KATJA VÄHÄVIHU

Heliotherapy and Narrow-band UVB Improve Vitamin D Balance

Studies in healthy subjects and in patients with atopic dermatitis and psoriasis

ACADEMIC DISSERTATION
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for public discussion in the Jarmo Visakorpi Auditorium,
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To Jukka, Lauri and Anna

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1. LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by Roman numerals I-IV:

I Vähävihu K, Ylianttila L, Salmelin R, Lamberg-Allard C, Viljakainen H, Tuohimaa P, Reunala T, Snellman E. Heliotherapy improves vitamin D balance and atopic dermatitis. *Br J Dermatol* 2008; **158**:1323-8.

II Vähävihu K, Ylianttila L, Kautiainen H, Tuohimaa P, Reunala T, Snellman E. Spore film dosimeters are feasible for UV dose monitoring during heliotherapy. *Photochem Photobiol* 2010; Jun 21 [Epub ahead of print].

III Vähävihu K, Ylianttila L, Kautiainen H, Viljakainen H, Lamberg-Allard C, Hasan T, Tuohimaa P, Reunala T, Snellman E. Narrow-band UVB course improves vitamin D balance in women in winter. *Br J Dermatol* 2010; **162**:848-53.

IV Vähävihu K, Ala-Houhala M, Peric M, Karisola P, Kautiainen H, Hasan T, Snellman E, Alenius H, Schauber J, Reunala T. Narrow-band UVB treatment improves vitamin D balance and alters antimicrobial peptide expression in skin lesions of psoriasis and atopic dermatitis. *Br J Dermatol* 2010; **163**:321-8.

2. ABBREVATIONS

AD	atopic dermatitis
CIE	Commission Internationale de l´Éclairage
D2	ergocalcipherol
D3	cholecalcipherol
HBD-2	human β-defensin 2
HT	heliotherapy
IARC	International Agency for Research on Cancer
MED	minimal erythemal dose
mRNA	messenger ribonucleic acid
NB-UVB	narrow-band ultraviolet B
1,25(OH)D	1,25-dihydroxyvitamin D, calcitriol
25(OH)D	25-hydroxyvitamin D, calcidiol
PASI	psoriasis area and severity index
PCR	polymerase chain reaction
PS	psoriasis
PTH	parathyroid hormone
PUVA	psoralen plus UVA phototherapy
RB meter	Robertson Berger meter
RIA	radioimmunoassay
SCORAD	severity scoring of atopic dermatitis
SED	standard erythema dose
SUP	selective ultraviolet phototherapy
UV	ultraviolet
UVA	ultraviolet A
UVB	ultraviolet B
UVC	ultraviolet C
DBP	vitamin D binding protein
VDR	vitamin D receptor

3. ABSTRACT

At present vitamin D insufficiency is common worldwide. In the Nordic countries and Britain this condition affects people especially during winter, when vitamin D synthesis induced by sunligt is zero. Solar UV exposure is crucial for vitamin D synthesis and as much as 90 % of all requisite vitamin D is formed in the skin. The desirable concentration of serum calcidiol (25(OH)D, 25-hydroxyvitamin D), which is the best indicator of vitamin D status, is still under debate but a concentration of 50 – 80 nmol/L is considered to be optimal for the skeleton. Calcitriol (1,25(OH)D, 1,25-dihydroxyvitamin D), the active form of vitamin D produced in the liver and kidney, but also in other tissues, such as the skin or prostate, is considered to be an autocrine or paracrine hormone, which regulates various cellular functions including cell growth. Due to this, vitamin D insufficiency seems to have far more extensive consequences for health than previously thought, ranging from well-known bone disease to prostate and other cancers and even to autoimmune diseases.

The amount of UVB exposure needed to induce vitamin D synthesis has been poorly studied. The present studies aim to examine the effects of natural sunlight during heliotherapy and artificial narrow-band UVB (NB-UVB) on vitamin D balance in winter.

Heliotherapy (HT) has long been used in the treatment of psoriasis (PS) and atopic dermatitis (AD), but its effect on vitamin D balance has not been previously examined. In the first study (I) we examined serum calcidiol concentrations in 23 patients with AD during a two-week HT course in the Canary Islands. At onset, 74 % of the AD patients had vitamin D insufficiency (serum calcidiol < 50 nmol/L). The median personal UVB dose received in the January HT group was 60 SED (standard erythema dose) and in the March group 109 SED. Serum calcidiol increased significantly both in the January (13.4 nmol/L) and March (24.0 nmol/L) groups. The calcidiol level remained elevated for at least 1 – 2 months. HT significantly improved SCORAD (severity scoring of atopic dermatitis), by 70 % in both groups, but only in the March group was there clear correlation between the increase in serum calcidiol and the improvement of SCORAD.

In the second study (II) we compared two methods, individual *Bacillus subtilis* spore film UV dosimeters and a Robertson Berger meter (RB meter) combined with personal diary records, in measurement of UVB doses received during a two-week HT course in the Canary Islands. The

mean personal UVB dose measured in a total of 21 AD patients with dosimeters was 75 SED in the January and 131 SED in the March group. The respective results from the RB meter combined with diary records were 63 SED and 119 SED. Serum calcidiol concentration increased by 9.7 nmol/L in the January and by 26.0 nmol/L in the March group. The increase in serum calcidiol correlated significantly with the UVB dose received. The results of the personal dosimeters and the RB meter combined with diary records showed a strong concordance correlation ($r = 0.63$), indicating that *s*pore film dosimetry is a reliable and easy way to measure personal UVB doses during heliotherapy.

In the third study (III) we examined whether NB-UVB exposures, used widely in the treatment of PS and AD and emitting UVB near the range of vitamin D synthesis (311-313 nm), improves vitamin D balance. Fifty-three healthy women received NB-UVB exposures either on the whole body (n = 19), on the head and arms (n = 9) or on the abdomen (n = 14). The exposures were given on seven consecutive days. Similarly, seven solar simulator exposures were given on the face and arms (n = 11). The cumulative UVB dose was 13 standard erythemal units (SED) in all regimens. At onset 77 % of the women suffered from vitamin D insufficiency and, in addition, six subjects from vitamin D deficiency (serum calcidiol < 25 nmol/L). Calcidiol concentration increased significantly in all study groups. When receiving NB-UVB exposures only on the head and arms, the increase in serum calcidiol was nearly the same as when the whole body was exposed, 11.0 nmol/L and 11.4 nmol/L, respectively. NB-UVB exposure on the abdomen increased calcidiol by 4.0 nmol/L and solar simulator exposures given on the face and arms by 3.8 nmol/L. The effect of NB-UVB exposures was long-lasting. After two months, serum calcidiol was still higher than initially in the group that was followed up, i.e. the group receiving NB-UVB exposures on the whole body.

In the fourth study (IV) we measured the changes in serum calcidiol after six (12.3 SED) and 15 NB-UVB (71.5 SED) treatments given during winter in 18 patients with psoriasis, 18 patients with AD and 15 healthy subjects. In addition, using skin biopsies we studied the effects of exposures on antimicrobial peptides, cytokines and chemokines by PCR techniques. The NB-UVB exposures were given three times a week for a total of 15 times on the whole body. Skin biopsies were taken before the treatment and after six exposures. Before treatment 89 % of the patients with PS, 94 % of the patients with AD and 53 % of the healthy subjects had vitamin D insufficiency. NB-UVB treatment produced a statistically significant ($p < 0.001$) increase in serum calcidiol, the increase being 59.9 nmol/L in PS, 68.2 nmol/L in AD and 90.7 nmol/L in healthy subjects. The PASI

(psoriasis area and severity index) and SCORAD improved significantly, but no correlation with the increase of serum calcidiol was found. The expression of two antimicrobial peptides, cathelicidin and human β-defensin-2 (HBD-2), was high in PS skin lesions. The expression of HBD-2 decreased in NB-UVB-treated PS and AD skin lesions, but the expression of cathelicidin increased. This effect might be mediated by improved vitamin D balance and the local cytokine network.

To conclude, the present studies in healthy subjects and patients with AD and PS show that vitamin D insufficiency is still prevalent in Finland in winter. Sunlight during heliotherapy or small doses of NB-UVB, which are clearly below the skin's erythemal threshold, effectively induced vitamin D synthesis, and, at the same time, improved the clinical course of AD and PS. NB-UVB exposures given on the whole body or on the head and arms were equally effective in increasing serum calcidiol. The NB-UVB exposures, given three times a week for a total of 15 times on the whole body, effectively corrected vitamin D insufficiency. Serum calcidiol increased up to 59.9 – 90.7 nmol/L and this high level persisted for at least one month. Suberythemal NB-UVB exposures seem to be a good alternative to correct vitamin D deficiency or insufficiency quickly and safely, but further studies are warranted to compare the effects and costs with dietary vitamin D supplementation.

TIIVISTELMÄ

D-vitamiinin vajaus on yleinen ilmiö maailmanlaajuisesti. Pohjoismaalaiset ja englantilaiset kärsivät D-vitamiinivajauksesta etenkin talviaikaan, koska silloin auringonvaloa ei ole riittävästi D-vitamiinisynteesiä varten. Jopa 90 % elimistömme D-vitamiinivarastoista on tuotettu ihossa auringon UVB säteilyn vaikutuksesta. D-vitamiini hydroksyloituu maksassa kalsidioliksi (25(OH)D), joka kuvastaa parhaiten elimistömme D-vitamiinitasoa. Kalsidiolin 50 -80 nmol/L:n pitoisuutta seerumissa pidetään tällä hetkellä tarpeellisena riittävän luustoaineenvaihdunnan takaamiseksi. Kalsidioli hydroksyloituu munuaisissa edelleen kalsitrioliksi (1,25(OH)D), joka on D-vitamiinin hormonaalisesti aktiivinen muoto. Viimeaikaisen tutkimustiedon mukaan myös useat muut kudokset, kuten iho ja eturauhanen, voivat tuottaa D-vitamiinin esiasteista kalsitriolia. Kalsitrioli toimii paikallisesti autokriinisen tai parakriinisen hormonin lailla ja säätelee solujen eri toimintoja, kuten kasvua. Näyttääkin siltä, että D-vitamiinilla on huomattavasti laajemmat vaikutukset terveyteen kuin mitä aikaisemmin on ajateltu. Luuston aineenvaihdunnasta huolehtimisen lisäksi riittävä D-vitamiinin saanti näyttää ehkäisevän mm. useita eri syöpätyyppejä ja mahdollisesti myös autoimmuunisairauksia.

Kirjallisuudessa on vain vähän tietoa siitä, kuinka paljon UVB säteilyä tarvitaan ihon D-vitamiinisynteesin käynnistämiseksi. Tässä työssä tutkimme, miten ilmastokuntoutuksen aikana saatu auringonvalo sekä ihotautien hoidossa käytettävä kapeakaistainen UVB valo (NB-UVB) vaikuttavat elimistön D-vitamiinitasapainoon.

Ilmastokuntoutus on vakiintunut hoitomuoto psoriaasi- ja atopiapotilaille. Aikaisemmin ei kuitenkaan ole tutkittu, onko sillä vaikutuksia elimistön D-vitamiinitasapainoon. Ensimmäisessä työssä (I) mittasimme seerumin kalsidiolitason ennen kahden viikon ilmastokuntoutusta Kanarian saarilla sekä ilmastokuntoutuksen jälkeen. Tutkimukseen osallistui 23 atooppista ihottumaa sairastavaa potilasta. Ennen ilmastokuntoutusta 74 %:lla potilaista oli D-vitamiinin vajausta eli seerumin kalsidiolipitoisuus oli alle 50 nmol/L. Tammikuussa kuntoutusmatkalla auringosta saatu UVB-annos oli keskimäärin 60 SED (standard erythema dose) ja maaliskuussa 109 SED. Molemmissa ryhmissä seerumin kalsidiolitaso nousi tilastollisesti merkitsevästi, tammikuun ryhmässä keskimäärin 13.4 nmol/L ja maaliskuun ryhmässä 24.0 nmol/L. Tämä vaste säilyi ainakin 1 -2 kuukauden ajan. Ihottuma parani molemmissa ryhmissä tilastollisesti merkitsevästi. Atooppisen ihottuman kliininen indeksi, SCORAD (severity scoring of atopic dermatitis), laski 70

%. Maaliskuun ryhmässä ihottuman paranemisen ja seerumin kalsidiolitason nousun välillä todettiin selvä korrelaatio.

