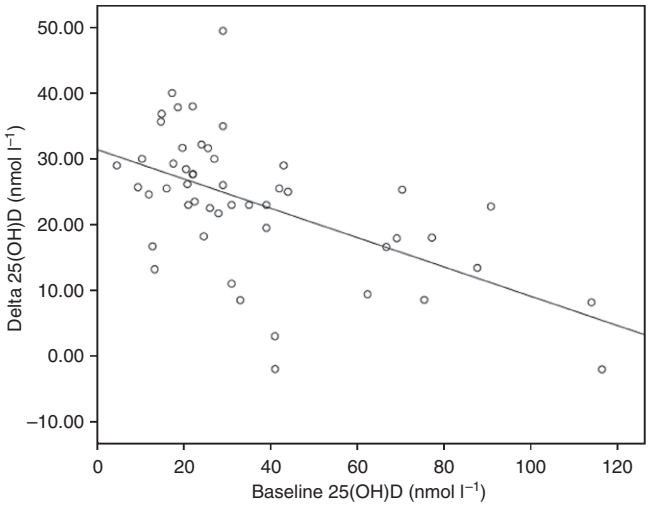

Figure 3. Relationship between the increase in vitamin D (Δ25(OH)D) and baseline 25(OH)D among the 50 participants (group 1) (P=0.000024; R^2=0.313).

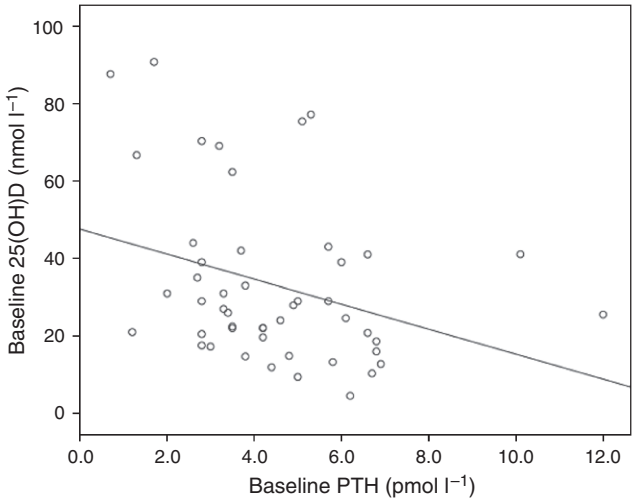

Figure 4. Relationship between baseline 25(OH)D and baseline parathyroid hormone (PTH) level (group 1) ($P = 0.025$; $R^2 = 0.104$; 48 participants were included, 2 outliers outside 3 standard deviations excluded).

DISCUSSION

In total, 67% of the 182 participants had 25(OH)D levels below 50 nmol l^{-1}, and 18% were below 25 nmol l^{-1}. The recommended level of 25(OH)D is estimated to be 50 nmol l^{-1} or higher (Brot *et al.*, 2001; Lips, 2004; Mosekilde *et al.*, 2005). However, it is debated whether higher 25(OH)D levels of 80–100 nmol l^{-1} are preferable (Holick, 1998; Heaney, 2005; Vieth, 2006).

To strengthen our findings, we included detailed information on lifestyle factors, collected information on intakes of calcium and vitamin D, included two serum samples from each subject, measured pigmentation, and conducted the study when the ambient UV radiation was negligible and the blood concentration of vitamin D was considered as the year's lowest in Denmark 56°N. Furthermore, we selected a homogeneous population of 28 non-sun worshippers (group 2) with limited past sun exposure, because differences in sun habits is a confounding factor when analyzing the influence of skin pigmentation, baseline total cholesterol, and other parameters except the influence of baseline 25(OH)D levels for which a wide range of baseline 25(OH)D levels are needed. UVB radiation was used as it fits the vitamin D action spectrum and uniform irradiation of large body surface areas can be obtained. A limitation of this study was the relatively small sample size, especially in the dark-skinned group. Furthermore, we found a high variance in the liquid chromatography-tandem mass spectrometry analysis of 25(OH)D of ~8.5%, despite including two serum samples from each subject and performing each 25(OH)D analysis twice.