Toisessa työssä (II) verrattiin kahta eri menetelmää auringonvalosta saadun UVB-annoksen mittaamisessa kahden viikon ilmastokuntoutuksen aikana Kanarian saarilla. Tutkimuksessa oli 21 atooppista ihottumaa sairastavaa potilasta, jotka käyttivät henkilökohtaisia annosmittareita (*Bacillus subtilis* spore film UV dosimeter). Tämän lisäksi he täyttivät yksityiskohtaista auringonottopäiväkirjaa, jonka tiedot yhdistettiin Robertson Berger mittarilla (RB-mittari) päivittäin mitattuihin UVB annoksiin. Henkilökohtaisilla annosmittareilla saatu UVB annos oli tammikuun ryhmässä keskimäärin 75 SED ja maaliskuun ryhmässä 131 SED. Vastaavat annokset RB-mittaria ja päiväkirjamerkintöjä käyttäen olivat 63 SED ja 119 SED. Seerumin kalsidiolitason nousu oli tammikuussa 9.7 nmol/L ja maaliskuussa 26.0 nmol/L. Nousut korreloivat tilastollisesti merkitsevästi saatuun UVB-annokseen. Molempien UVB mittausmenetelmien välillä oli vahva korrelaatio ($r = 0.63$), joten henkilökohtaisia annosmittareita voidaan jatkossa käyttää hyödyksi mitattaessa saatua UVB:n määrää ilmastokuntoutuksen tai muun auringonoton aikana.

Kolmannessa työssä (III) tutkimme, parantaako psoriasiksen ja atooppisen ihottuman hoidossa käytettävä kapeakaistainen UVB säteily (NB-UVB) elimistön D-vitamiinitasoa. NB-UVB säteilyn aallonpituusalue (311 – 313 nm) on lähellä D-vitamiinisynteesin kannalta optimaalista aallonpituusaluetta. Tutkimuksessa oli 53 tervettä naista, jotka saivat NB-UVB valotuksia joko koko keholle (n = 19), pään ja käsivarsien alueelle (n = 9) tai vatsan alueelle (n = 14). Valotukset annettiin seitsemänä peräkkäisenä päivänä ja jokainen ryhmä sai 13 SED:n kokonais-UVB-annoksen. Ennen valotuksia 41:llä (77 %) naisella oli D-vitamiinin vajaus, ja lisäksi kuudella oli D-vitamiinin puutos (kalsidioli < 25 nmol/L). Seerumin kalsidiolipitoisuus nousi tilastollisesti merkitsevästi kaikissa kolmessa tutkimusryhmässä. Kalsidiolipitoisuus nousi lähes yhtä paljon riippumatta siitä, annettiinko NB-UVB altistus koko vartalolle (nousu 11.4 nmol/L) vai ainoastaan pään alueelle ja käsivarsille (nousu 11.0 nmol/L). Vatsan alueen valotus nosti seerumin kalsidiolipitoisuutta 4.0 nmol/L ja aurinkosimulaattorilla annettu valo kasvojen ja käsivarsien alueelle 3.8 nmol/L. Seurantanäytteet otettiin koko keholle NB-UVB:tä saaneesta ryhmästä ja heillä seerumin kalsidiolipitoisuus oli vielä kahden kuukauden kuluttua kohonneella tasolla.

Neljännessä työssä (IV) tutkittiin, miten NB-UVB valohoito vaikuttaa 18 psoriaasipotilaan, 18 atopiapotilaan ja 15 terveen tutkimushenkilön D-vitamiinitasapainoon. NB-UVB-valotukset annettiin kolmesti viikossa yhteensä 15 kertaa koko vartalolle. Kalsidiolipitoisuudet määritettiin ennen valohoitoa sekä kuuden (12.3 SED) ja 15 valotuksen (71.5 SED) jälkeen. Lisäksi

ihonäytepaloista tutkittiin PCR tekniikkaa käyttäen, miten 6 valotusta vaikuttaa psoriaasi- ja atopia-leesioiden antimikrobiaalisiin peptideihin, sytokiineihin ja kemokiineihin. Ennen valotuksia 89 %:lla psoriaasipotilaista, 94 %:lla atopiapotilaista ja 53 %:lla terveistä tutkimushenkilöistä oli D-vitamiinivajaus. Seerumin kalsidiolitaso nousi 15 NB-UVB-valotuksen jälkeen tilastollisesti merkitsevästi. Nousu oli 59.9. nmol/L atooppisessa ihottumassa, 68.2 nmol/L psoriaasissa ja 90.7 nmol/L terveillä tutkimushenkilöillä. Ihottuman kliiniset indeksit, PASI (psoriasis area and severity index) ja SCORAD, paranivat tilastollisesti merkitsevästi, mutta korrelaatiota D-vitamiinitason nousuun ei voitu todeta.

Kahden antimikrobiaalisen peptidin, cathelicidinin ja human β-defensin-2 (HBD-2):n, ilmentyminen todettiin korkeaksi psoriaasi ihottumassa. Sekä psoriaasissa että atooppisessa ihottumassa kapeakaistainen UVB-valohoito vähensi HBD-2:n tuotantoa. Sen sijaan cathelicidinin tuotanto lisääntyi, mihin parantuneella D-vitamiinitasolla ja sytokiineilla saattaa olla osuutensa.

Yhteenvetona voidaan todeta, että nämä tutkimukset osoittavat suomalaisten edelleen yleisesti kärsivän talviaikaisesta D-vitamiinin vajauksesta. Ilmastokuntoutuksessa saatu auringonvalo sekä pieniannoksinen kapeakaista-UVB käynnistivät tehokkaasti ihon D-vitamiinisynteesin ja samalla paransivat atooppista ihottumaa ja psoriaasia. D-vitamiinisynteesiin riittää huomattavasti ihon punekynnystä pienempi UVB annos. Pään alueen ja käsivarsien alueelle seitsemän kertaa annettu kapeakaistainen UVB valotus oli yhtä tehokasta D-vitamiinituotannon kannalta kuin vastaava koko vartalon valotus. Erittäin tehokas elimistön D-vitamiinivajausta korjaava valohoito oli koko keholle annettu 15 NB-UVB:n sarja, joka nosti seerumin kalsidiolipitoisuutta 59.9 – 90.7 nmol/L. Kohonneet kalsidiolitasot olivat lähes ennallaan kuukauden kuluttua hoidon päätyttyä. Näin ollen pieniannoksinen NB-UVB-valotusten sarja voisi olla tehokas ja turvallinen vaihtoehto korjata D-vitamiinin puutosta. Jatkossa tarvitaan vielä tutkimuksia, jotka selvittävät NB-UVB-valotuksen tehokkuutta ja kustannuksia verrattuna tavanomaiseen, suun kautta annettavaan D-vitamiinikorvaushoitoon.

4. INTRODUCTION

Vitamin D is a precursor of a group of hormones which regulate calcium homeostasis and, according to recent studies, are also involved in the regulation of various cellular functions including cell growth (Solvsten 2000). Recently, an association between vitamin D insufficiency and various chronic diseases such as many cancers and autoimmune diseases has been described (Deluca and Cantorna 2001, Arnson et al. 2007, Holick 2007, Tuohimaa 2009, Bischoff-Ferrari 2010). Thus, it is alarming that vitamin D insufficiency seems to be a worldwide epidemic (Holick and Chen 2008). In the Nordic countries it affects people especially during the winter months, when UVB radiation from the sun is negligible (Lamberg-Allardt et al. 2001, Burgaz et al. 2007, Välimäki et al. 2007).

Vitamin D can be obtained by consuming foods rich in vitamin D, such as fish and vitamin D-fortified milk products, or by using vitamin D supplements. However, as much as 90 % of all requisite vitamin D is formed in the skin where solar UV exposure starts synthesis of vitamin D from its precursors (Holick et al. 1980, Lehmann 2005). The literature offers little data concerning the effect of sunshine or artificial UVB exposure on vitamin D balance. Since the same UVB wavelengths of sunlight are critical for vitamin D synthesis and at the same time increase the risk for skin malignancies, it is important to study the effects of solar and artificial UVB exposure on vitamin D balance.

Heliotherapy (HT) is one efficient treatment modality used for psoriasis (PS) and atopic dermatitis (AD) (Snellman et al. 1993, Autio et al. 2002). The UVB doses received during HT for AD have not been studied earlier and we assessed these doses in parallel with effects of HT on vitamin D balance and improvement of AD. Another treatment modality frequently used today for PS and AD is narrow-band UVB (NB-UVB) (Karvonen et al. 1989, Hjerppe et al. 2001). The output of NB-UVB lamps (311-313 nm) is close to the optimal wavelength for vitamin D synthesis, i.e. 297 nm (Holick et al. 1980). Previous *in vivo* studies concerning artificial UVB exposure and vitamin D synthesis are scarce and have mostly used broad-band UVB equipment (Guilhou et al. 1990, Chel et al. 1998, Chuck et al. 2001). Our study aimed to find out whether NB-UVB exposures could improve vitamin D balance in healthy subjects and in patients with PS and AD and thus might serve as one method of treatment for vitamin D insufficiency. Vitamin D has an impact on expression of antimicrobial peptides, such as cathelicidin and human β-defensin-2, which are important mediators

of innate immune defence in the skin (Wang et al. 2004). Accordingly we also studied the effect of NB-UVB exposures on the expression of antimicrobial peptides in the skin lesions of PS and AD.

5. REVIEW OF THE LITERATURE

5.1. Historical background of vitamin D

The discovery of vitamin D was closely tied to research on the cause and prevention of childhood rickets. The first scientific description of rickets was provided in the 17th century by Daniel Whistler (1645) and Francis Glisson (1650), as reviewed by Rajakumar et al. (2007). Rickets lead to deformities of the skeleton, such as bowed legs and deformed pelvis. It became an epidemic especially among city dwellers in Europe and Northern America with the growth of industrialism in the 19th century.

The link between sunlight and rickets was first recognized in 1822 by a Polish scientist, Jerdzej Sniadecki, as cited by Mozolowsky (1939). Sniadecki reported that children living in the inner city of Warsaw had a higher incidence of rickets than the children outside the city. He recommended direct exposure to sunlight to prevent and treat rickets. However, little attention was given to the environment as a cause of rickets. In 1890, Palm et al. found that rickets was widespread in the industrial centres of Great Britain, whereas people living with poor nutrition in China, Japan and India were seldom affected by the disease (Palm 1890). He urged the systematic use of sunbaths as a preventive and therapeutic treatment for rickets. However, it was difficult for people to believe that such a simple remedy as exposure to sunlight could cure this bone-deforming disease.

At the same time, in the late 19th century, phototherapy was introduced by Finsen who reported the successful use of UV radiation in the treatment of cutaneous tuberculosis (Finsen 1901). Since 1905, phototherapy has also been used in the treatment of psoriasis and atopic dermatitis (Albert and Ostheimer 2002). The effect of artificial UV light on rickets was also studied. Huldschinsky (1919) reported that exposure of children to radiation from a quartz mercury lamp (emitting UV light) for one hour three times a week was effective in treating rickets.

In parallel with observations of the relation between sun and rickets, scientists searched for specific foods that could prevent rickets. Already in the 19th century it was a common practice to give children cod liver oil to prevent and cure rickets. In Finland, Elias Lönnroth recommended cod liver oil for children in his book "Suomalaisen Talonpojan Koti-Lääkäri" published in 1839. The first scientific report considering rickets to be a nutritional deficiency disease was made by Mellanby in 1918 (Mellanby 1919). He reported that he could make beagle dogs rachitic and reverse the bone

disease with cod liver oil. At first it was thought that the antirachitic factor in cod liver oil was vitamin A (McCollum and Davis 1914), but in 1922 McColum et al. (1922) demonstrated that the antirachitic activity was separate from it. They exposed cod liver oil to heat and oxygen to destroy the vitamin A activity and showed that it still cured the disease. This new component in cod liver oil known to prevent rickets was designated as vitamin D as it was the fourth vitamin discovered after vitamins A, B and C.

The fact that both UV light and a dietary factor could have similar effects on the disease was difficult to reconcile. In 1922, Hess and Unger found that sunlight prevented rickets in rats with a rachitic diet. The following year Hess et al. (1925) and Steenbock et al. (1924) found that certain foods became antirachitic after UV irradiation and that irradiated milk could prevent rickets in children. Hess hypothesized that vitamin D was formed in the skin after UV exposure and that vitamin D was a sterol. Subsequently the precursor of vitamin D was identified as 7-dehydrocolesterol by the co-work of Hess, Windaus and Rosenheim (Wolf 2004). In further investigations in 1926 the chemical structure of vitamin D was identified (Wolf 2004), and in 1928 Adolf Windaus was awarded a Nobel prize for his studies on sterols and vitamin D.

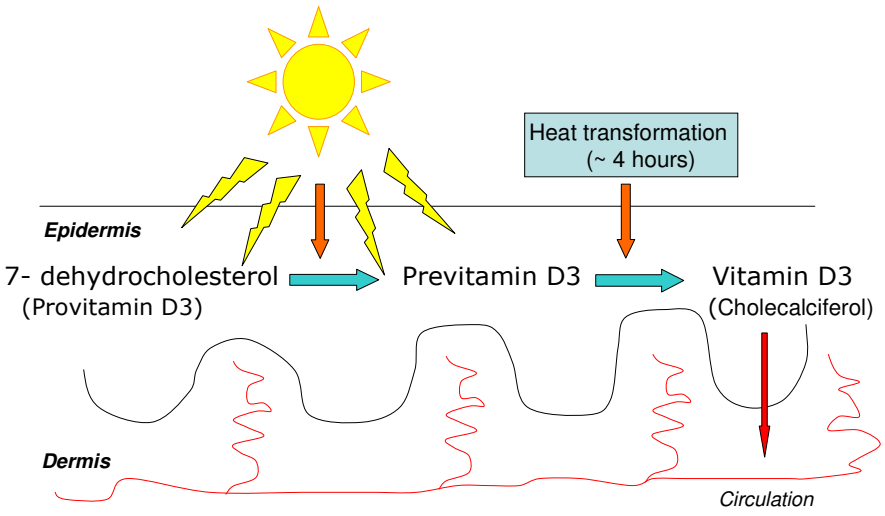
The discovery by Hess et al. (1925) and Steenbock et al. (1924) that UV-irradiated food, in particular whole milk containing butter fat, had an antirachitic potency led to tremendous advances in public health. In the USA as early as the 1930s milk products were irradiated to fortify milk with vitamin D. Once the structure of vitamin D had been characterised and a simple process developed for its synthesis, vitamin D was added directly to milk. This resulted in a rapid decline in the prevalence of rickets in children. In Finland, vitamin D supplements have been systematically given to children since the 1930s, first as cod liver oil. The dietary guidelines on vitamin D for children aged less than 3 years have changed over the years, from 60-100 µg in 1940 (Ala-Houhala et al. 1995) to 10 µg in 1996. Margarines and spreads have been fortified since 1940 with vitamin D (7.5 µg/100 g). As several studies showed vitamin D insufficiency in Finnish people especially in winter (Lamberg-Allardt et al. 2001, Välimäki et al. 2004) the Ministry of Social Affairs and Health recommended fortification of liquid milk products with 0.5 µg/100 g and margarines and spreads with 10 µg/100 g in 2003.

5.2. Vitamin D

5.2.1. Photosynthesis and dietary sources of vitamin D

Solar UV exposure is the major source of vitamin D3 and as much as 90 % of all requisite vitamin D3 is formed in the skin (Holick et al. 1980, Lehmann 2005). A total of 5 % of the solar UV light reaching the earth's surface is UVB radiation (290-315 nm), which induces photosynthesis of vitamin D3 in the skin (Figs. 1 and 3). The wavelength 297 nm has proved to be most effective in inducing vitamin D synthesis (Holick et al. 1980). Keratinocytes and fibroblasts contain 7-dehydrocholesterol in their cell membranes known as provitamin D3. The same molecule acts also as the precursor of cholesterol. Provitamin D3 effectively absorbs the high-energy UVB radiation penetrating into the skin. This energy is used to open the steroid ring system of provitamin D to form a 9,10-secosteroid known as previtamin D3 (Holick 1981 and 1994). Previtamin D3 is biologically inert and thermodynamically unstable. Due to heat its double bonds rearrange spontaneously to form vitamin D3 (cholecalciferol). This conversion occurs within four hours (Tian et al. 1993).

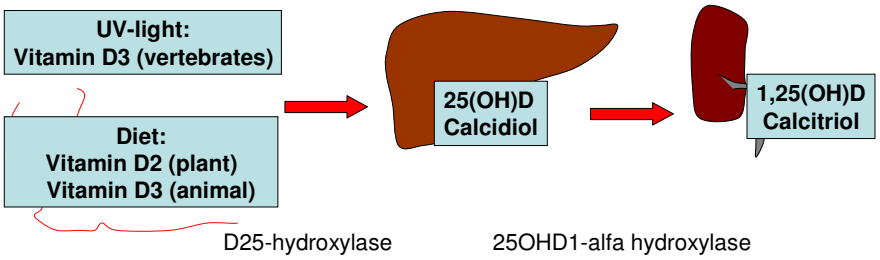
Figure 1. Photosynthesis of vitamin D3



Both previtamin D3 and vitamin D3 have the capacity to absorb UV radiation. This absorption, if sunlight exposure is prolonged, leads to isomerization of these molecules to form photoproducts, such as lumisterol and tachysterol (MacLaughlin et al. 1982) and 5,6-transvitamin D3, suprasterol I and II (Webb et al. 1989). These are inert in calcium metabolism but can later be converted back to vitamin D.

The newly synthesized vitamin D3 is biologically inert and is transported by a vitamin D binding protein to the liver to be hydroxylated to calcidiol (25(OH)D, 25-hydroxyvitamin D, Figs. 2 and 3) which is the major circulating form of vitamin D (Holick 1985, Holick 1994). This hydroxylation is catalysed by the 25-hydroxylase enzyme. The calcidiol concentration is used when analyzing the vitamin D balance of the body. A second hydroxylation is needed to convert calcidiol to calcitriol (1,25(OH)D, 1,25-dihydroxyvitamin D) which is conventionally regarded as the hormonally active form of vitamin D (Holick 1985 and 1994). However, in recent studies, also calcidiol seems to have hormonal activity (Lou et al. 2004, Tuohimaa et al. 2005). The second hydroxylation takes place in the kidney in the presence of 25OHD1-alfa hydroxylase enzyme, and this step is tightly regulated by a feedback system of calcitriol and by parathyroid hormone (PTH).

Figure 2. Metabolism of vitamin D.



In addition to the kidney, recent studies show that a number of other tissues like the skin and prostate cells are capable of locally hydroxylating calcidiol into calcitriol (Schwartz et al. 1998, Lehmann et al. 2004)

Factors influencing photosynthesis of vitamin D

Anything that decreases or prevents UVB photons from reaching the skin decreases the photosynthesis of vitamin D. Melanin and sunscreens are effective in absorbing UVB radiation (Clemens et al. 1982, Matsuoka et al. 1987). Thus an increase in pigmentation decreases cutaneous vitamin D production (Clemens et al. 1982). A proper application of SPF 8 sunscreen reduces the photosynthesis of vitamin D by at least 95 % (Matsuoka et al. 1987). Clothes (Matsuoka et al. 1992) and glass effectively hinder UVB rays from reaching the skin. Ageing decreases the thickness of the dermis and epidermis leading to a decline in provitamin D thus diminishing the photosythesis of vitamin D (Holick et al. 1989). The zenith angle of the sun varies with the latitude, season and time of day. The larger the zenith angle, the greater the propotion of UVB photons absorbed by the ozone layer and thus the smaller the number of photons reaching the earth's surface (Webb et al. 1988). At the latitude of Finland, only during summer months (May – Aug) is there enough UVB radiation to induce photosynthesis of vitamin D.

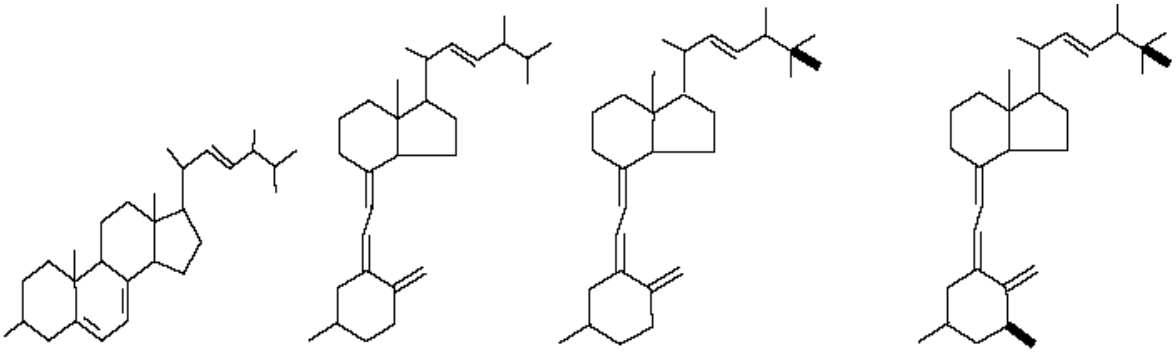
Dietary sources of vitamin D

'Vitamin D' is a generic name for a group of closely related secosteroids. The best-known forms of vitamin D are ergocalciferol (D2) and cholecalciferol (D3). The precursor of vitamin D2 is ergosterol which can be found in plant tissue, whereas vitamin D3 originates from the 7-dehydrocholesterol of vertebrates. Some wild-grown mushrooms and algae contain vitamin D2 (Outila et al. 1999). Vitamin D3 can be obtained from foods such as fish, liver and egg yolk. Farmed fish have a lower vitamin D3 content than wild fish (Lu et al. 2007).

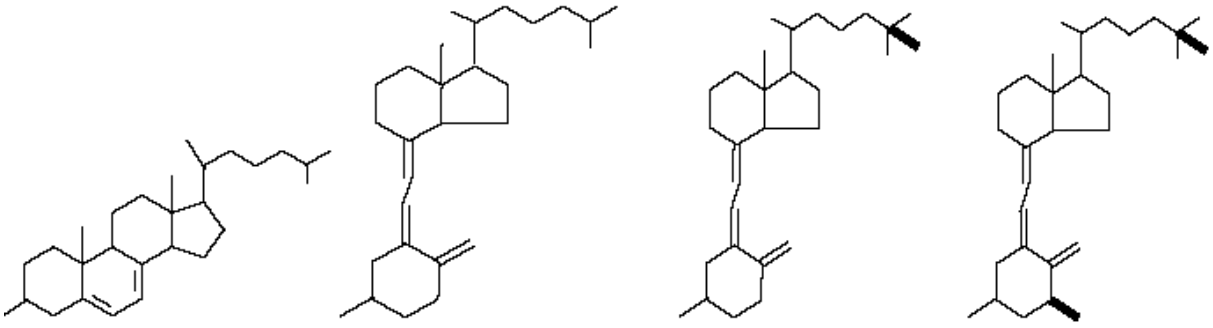
The two forms of vitamin D differ chemically in their side chains (Fig 3). Like the vitamin D3 that originates in the skin, dietary vitamin D2 and D3 are metabolized to form calcitriol (Fig 2 and 3). In the literature, a distinction is seldom made between calcidiol of D2 and D3 origin and neither did we make any distinction. Both forms are included in the assessment of serum calcidiol. Industrial vitamin D2 is produced by UVB irradiation of ergosterol obtained from yeast, while D3 is produced by UVB irradiation of 7-dehydrocholesterol obtained from lanolin from sheep's wool (Holick et al. 2008). Both are used in the fortification of food and in supplements. There are diverse opinions about the absorption of D2 and D3 and their efficacy in increasing serum calcidiol. Some consider

them equal in efficiency (Holick et al. 2008), while others consider vitamin D2 to have a markedly lower potency and shorter duration of action than vitamin D3 (Armas et al. 2004, Trang et al. 1998).

Figure 3. Chemical structure and the hydroxylated forms of vitamin D2 and D3.