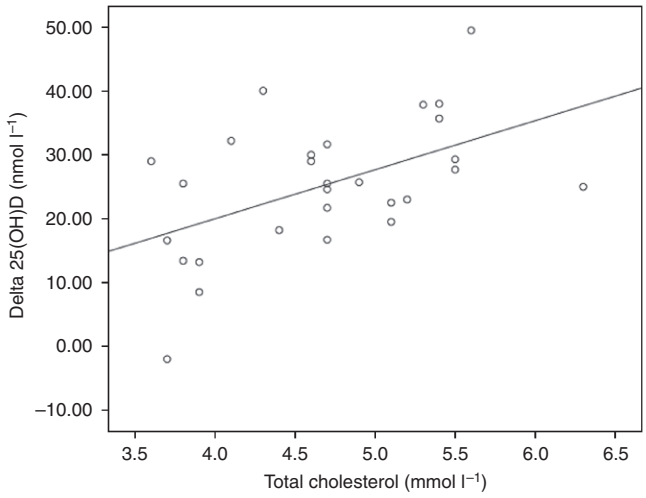

Figure 5. Relationship between the increase in vitamin D (Δ25(OH)D) and baseline total cholesterol in the 28 non-sun worshippers (group 2) with limited past sun exposure ($P = 0.005$; $R^2 = 0.265$).

Among the 50 participants in group 1, we found a significantly higher 25(OH)D production after UVB exposure in participants with a low baseline 25(OH)D level. Although not specifically exploring the mechanism, several studies have reported that individuals with low baseline vitamin D are the most effective at increasing serum levels of vitamin D after UVB exposure (Viljakainen *et al.*, 2006; Binkley *et al.*, 2007; Brustad *et al.*, 2007; Edvardsen *et al.*, 2007; Stephenson *et al.*, 2007; Carbone *et al.*, 2008). The reasons for this are unknown, but it may be because 25(OH)D inhibits 25-hydroxylase in the liver; the liver prompts hydroxylation of vitamin D_3 to 25(OH)D (Champe *et al.*, 2005).

Among the 28 non-sun worshippers in group 2, we found no significant relation between Δ25(OH)D and constitutive or facultative skin pigmentation. Furthermore, Table 2 shows

Table 2. PPF buttock and upper body and Δ25(OH)D for nine dark-skinned participants (skin types V–VI) and nine fair-skinned participants (skin types I–IV) matched according to baseline 25(OH)D level[1] (group 3)

	PPF buttock[2]		PPF upper body[3]		Baseline 25OH(D), nmol l^{-1}		Δ25OH(D), nmol l^{-1}	
Pair	**Dark[4]**	**Fair[5]**	**Dark**	**Fair**	**Dark**	**Fair**	**Dark**	**Fair**
1	11.9	7.6	12.6	8.6	10.3	9.4	30.0	25.7
2	13.0	3.8	10.8	5.7	11.9	14.7	24.6	35.7
3	17.4	2.0	17.7	3.4	12.8	16.0	16.7	25.5
4	8.6	3.2	6.8	4.1	13.3	17.6	13.2	29.3
5	9.1	1.5	9.0	1.7	17.3	22.0	40.1	38.0
6	6.6	4.0	7.4	6.7	18.6	22.1	37.9	27.7
7	9.6	5.2	12.6	5.6	24.1	24.6	32.2	18.2
8	5.7	4.0	7.6	4.9	25.6	26.0	31.7	22.5
9	8.6	5.2	9.3	6.4	41.1	39.0	−2.0	19.5
Mean (± SD)	10.1 (3.6)	4.1 (1.8)	10.4 (3.5)	5.2 (2.0)	19.4 (9.7)	21.3 (8.5)	24.9 13.5)	26.9 (6.7)
P-value	0.008		0.008		0.07		0.7	

25(OH)D, 25-hydroxyvitamin-D_3; PPF, pigmentation protection factor.
[1]Wilcoxon signed rank test was used in the paired test.
[2]Constitutive skin pigmentation.
[3]Facultative skin pigmentation.
[4]Dark-skinned, non-ethnic Danish participants, skin types V–VI (Fitzpatrick, 1988).
[5]Fair-skinned, ethnic Danish participants, skin types I–IV (Fitzpatrick, 1988).

no significant differences in Δ25(OH)D between the dark-skinned persons and the 25(OH)D baseline-matched fair-skinned persons (group 3) despite their highly significant difference in constitutive and facultative skin pigmentation. This shows that Δ25(OH)D is unrelated to skin pigmentation. A number of very small studies *in vitro* (Holick *et al.*, 1981) and *in vivo* (Clemens *et al.*, 1982; Chen *et al.*, 2007) indicate that melanin pigmentation diminish vitamin D production in the skin.