Provitamin D2	Vitamin D2	25(OH)D2	1,25(OH)D2
Ergosterol	Ergocalciferol	Calcidiol	Calcitriol



Provitamin D3	Vitamin D3	25(OH)D3	1,25(OH)D3
7-dehydrocholesterol	Cholecalciferol	Calcidiol	Calcitriol

5.2.2. Vitamin D and calcium homeostasis

In the past decades, our knowledge about vitamin D3 and its biological activity has significantly developed and its classification as a vitamin is no longer valid. It acts like a hormone. Vitamin D carries out its physiological functions through its active metabolites, calcitriol, and, according to recent studies, also through calcidiol (Lou et al. 2004, Tuohimaa et al. 2005). The effects of these calcipherol hormones are mediated through the nuclear vitamin D receptor, VDR, which functions as a transcription factor and is represented in nearly all tissues (Solvsten 2000). VDR is a member of the superfamily of nuclear receptor. The receptors belonging to this family are all constructed in a similar manner, with a DNA binding domain close to the N-terminal and a ligand binding domain at the carboxy terminal (Solvsten 2000). For example, thyroid hormone receptors belong to the same subfamily as VDRs. They are both completely dependent on an exogenous supply of precursors, the thyroid hormone receptors on a supply of dietary iodine and the VDRs on sun-induced or dietary vitamin D (Tuohimaa 2009). Because VDRs are found in most cells of the body, the biological responses to vitamin D vary according to the cell.

The main – or at least the best known - function of vitamin D is to maintain serum calcium and phosphorus homeostasis. Calcitriol, once formed in the kidney, is transported by a vitamin D binding protein (DBP) to the small intestine, where it interacts with VDRs resulting in an increase in the efficiency of intestinal calcium absorption (Holick 2000). Without vitamin D calcium absorption occurs only through passive diffusion and the small intestine absorbs approximately 10 – 15 % of dietary calcium (Holick 2000). In the presence of sufficient calcitriol nearly 30 % of the calcium is absorbed. It has been suggested that the threshold for maximum calcium absorption capability is 80 nmol/l (Heaney et al. 2003). During periods of growth the increased calcium demand results in an increased production of calcitriol and the efficacy of intestinal calcium transport can increase to 60 – 80 % (Holick 2000). If not enough dietary calcium is available, calcitriol interacts with osteoblasts resulting in a mobilization of calcium stores from the skeleton.

Vitamin D deficiency causes an increase in the serum parathyroid hormone (PTH) concentration. PTH increases tubular reabsorption of calcium in the kidney, stimulates osteoclastic activity and causes increased loss of phosphorus in the urine. This results in defective mineralization of bones. Rickets, osteomalasia, osteoporosis and bone fractures are the well-known outcomes of vitamin D3 insufficiency or deficiency (Ruohola et al. 2006, Holick and Chen 2008).

5.2.3. Vitamin D insufficiency, cancer and chronic diseases

Occurrence and prevention of vitamin D insufficiency

The desirable concentration of serum calcidiol, which is the best indicator of vitamin D status, is still under debate, but a concentration of 50 – 80 nmol/L is considered to be optimal for the skeleton (Dawson-Hughes et al. 2005, Holick 2007). According to Dawson-Hughes et al. (2005), vitamin D deficiency is regarded as a serum concentration of calcidiol below 25 nmol/L and insufficiency below 50 nmol/L. Some studies suggest values between 40 – 60 nmol/L to be optimal (Tuohimaa et al. 2004, IARC. 2008), but others propose considerably higher concentrations, even over 100 nmol/L (Grant and Holick 2005a).

The need for a consensus about optimal calcidiol concentration is obvious, since at present vitamin D insufficiency is common worldwide (Holick and Chen 2008). In the Nordic countries and Britain this condition affects people especially during winter (Lamberg-Allardt et al. 2001, Burgaz et al. 2007, Hyppönen and Power 2007, Välimäki et al. 2007). Also working indoors during summer months hinders replenishment of vitamin D stores. It has been estimated that approximately one billion people worldwide are vitamin D deficient or insufficient (Holick 2007). However, when considering optimal calcidiol concentrations, many factors have to be taken into account. One problem arises in methodology, as calcidiol assessments may show huge variations between different laboratories (Hollis 2008, Wagner et al. 2009). There is also some evidence that both low and high concentrations of calcidiol may enhance development of certain diseases (IARC 2008, Tuohimaa et al. 2004 and 2009). Thus more studies are needed to confirm the desirable upper limit. Meanwhile a physiological concentration between 50 -80 nmol/L, which enhances the basic and best known function of vitamin D, calcium homeostasis, seems to be a safe goal, when considering optimal calcidiol concentrations.

At present, in Finland, the recommendation for dietary vitamin D intake is 7.5 µg a day for individuals aged 3-60 years (National Instititute for Health and Welfare, www.thl.fi). For children under 3 years old and persons over 60 years old as well as pregnant and lactating women and other risk groups the recommended intake is 10 µg. This (7.5 µg) can be achieved if the diet contains e.g. three glasses (600ml) of fortified milk and 5-6 slices of bread with fortified spreads daily and, in addition, at least two portions of fish dishes a week. For children less than three years old vitamin D supplements are recommended throughout the year.

Viljakainen et al. (2006) showed in a recent study that to maintain the serum calcidiol level at 45 – 60 nmol/L in elderly people in the absence of sunlight a daily intake of 15 µg of vitamin D is needed. The study found that the vitamin D balance attained equillibrium after six weeks of supplementation. Consumption of 20 µg of vitamin D per day leads to a concentration of 70 nmol/L (Viljakainen et al. 2006, Nelson et al. 2009). As vitamin D is fat-soluble, the safety is a concern. Dietary vitamin D can accumulate in the body, whereas the production of vitamin D in the skin following sun exposure is such a well-regulated system that overdosing is not possible. Vitamin D intoxication has been described with serum concentrations exceeding 200 nmol/L (Vieth 1999). A daily intake of 50 µg has been proposed as the safe upper limit for vitamin D (Scientific Committee on Food 2002) However, Vieth et al. (2001) demonstrated that after three months of daily use of D3 supplements with doses of 25 µg or 100 µg, serum calcidiol concentrations plateaued at 69 nmol/L and 96 nmol/L, respectively, and no significant changes in serum calcium and urinary calcium excretion were observed. Even a daily dose of 250 µg did not cause toxic effects in adults (Hathcock et al. 2007). In school children a daily dose of 50 µg was well tolerated and no side effects were observed in a one-year study (Maalouf et al. 2008). No long term studies are available about other possible side-effects of vitamin D supplements when high doses are used for many years.

Vitamin D insufficiency, cancers and chronic diseases

Besides the cells of the small intestine and the osteoblasts, VDRs have been found in a number of other tissues and cells, including the skin, prostate, breast, colon, brain, heart, pancreas, muscle and immune cells (Holick 2004 and 2007). Epidemiological studies suggest that vitamin D and exposure to UVB light reduce the risk of nearly 20 types of cancer (Grant and Holick 2005b, Grant 2007). Most evidence is found concerning colorectal, prostate and breast cancers (Guyton et al. 2003). Interestengly, the risk of prostate cancer associated with serum calcidiol is U-shaped (Tuohimaa et al. 2004), in other words, both low and high concentrations increase the risk of prostate cancer. A similar discovery has been made concerning ageing (Tuohimaa 2009). However, despite increasing evidence on the connection between cancers and vitamin D levels, in a recent report of the IARC Working Group (International Agency for Research on Cancer, 2008) only the connection to colorectal cancers was confirmed.

In addition to osteoporosis and cancers, associations between vitamin D and a number of other diseases have been described. As reviewed by Lee et al. (2008), Tuohimaa (2009), and Bischoff-Ferrari (2010), it has been demonstrated that vitamin D insufficiency can increase the risk of muscle weakness (Bischoff-Ferrari et al. 2004, Montero-Odasso and Duque 2005), respiratory infections (Laaksi et al. 2007), autoimmune diseases (Ponsonby et al. 2005, Van Etten et al. 2003), diabetes (Hyppönen et al. 2001), hypertension (Krause et al. 1998, Forman et al. 2008, Margolis et al. 2008), cardiovascular diseases (Scragg et al. 1990, Zittermann and Koerfer 2008) and congestive heart failure (Zittermann et al. 2006). Melamed et al. (2008) have reported that there is an independent association between low calcidiol values and deaths from all causes in the general population. In addition, numerous studies suggest that vitamin D is involved in brain function, as reviewed by Garcion et al. (2002), de Abreu et al. (2009) and Tuohimaa et al. (2009). For example, vitamin D insufficiency has been associated with an increased risk of multiple sclerosis (Schwartz 1992), seasonal affective disorder (SAD) (Gloth et al. 1999), schizophrenia (Mackay-Sim et al. 2004, McGrath et al. 2004), Parkinson's disease and Alzheimer's disease (Evatt et al. 2008, Oudshoorn et al. 2008). However, all these connections need to be confirmed by further studies.

5.3. Solar UV exposure

5.3.1. Solar UV radiation

The spectrum of solar UV radiation is divided into UVA, UVB and UVC regions according to the wavelength of the photons emitted by the sun. Radiation in the UVB and UVC regions of the spectrum is capable of damaging biological organisms. The high energy UVC photons (wavelengths shorter than 290 nm) are almost completely absorbed by the ozone layer above the earth. UVB radiation (wavelengths between 290 and 320 nm) is only partially absorbed by the ozone layer. The smaller the zenith angle, the smaller the portion absorbed, and thus near the equator at noon the UVB exposure is at its strongest. UVA rays (wavelengths greater than 320 nm) are not absorbed by ozone and they comprise 95 % of the UV radiation reaching the earth's surface, while only 5 % consists of UVB radiation.

The intensity of UV radiation is called irradiance and it varies largely depending on factors such as latitude, time of day, time of year, cloud cover, haze (aerosols), ozone and elevation above sea level (Webb 2006). As the wavelength decreases, the photons have higher energy, but there are fewer of

them because of absorption by ozone. The irradiance is expressed as watts per square meter. The larger the number of photons is, and the higher the irradiance, the greater is the damage to biological organisms. On the other hand, the sensitivity of biological organisms increases as the wavelength shortens. The relationship of UV radiation to biological organisms can be taken into account by an "action spectrum". The action spectrum is always specific for a given organism. For example, the action spectra for sunburn of the skin (CIE action spectra) (Commission Internationale de l'Eclairage (CIE). 1999, Diffey et al. 1997, McKinlay and Diffey 1987) and for vitamin D production in the skin (Commission Internationale de l'Eclairage (CIE) 2006) have been defined. To define an action spectrum, the amount of damage (or other end stage, e.g. vitamin D production) is experimentally determined in a given organism as a function of wavelength or photon energy. In order to determine the wavelengths where greatest damage occurs, the two functions, for example the values in the action spectra for sunburn of skin and those in the irradiance curve, are multiplied together. This process is called weighting. When weighting the physical dose for erythema in this case, it is called the CIE erythema weighted dose.

5.3.2. Measurement of solar UVB doses

When measuring UVB doses, the time component is taken into account, and thus the unit is the irradiance in a given area during a given time. In physical terms exposure is denoted in units J/m² or J/cm² , where J = Ws. However, when measuring the UVB dose received by the skin the physical dose is often weighted for erythema (J/cm² CIE). The unit for the vitamin D weighted dose is kJ/m² D-vit.CIE.

There are three units representing erythema weighted doses: CIE erythema weighted mJ/cm² or J/m2, minimal erythemal dose (MED) and standard erythema dose (SED). The literature still often uses the unit MED, which is the smallest dose to cause erythema with well-defined borders at 24 h after irradiation of the skin. The MED unit has the advantage over radiometric units in that it is directly related to the biological consequences of exposure in a given individual. However, the MED unit is subjective, since the erythemal response varies conciderably between individuals. To overcome this problem the standard erythema dose (SED) was established (Commission Internationale de l'Eclairage (CIE). 1999, Diffey et al. 1997). 1 SED is equivalent to 100 J/m2 and 10 mJ/cm² CIE erythema weighted irradiance. According to this definition the MED in skin types I – IV lies between 150 and 600 J/m2 CIE, which is equivalent to 1.5 – 6 SED. As an example, the

ambient exposure on a clear European summer day gives about 30 – 40 SEDs. Four SEDs will produce moderate erythema on unexposed, white skin, but only minimal erythema on previously exposed skin (Diffey et al. 1997).

Measurement of personal solar UVB doses is challenging, since no method exists which could take into account all the variables, such as the effect of motion, shadows, scattering, clothing, sunscreen, etc. One part of the body may receive direct sunshine whereas another part may be in shadow or covered. The ambient UVB dose, i.e. all the available UVB radiation in the surroundings, can be measured with different types of radiometers. Broadband radiometers measure the irradiance in a broad spectral band. For example, the Robertson Berger-type UV meter (RB meter) measures the erythemally effective irradiance and was introduced by Berger in 1976 (Berger 1976, Robertson 1968). Another type of radiometer is a narrowband meter known as a spectroradiometer, which reads the spectral irradiance and radiance of the source from wave band to wave band. Both types of radiometer measure the UV irradiation continuously and give readings for short intervals, for example the RB meter gives the results for ten minute intervals. With the help of personal sun behaviour diaries estimation of personal UVB doses can be made.

Since the late 1970´s, personal UVB dosimeters have been used to determine the UV-doses received from the sun. Several models based on different mechanisms are nowadays available, such as chemical (Davis et al. 1976), biological (Berces et al. 1999) and electronic (Thieden et al. 2004) dosimeters. The advantage of electronic dosimeters is that the exposure times are included in the data while other types of dosimeters can only measure the cumulative dose received by the dosimeter. Biological dosimeters are based on UV-induced DNA damage and they can comprise either biomolecules (uracil and DNA, bacteriophages) or spores, like *Bacillus subtilis,* (Berces et al. 1999). The mechanism of *Bacillus subtilis* spore film dosimeters is based on the fact that the spectral responsivity profile of the bacterial spore (Quintern et al. 1992) together with an optical filter (Quintern et al. 1997) is similar to the erythemal sensitivity of human skin after UVB exposure. In others words, within the bacterial spores the DNA is damaged similarly by UVB radiation as in human skin. To optimize the similarity between the responsivity of human skin and the spore film, the dosimeter system has been modified by an organic filter system (Quintern et al. 1997). Vitamin D has also been introduced as a biological dosimeter (Galkin and Terenetskaya 1999, Terenetskaya 2004). Common for all biological dosimeters is that they all record cumulative UVB exposure and eventually reach saturation.

5.4. Heliotherapy and phototherapy for psoriasis and atopic dermatitis

Heliotherapy

It has been known for hundreds of years that exposure to sun improves clinical symptoms of several skin diseases, such as psoriasis (PS) and atopic dermatitis (AD) (Fry 1988). In the Nordic countries these patients are known to have fewer symptoms during summer than in winter, when no sunshine is available. Travelling to sunny countries during the winter months often relieves their symptoms.

The Finnish associations for dermatological patients (Psoriasisliitto ry and Iholiitto ry) have arranged heliotherapy courses for patients with PS and AD in the Canary Islands since the 1970´s. The patients are referred by a dermatologist and during the heliotherapy course specially trained nurses guide patients on how to sunbathe and treat their skin. The treatment results have been good (Snellman et al. 1993, Autio et al. 2002). Snellman et al. (1993) studied the effect of a four-week heliotherapy course on 361 Finnish patients with psoriasis and found that it reduced the psoriasis severity index by at least 75 % in 84 % of patients. In another study of 216 Finnish patients with atopic dermatitis the mean SCORAD (Severity Scoring of Atopic Dermatitis. Consensus report of the European Task Force on Atopic Dermatitis 1993) index was reduced by 70 % after two weeks of heliotherapy and three months later it was still 45 % lower than initially (Autio et al. 2002). Also the Dead Sea has been found to be suitable for heliotherapy throughout the year. Heliotherapy courses for two and four weeks at the Dead Sea effectively cleaned PS plaques (Hodak et al. 2003, Ben-Amitai and David 2009). The safety of treatment using sun exposure is a subject of concern. A climatotherapy study conducted at the Dead Sea with over 1000 Israeli participants with PS showed that the risk for malignant skin diseases did not increase, but those patients who received heliotherapy experienced more solar damage, like elastosis, poikiloderma and facial wrinkles than the control patients (David et al. 2005). However, in a Danish study with 1738 patients with PS subjected to climatotherapy at the Dead Sea an increased risk for non-melanoma skin cancers was shown (Frentz 1999). These patients had a history of previous good response to phototherapy or sun exposure on PS plaques.

Phototherapy

Artificial light was introduced for the treatment of PS and AD in the early 20th century (Albert and Ostheimer 2002) and it has been a routine treatment modality for PS for over 50 years. Today it is widely used in the treatment of PS, AD and other inflammatory skin diseases. The spectral range of the UV lamps used earlier times varied from a broad band including also UVC rays in the early 1950´s to broadband UVB, UVA, SUP (selective ultraviolet phototherapy) and narrow-band UVB. Most studies concerning the development of phototherapy are carried out with PS patients. In 1953, John Ingram described the combination of broad-band UVB radiation, dithranol and tar bathing in the treatment of psoriasis (Ingram 1954). In 1974, the combination of oral psoralen intake and subsequent UVA irradiation (PUVA) was reported (Parrish et al. 1974). More recently, special cabins with lamps emitting UVB between 280 and 320 nm or UVA for PUVA phototherapy have been available and widely used in the treatment of psoriasis.

In the late 1970s, the wavelength of 313 was shown to be the most effective as regards PS (Fischer 1976). Parrish and Jaenicke (1981) reported that the same wavelengths, around 311 nm, were most effective both in causing erythema and in clearing PS. NB-UVB equipment containing TL-01 fluorescent lamps emitting a major peak at the wavelength of 311nm (± 2nm) was developed (Karvonen et al. 1989). The early 1990s saw the break-through of NB-UVB lamps and several studies have shown that NB-UVB phototherapy is superior in efficacy to broad-band UVB or PUVA as a treatment of PS (Green et al. 1988, Karvonen et al. 1989). Three exposures a week are suggested as the most effective exposure protocol (Dawe et al. 1998). At present, NB-UVB cabins with TL-01 lamps (Waldmann UV 7001) are available in nearly all central hospitals in Finland and also in some private clinics. In addition to PS, NB-UVB is widely used in the treatment of AD (Hjerppe et al. 2001, Krutmann 2000) and other inflammatory skin diseases.

As regards efficacy, safety and cost effectiveness, NB-UVB phototherapy appears to be the first-line treatment for the control of generalized PS (Feldman et al. 2003), since, in long-term, PUVA treatment can increase the risk for non-melanoma skin cancers (Stern and Lunder 1998). The safety of NB-UVB treatment has been shown in a recent study with 3687 patients; no significant association between NB-UVB treatment and skin cancers was observed (Hearn et al. 2008).

5.5. UV exposure, immune functions and vitamin D

Since the 1970s it has been known, that UV exposure on the skin is immunosuppressive and this effect is mediated mostly via T cells as reviewed by Schwarz (Schwarz 2002 and 2010). Numerous studies have shown that the regulatory T cells induced by UV radiation suppress the immune system in an antigen-specific manner (Schwarz 2010). Different subtypes of regulatory T cells are involved in this immunosuppression. In other words, the UV exposure depresses the adaptive immune system. T cells are the critical cellular mediators of the vast majority of inflammatory skin diseases, and suppression of T cell function is one important mechanism causing improvement of PS and AD after UVB treatment. For long it was hard to understand why UVB exposure did not increase skin infections as a consequence of immunosuppression. The breakthrough in understanding was the discovery that UVB exposure increases expression of antimicrobial peptides (Glaser et al. 2009) and that calcitriol is a direct inducer of antimicrobial gene expression (Wang et al. 2004). Antimicrobial peptides are small cationic peptides synthesized by epithelial and immune cells and they belong to the innate immune system.

The innate immune system is needed when the stratum corneum layer of the skin, the first line defence of skin against microbial agents, is injured opening the way to invasion by microbial agents. The innate immune system helps to prevent further invasion (Ong et al. 2002). Immediately, the neutrophils and macrophages start to produce reactive oxygen intermediates and to phagocytize the microbial agents to kill them. One important part of the innate immunity system consists of antimicrobial peptides, which mount a chemical defence shield against microbial agents (Schroder and Harder 2006). Cathelicidins and β-defensins are the best characterized antimicrobial peptides with antimicrobial activity against bacterial, fungal and viral pathogens (Braff and Gallo 2006). The expression of human β-defensin-2 (HBD-2) and LL-37, a cathelicidin, is triggered by injury or inflammation of the skin and also by vitamin D (Schauber et al. 2007). The association between expression of cathelicidin and vitamin D was first described in 2005 (Mallbris et al. 2005). The discovery that UVB exposure triggers expression of several antimicrobial peptides including HBD-2 (Glaser et al. 2009) and the finding of Schauber et al. (Schauber et al. 2007) and others (Liu et al. 2006, Liu et al. 2007, Wang et al. 2004) that vitamin D is a direct regulator of several antimicrobial innate immune responses, answered the question of why bacterial skin infections are not increased, but even cured after UVB exposure. Overall, UVB exposure induces the innate immune system by its effect on vitamin D, but suppresses the adaptive immune system by its effect on T cells (Schwarz 2010).

5.6. Studies on artificial UVB exposure and vitamin D synthesis

It has long been known, that in addition to sunlight, also artificial UVB exposure is able to induce vitamin D synthesis in human skin, as summarized in Table 1 (page 32). It should be noted that the calcidiol concentrations cannot be directly compared due to variations in the laboratory methods. These studies have mainly been conducted using broadband UVB equipment or tanning beds and the UVB doses received have seldom been defined. Vitamin D response to NB-UVB exposures has not been studied before and, similarly, the previous heliotherapy studies in PS and AD have not looked at what happens to serum calcidiol.

However, *in vitro* studies have shown that NB-UVB is capable of inducing vitamin D synthesis (Lehmann et al. 2000). Lehmann et al. (2000 and 2001) made the important discovery that exposure of cultured human keratinocytes to NB-UVB or UVB irradiation at 300 nm can convert vitamin D3 into calcitriol. Using a microdialysis technique they showed that UVB irradiation at 300 nm resulted in an increased calcitriol concentration in extracellular fluid of exposed skin *in vivo,* as well (Lehmann et al. 2003). They concluded that keratinocytes seem to have an autonomous pathway to metabolize vitamin D into calcitriol locally.

Table 1. Previous studies on UVB exposure and serum calcidiol concentration in man.

Author and subjects	Age (mean or range)	Light source and exposed skin area	Exposures	Calcidiol at start/at end *nmol/L*
Corless et al. (1978)		UV (Westinghouse		
Geriatric (*n* = 6)	84	FS20) in the dayroom). Face, forearms, hands, legs below knees	3 hours daily for 8 weeks	11.1/39.1
Snell et al. (1978)		UV (Vitalux)	4 weeks, frequency not	
Geriatric (*n* = 12)	81		stated	9/24
Davie M et al. (1982)		UV	3 times a week for 10	
Epileptic (*n* = 8)	22	5 % of skin = 600 cm²	weeks	6/35
Control (*n* = 8)	21			15/35
Toss G et al. (1982)		UV (Westinghouse FS)	once a week for 3	
Geriatric (*n* = 12)	81	large skin area	months	~26/56
Mawer EB et al. (1984)		UV (emission spectrum	daily for 3 weeks	
Psoriasis (*n* = 8)	20 – 57	300 -380 nm)		30/113
Controls (*n* = 5)	20 - 57	whole body		53/138
Brazerol WF et al. (1988)		UVB (Westinghouse	2 times a week for 6	
Black adults (*n* = 7)	22 – 35	FS-40)	weeks, suberythemal	29.5/101.75
White adults (*n* = 13)	22 - 35	Whole body	doses	68.0/132.21
Guilhou JJ et al. (1990)		UVB (Waldman 8000)	3-5 times a week for 3	
Psoriasis (*n* = 20)	18 - 71	whole body	weeks, mean cumul.	45/135
Controls (*n* = 11)	21 - 41		dose 1.40 J/cm²	60/170
Falkenbach A et al. (1993)		UVB (solarium, two	10 times within 12 days	
Healthy males (*n* = 24)	21 - 37	different) whole body, suberythemal doses	group 1 group 2	115.5/195.7 123.7/220.5
Chel VG et al. (1998)		UVB	½ MED 3 times a week	
Psychogeriatric (*n* = 14)	85	1000 cm² of lower back	for 12 weeks	< 30/60
Chuck A et al. (2001)		subliminal UVB on	daily	
Geriatric (*n* = 7)	83	face and hands	- 30 min (0.33 SED) for 6 months	14/25
			- 60 min (0.54 SED) for 6 months	25.1/36.5
Gronowitz E et al. (2005)		UVB	1 – 3 times a week for 6	
Cystic fibrosis (*n* = 9)	9 - 40		months	55/125

6. AIMS OF THE STUDY

Vitamin D insufficiency is common worldwide and in Finland this condition affects people especially during winter. The objectives of this thesis were to study the effects of heliotherapy and narrow-band UVB (NB-UVB) on vitamin D balance in winter. Healthy subjects and patients with atopic dermatitis and psoriasis were examined. An additional target was to investigate the effects of NB-UVB treatment on antimicrobial peptides and cytokines in the skin lesions.