Conversely, Brazerol *et al.* (1988) found a similar vitamin D increase in a fair-skinned group ($n=13$) compared with a dark-skinned group ($n=7$) exposed to suberythemal whole-body UVR twice a week for 6 weeks. Furthermore, a study of Rockell *et al.* (2008) found that only facultative skin color is a determinant of vitamin D production and claims that constitutive skin type is of no significant importance. In addition, Marks *et al.* (1995) reported no significant relations between skin type and vitamin D production. There is an obvious lack of agreement about the role of skin pigmentation in vitamin D production after UVB exposure, and skin pigmentation was not measured in any of the studies. However, several studies have reported a lower level of vitamin D among dark-skinned than among fair-skinned individuals (Harris and Dawson-Hughes, 1998; Nesby-O'Dell *et al.*, 2002), and it might be possible that vitamin D insufficiency among certain ethnic groups results from other factors than skin pigmentation such as behavior or diet.

We found a remarkable difference in sun habits between the dark-skinned group, who were non-sun worshippers with limited levels of sun exposure, and the fair-skinned group, in which the main part was sun worshippers with excessive levels of sun exposure. These differences in sun habits may explain why dark-skinned persons are reported to have lower vitamin D levels than fair-skinned persons (Harris and Dawson-Hughes, 1998; Nesby-O'Dell *et al.*, 2002, Young, 2006). Although we found no differences in vitamin D formation between dark- and fair-skinned persons during wintertime, when melanin is located in the basal layers of epidermis, a relation could exist in the summertime, when melanin moves further up in epidermis due to sun exposure. Another explanation could be that 3 standard erythema doses (SEDs) administered four times to 24% of the body surface area is sufficient to reach a state of saturation.

Notably, we also found a significant positive relation between Δ25(OH)D and baseline total cholesterol levels. The synthesis of vitamin D starts in the bowel epithelial with the oxidation of cholesterol from food or bile to pro-vitamin D_3 (7-dehydrocholesterol), which is then transported to the skin, mainly the epidermis, wherein it is isomerized to pre-vitamin D_3 (cholecalciferol) by UVB radiation (Champe *et al.*, 2005). A recent study also reports a significant positive relation between baseline vitamin D and total cholesterol level (Pérez-Castrillón *et al.*, 2007). However, there is no indication of a fall in vitamin D status after statin therapy (Dobs *et al.*, 1991; Pérez-Castrillón *et al.*, 2007). Statin therapy inhibits the enzyme hydroxymethylglutaryl-CoA (HMG-CoA) reductase (Champe *et al.*, 2005), which is believed to increase the level of 7-dehydrocholesterol, a precursor of vitamin D (Pérez-Castrillón *et al.*, 2007). It should be noted that none of the participants in our study had any intake of cholesterol-lowering medicine.

Our study shows that both fair-skinned and dark-skinned persons without any underlying medical condition should be able to produce sufficient vitamin D from a few low doses of UVB (3 SEDs are equivalent to ~30 minutes of sun exposure in the middle of a clear summer day in Denmark, 56°N). However, due to its skin-carcinogenic effect, we would not recommend UVB treatment to be used as a source of vitamin D to the general population, because sufficient vitamin D levels can be re-established with vitamin D supplements (Gilchrest, 2007; Hathcock *et al.*, 2007; Thieden *et al.*, 2008).

In conclusion, we found that baseline vitamin D level is an important determinant of vitamin D production after UVB treatment. We also found that constitutive or facultative skin pigmentation in winter is of no importance for the vitamin D production in the skin. Furthermore, this study revealed a relation between total cholesterol and vitamin D production, indicating that a low natural cholesterol level might be problematic.

MATERIALS AND METHODS

Design

This is an open and controlled clinical trial, conducted at Bispebjerg University Hospital, Denmark 56°N, from January to March 2008, when the ambient UVB radiation is negligible and the low outdoor temperature prohibits solar exposure, apart from the hands and face. The participants were recruited by advertising on websites between December 2007and January 2008.

Participants and procedure

The initial screening of 182 persons. We screened 182 healthy persons (106 women and 76 men, mean age 29.8 years, range 18–51 years) for 25(OH)D level in January and February to find a suitable population of participants with a wide range of baseline 25(OH)D levels (Figure 1) and a wide variation in skin pigmentation. Vitamin D sufficiency is defined as 25(OH)D levels $\geqslant 50\,\text{nmol}\,\text{l}^{-1}$, vitamin D insufficiency as 25(OH)D levels $<50\,\text{nmol}\,\text{l}^{-1}$, and vitamin D deficiency as 25(OH)D levels $\leqslant 25\,\text{nmol}\,\text{l}^{-1}$.