Specific research aims were:

1. To study the effect of heliotherapy on vitamin D balance and on healing of skin in patients with atopic dermatitis (I).

2. To evaluate the feasibility of spore film dosimeters in the assessment of the personal UVB doses received during heliotherapy and comparing the results with vitamin D changes (II).

3. To examine how NB-UVB exposures given in winter on various body areas of healthy subjects induce vitamin D synthesis (III).

4. To study the effects of conventional NB-UVB phototherapy on vitamin D balance in patients with psoriasis and atopic dermatitis (IV).

5. To examine whether NB-UVB treatment alters antimicrobial peptide and cytokine expression in skin lesions of patients with psoriasis and atopic dermatitis and whether the changes correlate with the increase in vitamin D (IV).

7. MATERIALS AND METHODS

7.1. Subjects

Altogether 137 subjects volunteered and 127 completed the four studies (I - IV) of the present thesis. The studies were implemented during winter months (November – March) in the Canary Islands, Spain (I, II), Päijät-Häme (Lahti) and Kanta-Häme (Hämeenlinna) Central Hospitals (III) and in the University Hospital of Tampere (IV), Finland. The ethics committees of these hospitals approved the study protocols and all subjects gave informed consent to participate. Inclusion criteria were no phototherapy, solarium, sun holidays or vitamin D supplementation in the two preceding months. The demographics of the atopic dermatitis (AD) and psoriasis (PS) patients and the healthy subjects are compiled in Table 2.

Table 2. Demographics of the study subjects completing the four studies (I-IV)**.**

Study	Subjects *n* (women)	Mean age in years (range)	Type of exposure (study period)	Mean calcidiol at onset of the study *nmol/L* (range)	Mean dietary vitamin D intake *µg/d* (range)
I					
Patients with AD	23 (16)*	36 (21 – 57)	HT for two weeks (Jan or Mar 2005)	53.8 (27.4 – 143)	5.3 (1.3 – 10.8)
II					
Patients with AD	21 (15)**	37 (21 – 57)	HT for two weeks (Jan or Mar 2005)	54.4 (27.4 – 143)	5.4 (1.3 – 10.8)
III					
Healthy subjects	53 (53)	41 (21 – 61)	NB-UVB or solar simulator daily x7 (Dec - Mar 2004 - 2006)	39.1 (16.4 - 81.6)	6.6 (0.2 – 18.5)
IV					
Patients with PS	18 (8)	48 (19 – 67)	NB-UVB three times a week x 15 (Jan - Mar 2008 and 2009)	36.8 (15.7 - 58.5)	-
Patients with AD	18 (9)	33 (19 – 50)		32.2 (15.1 - 53.2)	
Healthy subjects	15 (15)	42 (24 – 56)		60.5 (35.2 - 112.4)	

* The number of women was stated incorrect in Study I. The corrected number is shown in this table.

** The same patients participated also in Study I.

Heliotherapy and dosimeter studies (I, II)

Twenty-three adult patients with AD received a two-week course of HT in Puerto Rico in the Canary Islands either from January 24 to February 4 in 2005 (n = 11) or from March 12 to March 26 in 2005 (n = 12) (I). Twenty-one of them were included in the dosimeter study (II). The median SCORAD at the onset of Study I was 34 in the January group and 30 in the March group. In the HT study, four patients had high initial calcidiol concentrations and were therefore analysed separately.

NB-UVB study in healthy women (III)

Fifty-three healthy healthcare workers received a seven-day NB-UVB or solar simulator course in winters (December – March) 2004 – 2006. The study was conducted in the Department of Dermatology in Päijät-Häme and Kanta-Häme Central Hospitals in Finland.

NB-UVB study in patients with PS and AD and in healthy subjects (IV)

Eighteen adult patients with PS, 18 with AD and 15 healthy subjects received a total of 15 NB-UVB exposures three times a week during January-March in 2008 and 2009 in the Department of Dermatology of Tampere University Hospital. The mean PASI (psoriasis area and severity index) (Fredriksson and Pettersson 1978) at the onset of the study was 8 and the mean SCORAD was 37. The PASI and SCORAD indices (I, IV) were determined by the author and two co-authors.

7.2. Methods

7.2.1. Heliotherapy and measurements of solar UVB (I, II)

Heliotherapy was arranged in Puerto Rico, Gran Canaria, Spain (30°N, 15°W). The treatment protocol was the same as usually used for AD patients receiving HT. On the first day the time to sunbathe was 15 min for patients with skin type II or severe AD and 30 min for the remaining patients. The sunbathing time was gradually increased by 15 min up to two hours daily. Half of the sunbath was taken on the back side and half on the front site of the body in a lying position and

without sunscreens. At other times, strict UV protection with clothes and sunscreens was recommended.

The ambient UVB dose during the two-week HT course was measured by a Robertson Berger type broadband meter (RB meter) (Solar Light Model 501 UV-meter s/n 4845, Glenside, United States). The RB meter was placed on the roof of a sunny terrace near the place where the patients were sunbathing, thus measuring the maximal available UVB dose. It gives readings at ten-minute intervals, and the readings are expressed in standard erythema doses (SED) as described in Studies I and II. The RB meter results were also weighted for vitamin D (kJ/m² D-vit. CIE) (II). The patients recorded their sunbathing behaviour in a specific diary. The records included clothing, exact times of sunbathe and times of other outdoor activities and sunscreen applications. For estimation of unprotected skin area at each moment of day, specific clothing coverage coefficients and sunscreen protection coefficients were established (I, II). The RB meter results were weighted using these coefficients to obtain an estimate of the personal UVB dose.

The personal UVB dose was additionally determined by *Bacillus subtilis* spore film dosimeters (VioSpor blue line III, BioSense, Bornheim, Germany). The dosimeters are capsules 32 mm in diameter and they were attached either on the chest or upper arm. The working principle is based on UV-induced DNA damage to a film of dried bacterial spores (*Bacillus subtilis*) as described earlier (page 30). The analysis of the exposed dosimeters was performed by the manufacturer. One dosimeter was worn from morning till evening on each 13 days of HT. In addition, two dosimeters were used to measure single-day UVB doses on days one and two, as described in Study II.

7.2.2. NB-UVB and solar simulator exposures (III, IV)

NB-UVB study in healthy women (III)

Seven NB-UVB or solar simulator exposures were given to healthy women (n = 53) on consecutive days. The UVB dose was 1 SED on the first day and thereafter 2 SED on each day. Accordingly, the cumulative UVB dose was 13 SED. NB-UVB exposures were given on different body areas in three groups: whole body (n = 19), head and arms (n = 9) or abdomen (n = 14). The whole-body NB-UVB exposure was given using a Waldmann UV 7001 cabin equipped with 20 TL01 tubes. The head and arms were exposed using a similar Waldmann UV 7001 cabin equipped with 40 TL01

tubes. The non-exposed parts of the body were carefully covered by clothes. The abdomen was irradiated with a Waldmann UV 801KL panel equipped with four TL01 tubes. Solar simulator exposures were given on the face and arms (n = 11) with a panel equipped with a broad band UVB lamp (Philips HB411 panel, HPA 400). The spectral irradiances of the lamps used are shown in Fig. I, Study III.

NB-UVB study in patients with PS and AD and in healthy subjects (IV)

Fifteen NB-UVB exposures were given three times a week for patients with PS (n = 18) and AD (n = 18), and for healthy subjects (n = 15) with a Waldman cabin equipped with 20 TL01 tubes. The initial dose was 1 SED corresponding a physical dose of 0.13 J/cm. This was gradually increased up to 9.5 SED (1.19 J/cm²). The cumulative UVB dose was 12.3 SED (1.54 J/cm²) after six exposures and 71.5 SED (8.88 J/cm²) after 15 exposures. The spectral irradiances of the lamps used are shown in Fig. I, study III.

7.2.3. Serum calcidiol measurements (I – IV))

Blood samples were taken before, during, and after sun or NB-UVB exposures as described in Studies I -IV. The samples were protected from light, centrifuged and the serum was frozen at -20°C. The serum 25(OH)D concentration was measured as duplicates by radioimmunoassay. The dietary intake of vitamin D was determined by a semi-quantitative food frequency questionnaire (I, III)

7.2.4. Antimicrobial peptides, cytokines and chemokines in skin lesions of psoriasis and atopic dermatitis during NB-UVB treatment (IV)

Punch biopsies from skin lesions were taken from seven patients with PS and eight patients with AD. Seven healthy subjects served as controls. The biopsies were taken before the treatment and after six NB-UVB exposures from the same representative skin lesion and they were immediately frozen and stored at -70 °C. Total mRNA was extracted from the biopsies. The transcript levels of two antimicrobial peptides, cathelicidin and HBD-2 (human β-defensin 2), and the mRNA

expression levels of different cytokines and chemokines in the PS and AD lesions were analyzed by real-time quantitative PCR. The methods are described in detail in Study IV.

7.2.5. Statistical analyses (I – IV)

In Study I, the significance of the changes in serum calcidiol concentrations and SCORAD were analysed by Wilcoxon´s sign rank test. Spearman´s rho was used for the correlations between the personal UV dose received and changes in serum calcidiol concentrations as well as changes in SCORAD.

In Study II, the concordance correlation coefficient (CCC) was used to calculate the agreement between measurements. Confidence intervals for the concordance correlations were obtained by bias-corrected and accelerated bootstrapping (5000 replications). The Pearson method was used in the calculation of the correlation coefficients (r).

In Study III, the changes in serum calcidiol concentration in the four study groups were analysed using the permutation test. The changes in serum calcidiol concentration between the study groups were analysed by a bootstrap-type analysis of covariance (ANCOVA) with the baseline values as covariables. The 95 per cent confidence intervals (95% CI) were obtained by bias-corrected and accelerated bootstrapping (5000 replications), because of the skewed distribution of the variables.

In study IV, the changes in serum calcidiol concentration in the three study groups, PASI and SCORAD were analysed using a permutation test with the Monte-Carlo p-value. Confidence intervals for the changes were obtained by bootstrapping (5000 replications). Statistical analysis of cathelicidin and HBD2 expression in PS and AD skin lesions before and after NB-UVB treatment was compared with healthy controls with the Mann-Whitney test. Comparison of cathelicidin and HBD2 expression in the untreated and NB-UVB treated skin lesions was performed with the Wilcoxon matched pairs test. Statistical analysis of cytokine expression in PS skin vs. AD skin was made with the non-parametric Mann-Whitney test.

8. RESULTS

8.1. Effect of heliotherapy on vitamin D balance in patients with atopic dermatitis (I)

The UVB dose received during HT was 60 SED in January and 109 SED in March (I; Table 1). The duration of sunbathing was equal, on average 22 h, in both groups. 74 % of the AD patients had mild or moderate vitamin D insufficiency (calcidiol < 50 nmol/L). Four patients (17 %) had a serum calcidiol concentration > 80 nmol/L and were therefore analyzed separately. The vitamin D response to heliotherapy is shown in Table 3.