Group 1: 50 participants with a wide range in baseline 25(OH)D levels. We selected 50 participants (Table 1) with different levels of baseline 25(OH)D: 25 with 25(OH)D levels evenly distributed from 25 to $50\,\text{nmol}\,\text{l}^{-1}$, 15 with 25(OH)D $<25\,\text{nmol}\,\text{l}^{-1}$, and 10 with 25(OH)D $>70\,\text{nmo}\,\text{l}^{-1}$ (Figure 2). All participants completed a standardized questionnaire that ascertained demographic characteristics, chronic diseases, medication, dietary supplements, sun habits, sun holidays, sun-bed use, and other lifestyle variables that could cause differences in vitamin D level. Weight and height were recorded and the body mass index was calculated. All 50 participants were used for the investigation of the influence of baseline 25(OH)D level on vitamin D production after UVB radiation.

Group 2: a homogeneous population of 28 non-sun worshippers. By a questionnaire about sun habits, it was possible to subdivide the 50 participants (group 1) into 28 non-sun worshippers (group 2) and 22 sun worshippers. The non-sun worshippers were

defined as persons who preferred to sit in the shadow, did not sunbathe (without clothes), and reported limited sun exposure the previous summer. The sun worshippers were defined as persons who preferred to sit in the sun (with clothes), preferred to sunbathe (without clothes), and reported excessive sun exposure the previous summer. The 28 non-sun worshippers were considered to be a relatively homogeneous population and were therefore included in the analysis of the influence of skin pigmentation, cholesterol, and other variables. Uncontrollable sun exposure the previous summer is a confounding factor when analyzing these parameters.

Group 3: nine matched pairs. A total of 18 participants (Table 1) consisting of 9 pairs of dark- (skin types V–VI (Fitzpatrick, 1988)) and fair-skinned participants (skin types I–IV (Fitzpatrick, 1988)) were selected from group 2. The nine pairs were matched according to ''identical'' baseline 25(OH)D levels (Table 2), and the group was created to confirm the lack of relation between skin pigmentation and vitamin D production found in group 2.

Inclusion criteria. Inclusion criteria for all participants were: (1) age 18–51 years; (2) willingness to avoid sun-bed exposure; (3) holidaying south of 45° latitude after 1 November 2007; and (4) avoiding supplementation of vitamin D 2 months before the study and during the study period.

Exclusion criteria. The participants were not admitted to the study if they had (1) sun-bed use or holidays in sunny countries (at latitudes below 45°) after 1 November 2007; (2) supplementary vitamin D ingestion above the level in a multivitamin tablet (10 μg per day); (3) skin disease; (4) psychological disease; (5) drug addiction; (6) intake of medication which causes photosensitive skin; (7) intake of cholesterol-lowering medicine; or (8) if they were pregnant.

Ethics. The ethics committee approved the study protocol (H-B-2007–100) and the study was carried out in accordance with the Declaration of Helsinki. All participants gave a written informed consent.

Skin type, skin pigmentation, and skin redness percent

To follow skin response to the UVB radiation, the redness percent and PPF was measured on the back, abdomen, and buttocks at baseline and before every UVB session by a skin reflectance meter (UV-Optimize, Scientific, Chromo-light, Espergaerde, Denmark, measuring range 1.0–24.0 (Wulf, 1986)). Self-reported skin type according to Fitzpatrick was also registered at baseline (Fitzpatrick, 1988). PPF is objectively measured skin type (predicted number of SED to MED (minimal erythema dose)).

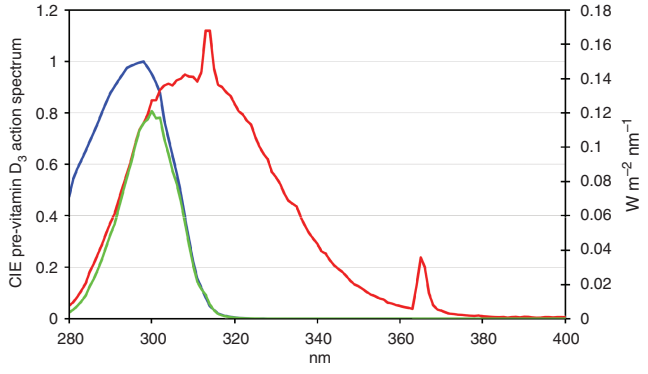

Figure 6. Red line: UV spectrum for a TL 12 UV lamp. Blue line: Action spectrum for the production of pre-vitamin D_3 in humans (CIE 174 Technical Report, 2006). Green line: CIE pre-vitamin D_3 weighted UV action spectrum of the TL 12 UV lamp.

Irradiation

A broadband UVB radiation source consisting of a bank of Philips TL12 tubes (280–350 nm, Philips, Eindhoven, The Netherlands) was used for irradiation (Figure 6). The UV source could irradiate all body surface areas with equal intensity. The UV-intensity was controlled weekly during the treatment period using a Sola-Hazard spectroradiometer (Solatell, Cornwall, UK). The participants had four UVB exposures to the chest and back (24% body surface (Augustsson *et al.*, 1992)). Each exposure of 3 SEDs (equivalent to a physical dose of 109.8 mJ cm^{-2}) was given during 10 minutes with 2 or 3 days' interval (that is: Mon, Wed, Fri, Mon). 1 SED is defined as 10 mJ cm^{-2} at 298 nm using CIE (Commission on the Environment) erythema action spectrum and it is the UV dose that elicits just perceptible erythema in the most sensitive person in a group of very sun-sensitive healthy persons (Lock-Andersen *et al.*, 1996).

Biochemical analyses

Blood samples were analyzed at baseline for ionized calcium, parathyroidea hormone, alkaline phosphatase, total cholesterol, and 25(OH)D. 25(OH)D levels were both measured at baseline and at 2 days after the fourth and last UVB session. The blood samples were taken by venipuncture and were centrifuged (5,000 × *g* in 10 minutes) within 2 hours of sampling.

Vitamin D. The serum samples for 25(OH)D analysis were frozen, stored at −80 °C, and sent on dry ice to the biochemical laboratory for 25(OH)D analysis by liquid chromatography-tandem mass spectrometry. To minimize the variance of 25(OH)D, two serum samples from each subject were included and each 25(OH)D analysis was performed twice. The analysis was calibrated with five in-house-prepared calibrators. The total relative SD for double determinations was 8.5% and the interseries relative SD were 14.1% at 20 nmol l^{-1}, 4.4% at 50 nmol l^{-1}, and 4.9% at 222 nmol l^{-1}.

Parathyroid hormone. Serum PTH was determined on Immulite 2500 biochemistry analyzer (Diagnostic Products Corporation, Los Angeles, CA). It is based on a chemiluminescence immunometric assay with a detection limit of 0.3 pmol l^{-1} and is specific for PTH. The intraseries variance is 15.0% at the 2.4 pmol l^{-1} level, 10% at 6.3 pmol l^{-1}, and 12.0% at 22.8 pmol l^{-1}.

Ionized calcium. Ionized calcium was measured in serum on an ABL700 blood gas analyzer (Radiometer a/s, Brønshøj, Denmark) using the E733 ion-selective electrode. The intraseries variance is 0.7% and the interseries variance is 1.2% at 1.6 mmol l^{-1}.

Alkaline phosphatase. Serum alkaline phosphatase was measured on a VITROS 5.1 FS (Ortho Clinical Diagnostics, Raritan, NJ) using VITROS ALKP slides and VITROS calibrator kit 3. The intraseries variance is 1.8% and the interseries variance is 1.5% at 107 U l^{-1}.

Total cholesterol. Serum total cholesterol was measured on a VITROS 5.1 FS (Ortho Clinical Diagnostics, Raritan, NJ) using VITROS slides methods and VITROS calibrator kit 2. The intraseries variance is 1.1% and the interseries variance is 1.6% at 3.7 mmol l^{-1}.

Statistical analysis

The data were statistically handled using SPSS 17.0 for Windows (SPSS, Chicago, IL). The relationship between Δ25(OH)D and sex, age, height, weight, number of fish meals per week, body mass index, baseline 25(OH)D level, biochemical parameters, PPF, and redness percent was examined using multiple linear regression analysis with all independent variables entered into a forward model. Comparing Δ25(OH)D and sex, we used Mann–Whitney test. Comparing the dark-skinned group with the fair-skinned group (matched pairs) according to baseline 25(OH)D level, we used Wilcoxon signed rank test. The significance limit was $P<0.05$. Data were considered outliers if they were outside 3 SD of the regression line.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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