Table 3. Present studies (I, III and IV) on UVB exposure and serum calcidiol concentration in man.

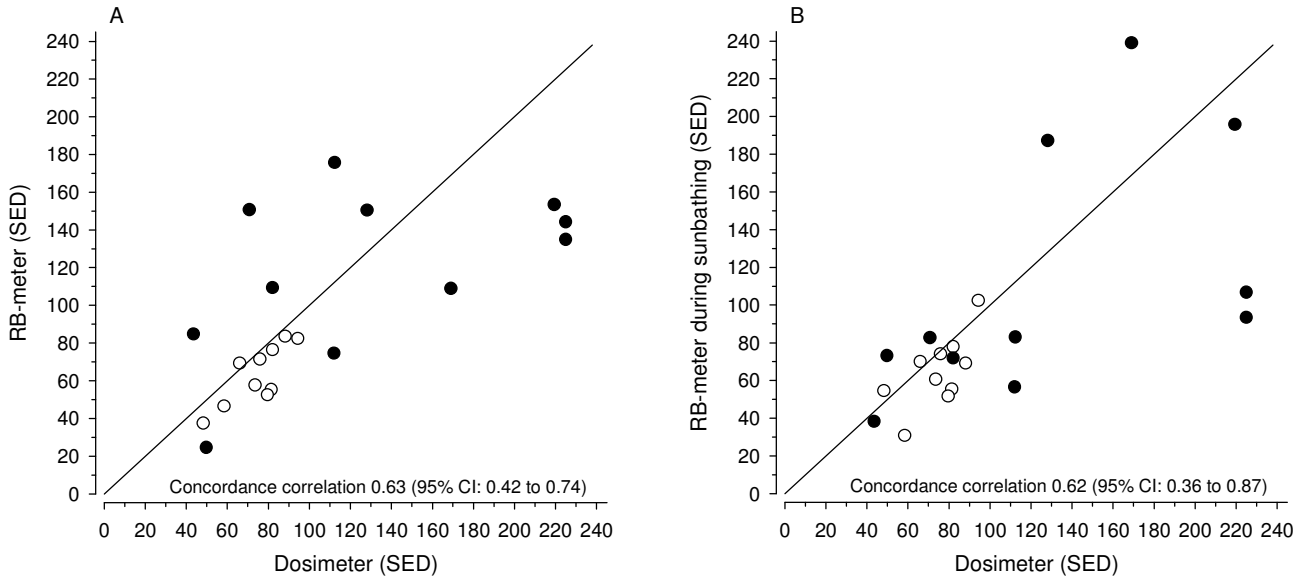
Study and subjects	**Age (mean)**	**Light source and exposed skin area**	**Exposures**	**Calcidiol** *nmol/L* (*mean ± SD*)		
				at start	**at end**	***P*-value**
Study I (2007)		Sun in Canary Islands:	¼ - 2 hours for 13 days			
With AD (*n* = 19)		Whole body	Mean personal UVB			
	36	Jan (*n* = 8)	dose: 60 SED (Jan) and	42.9 ± 7.8	56.3 ± 9.1	0.008
		Mar (*n* = 11)	109 SED (Mar)	41.1 ± 9.7	66.8 ± 21.2	< 0.001
Study III (2010)		NB-UVB:	2 SED daily for 7 days			
Healthy adults (*n* = 53)	41	Whole body (*n* = 19)	Cumulative NB-UVB	44.3 ± 15.1	55.7 ± 16.2	< 0.001
		Head and arms (*n* = 9)	dose: 13 SED	37.8 ± 15.1	48.8 ± 18.7	0.0046
		Abdomen (*n* = 14)		35.1 ± 9.8	39.2 ± 8.7	0.011
		Solar simulator:				
		Face and arms (*n* = 11)		38.1 ± 13.1	41.9 ± 12.6	0.038
Study IV (2010)		NB-UVB:	Gradually increasing			
With PS (*n* = 18)	41	Whole body	doses 3 times a week 15	36.8 ± 12.5	96.8 ± 20.0	< 0.001
With AD (*n* = 18)			times	32.2 ± 29.5	100.4±26.7	< 0.001
Healthy controls (*n* = 15)			Cumulative NB-UVB	60.5 ± 21.8	151.1±63.6	< 0.001
			dose: 71.5 SED			

The median increase in serum calcidiol was 13.4 nmol/L in January (n = 11) and 24.0 nmol/L in March (n = 12) (I; Table 3). Both increases were statistically significant. At day 6 the increase in serum calcidiol, 4.8 nmol/L in January and 13.3 nmol/L in March, did not reach statistical significance. The effect of HT on calcidiol levels was long lasting (I; Fig 1) since the follow up samples showed significantly higher serum calcidiol values one and two months after HT as compared with the initial values. Both the increase in serum calcidiol (r = 0.63, I; Fig 3a) and the clinical improvement of AD (r = 0.67, I; Fig. 3b) showed a positive correlation with the UVB dose received in March. The SCORAD decreased from 34 to 9 (p = 0.008) in January and from 30 to 9 (p = 0.002) in March showing alleviation of AD.

8.2. Measurement of personal UVB doses during heliotherapy (II)

The mean personal UVB dose received during two weeks of heliotherapy in the Canary Islands and measured by spore film dosimeters was 75 SED in January and 131 SED in March. The dosimeter results showed a strong concordance correlation with the doses obtained from the RB meter results combined with the all-day diary data and with RB meter results during sunbathing hours only, as seen in Fig 4. The mean sunbathing time during HT was 24.3 hours in January and 21.6 hours in March. The vitamin D-weighted dose during sunbathing hours was 10.4 kJ/m² D-vit. CIE in January and 17.6 kJ/m² D-vit. CIE in March.

Figure 4. Personal UVB dose measured with a RB meter combined with diary records (A) and ambient solar UVB dose recorded by the RB meter during sunbathing hours (B) against measurements with *B. subtilis* spore film dosimeters with the line of equality. Patients received heliotherapy either in January (empty circles) or in March (black circles).



There was a clear correlation between the increase of serum calcidiol concentration and the UVB dose received (II; Fig 2A and B). The correlation was at its strongest when comparing the increase of serum calcidiol with the vitamin D-weighted UVB dose (II; Fig II C).

8.3. Effect of NB-UVB exposures on vitamin D balance in healthy women (III)

At the onset 77 % of the healthy women in the study had mild or moderate vitamin D insufficiency (calcidiol < 50 nmol/L). 11 % had vitamin D deficiency (calcidiol < 25 nmol/L). The initial vitamin D parameters are shown in Table 2.

The NB-UVB exposures were given on seven consecutive days either on the whole body (n = 19), on the head, arms and hands (n = 9) or on the abdomen (n = 14). The mean increases in serum calcidiol were 11.4 nmol/l, 11.0 nmol/L and 4.0 nmol/L, respectively (III; Table 2). All increases

were statistically significant (III; Table 2). As few as three NB-UVB exposures caused a statistically significant increase in serum calcidiol in the first two groups, 7.3 nmol/L and 6.8 nmol/L, respectively. The highest mean serum calcidiol was seen two weeks after the whole-body NB-UVB exposures (III; Fig 3). Six weeks later serum calcidiol still was markedly higher than at the onset of the study.

8.4. Effect of NB-UVB treatment on vitamin D balance in patients with psoriasis and atopic dermatitis and in healthy subjects (IV)

At the onset 89 % of the patients with PS, 94 % of the patients with AD and 57 % of the healthy controls had vitamin D insufficiency (calcidiol < 50 nmol/L). Of these subjects 5/16 with PS and 7/17 with AD had vitamin D deficiency (calcidiol < 25 nmol/L).

NB-UVB exposures given three times a week a total of 15 times markedly increased mean serum calcidiol as seen in IV; Fig 1. The increase was 68.2 nmol/L in AD (n = 18), 59.9 nmol/L in PS (n = 18), and 90.7 nmol/L in healthy controls (n = 15). The serum calcidiol persisted at this elevated level for at least one month in the PS and AD patients but the concentration showed some decrease in the healthy subjects (IV; Fig 1). The clinical improvement of PS and AD was statistically significant, but it did not correlate with the increase of serum calcidiol. The mean SCORAD improved from 37.1 to 14.2 ($p < 0.001$) and PASI from 8.0 to 3.8 ($p < 0.001$).

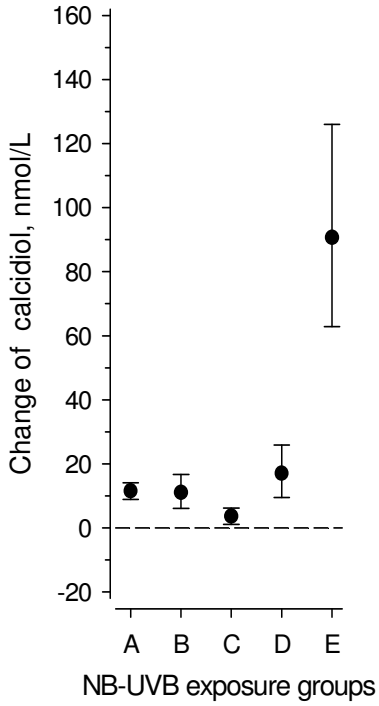
8.5. Comparison between serum calcidiol results in studies I – IV

Serum levels of calcidiol significantly increased in all the study groups after heliotherapy and NB-UVB exposures (Fig 5A and B). The most pronounced increase was seen in the healthy subjects receiving NB-UVB exposures three times a week for a total of 15 times (IV). When receiving NB-UVB exposures only on the head and arms, the increase in serum calcidiol was nearly the same as when exposing the whole body (III). The NB-UVB exposure protocol three times a week (IV) compared with exposures given on consecutive days (III) proved to be more effective in increasing serum calcidiol (Fig. 5A). The increase in serum calcidiol in patients with AD was more pronounced after 15 NB-UVB exposures than after 13 days of heliotherapy (Fig. 5B), although both therapies gave a statistically significant improvement in SCORAD.

Figure 5. **(A)** Change in serum calcidiol in healthy subjects after a seven-day NB-UVB course (A – C, Study III) and after six (D) and fifteen (E) NB-UVB exposures given three times weekly (Study IV).

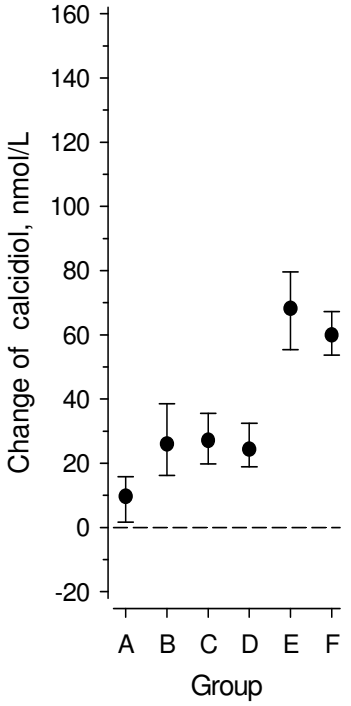
(B) Change in serum calcidiol in patients with atopic dermatitis after 13 days of heliotherapy and in patients with atopic dermatitis (AD) and psoriasis (PS) after 6 and 15 whole body NB-UVB exposures. The NB-UVB exposures were given three times a week.

A.



A: daily x 7 whole body (13 SED)
B: daily x 7 head and arms (13 SED)
C: daily x 7 abdomen (13 SED)
D: three times a week x 6 whole body (12.3 SED)
E: three times a week x 15 whole body (71.5 SED)

B.



A: AD: 13-day heliotherapy in January
B: AD: 13-day heliotherapy in March
C: AD: 6 NB-UVB (12.3 SED)
D: PS: 6 NB-UVB (12.3 SED)
E: AD: 15 NB-UVB (71.5 SED)
F: PS: 15 NB-UVB (71.5 SED)

8.6. Effect of NB-UVB exposures on antimicrobial peptides, cytokines and chemokines in skin lesions of psoriasis and atopic dermatitis (IV)

Expression of cathelicidin and HBD-2

Biopsies were taken before and after six NB-UVB exposures from PS (n = 7) and AD (n = 8) patients and from healthy controls (n = 7). Both in PS and AD lesions the mRNA expression levels of cathelicidin and HBD-2 (human β defensin 2) were elevated before treatment compared with healthy skin (IV; Figs. 2 and 3). After 6 NB-UVB exposures the expression of cathelicidin increased markedly in PS and slightly in AD lesions, but no statistical significance was observed. In contrast, HBD-2 expression significantly decreased in PS lesions. A minor decrease in expression of HBD-2 was seen in AD lesions.

Expression of cytokines and chemokines

Before NB-UVB treatment, expression of IL-1β, IL-17A and IFN-γ was significantly higher in PS lesions compared with AD lesions (IV; Fig 4). In contrast, expression of CCL17 (TARC) was significantly higher in AD lesions. IL-10, TGF-β1, TNF-α and CCL20 (MIP-3a) were expressed similarly in both diseases. IL-4 expression was basically negative in PS and AD lesions. After 6 NB-UVB treatments a marked, but not statistically significant, reduction in expression of IL-1β and IL-17A was observed in PS lesions. NB-UVB-treatment did not change the expression of the other cytokines examined.

9. DISCUSSION

9.1. Serum calcidiol in study subjects before heliotherapy and narrow-band UVB exposures in winter

Our studies were carried out in winters 2005 – 2008, and showed that 83 % of the 127 healthy subjects and patients with atopic dermatitis or psoriasis had vitamin D insufficiency at the onset of the study, i.e. their serum calcidiol was below 50 nmol/L. These low levels occurred even in those whose ingested vitamin D met the current recommendations for vitamin D intake, 7.5 μg daily (National Instititute for Health and Welfare, www.thl.fi). These findings agree with earlier studies on low vitamin D concentrations in the Finnish population (Lehtonen-Veromaa et al. 1999, Kauppinen-Mäkelin et al. 2001, Lamberg-Allardt et al. 2001, Välimäki et al. 2004 and 2007). In winter, when UVB radiation from the sun is negligible, calcidiol concentrations do not exceed the optimal concentration needed for normal bone turnover, 50 – 80 nmol/L (Dawson-Hughes et al. 2005). In line with this, in a recent study, 85 % of 182 Danes showed vitamin D insufficiency in winter (Bogh et al. 2010). However, in that study as and in our studies, one inclusion criteria was abstinence from vitamin D supplements for at least two months prior to the study and this may have affected the results. Many people consume vitamin D supplements in winter, so that our vitamin D insufficiency figures do not represent the situation in the whole population of Finland. Vitamin D insufficiency is common also in the other Nordic countries and in Britain especially in winter (Brustad et al. 2004, Hyppönen and Power 2007). Recently this condition has been found to be a worldwide health problem (Holick and Chen 2008). Today vitamin D insufficiency is believed to have much more extensive effects on health than previously thought, ranging from bone disease to prostate and other cancers and autoimmune diseases (Deluca and Cantorna 2001, Arnson et al. 2007, Holick 2007, Tuohimaa et al. 2007). Thus, in primary care, vitamin D insufficiency should be actively screened and treated.

Our studies aimed to monitor the influence of natural sunlight during heliotherapy or UV radiation from a NB-UVB cabin on the vitamin D balance by measuring serum calcidiol concentrations before, during, and after the exposures. In parallel we also evaluated the curative effect of these treatments on AD and PS. Earlier data about UVB exposure and the photosynthesis of vitamin D is scant, especially concerning the UV doses needed to induce vitamin D synthesis. Experimental *in vivo* studies on this matter are very scarce. This is surprising as the basic physiological phenomenon

to maintain bone health has been familiar since the time when vitamin D was first discovered. In contrast, the effect of supplements on vitamin D balance has been well established. Several studies have shown that 15 – 20 µg of vitamin D is needed daily to achieve a concentration of 60 – 80 nmol/L when no sunshine is available (Vieth et al. 2007, Viljakainen et al. 2009). However, it is not known whether all the positive associations between sunlight and health are mediated solely via the hormonally active metabolites of vitamin D or, whether there are other, as yet undefined, mechanisms involved. Thus it is not known whether ingested vitamin D leads to identical health benefits as exposure to the sun.

9.2. Heliotherapy increases serum calcidiol concentration in patients with atopic dermatitis

Two weeks of heliotherapy in the Canary Islands in either January or March 2005 gave a statistically significant improvement in vitamin D balance (I). The mean increase in serum calcidiol was 13 nmol/L (from 44 to 56 nmol/L) in January and 24 nmol/L (from 42 to 62 nmol/L, Fig. 5B) in March. The estimated UVB doses received were 60 and 109 SED, respectively. The sunbathing time was gradually increased from 15 minutes up to two hours daily. The sunbaths were taken without sunscreens to optimize the healing effect of UVB on skin inflammation. Afterwards the use of clothes and potent sunscreens was recommended. Sunscreens with SPF 15 are known to block vitamin D synthesis in the skin, if properly administered (Matsuoka et al. 1987). We were surprised to find that the increase in serum calcidiol was not more pronounced. The daily sunbathing time was quite long, two hours, which might have led to degradation of the newly formed vitamin D or at least to formation of inactive metabolites (Webb et al. 1989). Degradation effectively inhibits over dosage of vitamin D formed after exposure to sunlight. Actually, the formation of inactive metabolites for later utilisation could explain one interesting observation in the present heliotherapy study: the calcidiol concentrations remained elevated for at least one to two months. There may also be an upper limit to calcidiol formation. In the NB-UVB study (III), when only the head and arms were exposed, the increase in serum calcidiol was nearly the same as after exposing the whole body (Fig 5A). Perhaps the daily exposures on the whole body were not ideal as regards synthesis of vitamin D. However, there was a clear correlation between the UVB dose received and the increase in serum calcidiol. A recent heliotherapy study in patients with PS, in contrast, did not find such a correlation (Osmancevic et al. 2009b). In that study, the mean improvement in serum calcidiol was conciderably more than in our study, from 57.2 nmol/L to 104 nmol/L, during 15 days of

heliotherapy. This may be due either to differences in the duration of heliotherapy or in the treatment protocols, which in PS tend to include more sunbathing than in AD.

The heliotherapy was effective in improving AD. The median decrease in SCORAD was 70 %, which is in agreement with the results of an earlier study (Autio et al. 2002). We detected a clear correlation between the increase in serum calcidiol and the improvement of SCORAD in the March group but in the January group there was no correlation. The question of whether UV-induced calcidiol synthesis might have any influence on the healing of AD remains to be elucidated by further studies.

9.3. Narrow-band UVB exposures increase serum calcidiol in healthy subjects, as well as in patients with atopic dermatitis and psoriasis

Earlier *in vitro* studies have shown that the optimal wavelength for vitamin D synthesis is 297 nm (Holick et al. 1980). Lehmann et al. (2007) showed in cultured keratinocytes that the wavelength of 300 nm is twice as effective in inducing vitamin D synthesis as the wavelength of 311 nm. We showed *in vivo*, that NB-UVB efficiently enhanced vitamin D synthesis both in healthy subjects and in patients with PS and AD. Fifteen NB-UVB exposures given on the whole body increased the serum calcidiol concentration by 91 nmol/L (from 60 to 151 nmol/L), 60 nmol/L (from 37 to 97nmol/L) and 68 nmol/L (from 32 to 100 nmol/L), respectively. The effect on vitamin D balance, like that caused by heliotherapy, was long-lasting. One month later the serum calcidiol concentration was still at the same elevated level in both PS and AD patients. In the healthy subjects the concentration slightly decreased during the follow-up period, but still remained higher than in the PS and AD patients. This may imply that the decrease just led to more physiological concentrations.

In agreement with our results, Czarnecki (2008) treated seven PS patients with eighteen NB-UVB exposures and found a markedly increased serum calcidiol concentration in all patients. In a second NB-UVB study a mean number of 28 NB-UVB exposures in 42 patients with PS increased serum calcidiol from 88 nmol/L to 138 nmol/L (Osmancevic et al. 2009a). The increase was not as pronounced as in our 18 PS patients, who were exposed for a total of 15 times to NB-UVB. However, the differences in the initial vitamin D values seem to have an impact on these results. It has been shown that the lower the baseline calcidiol concentration, the higher the increase in serum

calcidiol after UVB exposure (Bogh et al. 2010). However, we could not show such a correlation, possibly because our series was small and most of our subjects had vitamin D insufficiency, i.e. rather similar calcidiol concentrations. Some other recent studies using a broadband UVB source in PS patients have also shown marked increases in serum calcidiol (Prystowsky et al. 1996, Armas et al. 2007, Osmancevic et al. 2007). Surprisingly, also tanning beds, which emit minimal amounts of UVB, have been shown to increase serum calcidiol (Thieden et al. 2008, Moan et al. 2009). This focuses attention on the fact that only very small doses of UVB are needed to enhance vitamin D synthesis, as shown also in our studies.

One interesting finding was that just seven NB-UVB exposures on the head and arms (estimated 20 - 25 % of body surface area, III) was as effective as exposure of the whole body in increasing serum calcidiol (Fig. 5A). Both modalities led to a mean increase of 11 nmol/L in serum calcidiol concentration. The reason for this could be evolutionary; the face and arms are the areas most likely to be exposed to sunlight and so might have developed an enhanced capacity to produce vitamin D. This idea is contradicted by the fact that the abdominal area, in relation to the exposed body surface area (6 -10 %), seemed to be as effective in increasing serum calcidiol as the area of head and arms (20 – 25 %). It could also be that there is an upper limit for calcidiol formation per time unit. Our study is unique in comparing synthesis of vitamin D in different body areas *in vivo.* Additional studies are urgently needed in this important area.

Another interesting finding in our study was the observation that exposure of the body three times a week to NB-UVB seemed to be more effective in increasing the serum calcidiol concentration than the daily exposures. Seven exposures with an initial dose of 1 SED and later 2 SED daily (13 SED, III) caused an increase of 11.4 nmol/L in serum calcidiol. When the schedule was six exposures three times a week, starting with a dose of 1 SED and gradually increasing the dose to 3.3 SED (12.3 SED, IV), the increase in serum calcidiol was 17 nmol/L. The results of Bogh et al. (2010) support our finding of better efficacy when giving fewer exposures in a week. They exposed the chest and back (24 % body surface area) of 50 subjects to broadband UVB and showed that four exposures of three SED during one week increased the mean serum calcidiol by 23 nmol/L. This increase was more pronounced than what we could show with seven NB-UVB exposures on nearly as a large body surface area (head and arms 20 – 25 %). However, the different radiation sources and doses given could also have affected the results.

One SED from a NB-UVB lamp and 1 SED from sunlight are not comparable as regards induction of vitamin D synthesis (Young 2010). Sunlight includes the whole spectrum of UV radiation, whereas NB-UVB contains just the wavelengths most effective for vitamin D synthesis. This difference was obvious in our studies, too. Consequently, direct comparisons between the effects of artificial and natural UVB sources should be avoided. However, independently, the heliotherapy as well as the NB-UVB and solar simulator exposure studies showed that small doses, ranging from 1 to 2 SED, were able to induce vitamin D synthesis. In heliotherapy, already six days of treatment led to an increase in serum calcidiol. In the first week of heliotherapy the daily solar UV dose ranged from 1 to 3 SED in January. Similarly, a small number of NB-UVB exposures (2 SED) clearly below the minimal erythemal dose caused an increase in serum calcidiol. Our results support earlier recommendations that even short solar exposures three times a week received on the hands and arms only, are enough to maintain a sufficient vitamin D balance (Holick 1981). However, at the latitude of Finland (60 - 70ºN), a solar UVB dose of 2 SED is received in about 30 min at midday around midsummer. Before noon or later in the afternoon, as well as earlier and later in summer, longer exposures are needed. This is supported by a recent British study with 120 participants (Rhodes et al. 2010). In that study, an exposure of 1.3 SED (corresponding to 13 minutes of midday sunshine at latitude 50-60ºN in summer) to a solar simulator three times a week for six weeks while wearing a T-shirt and shorts (35 % body surface area) could not increase serum calcidiol to optimal levels. In 90 % of participants a serum calcidiol concentration of 45 nmol/L was reached but only one out of four reached a concentration of 80 nmol/L.

9.4 Measurement of personal solar UVB doses during heliotherapy

We measured personal UVB doses received during the two-week heliotherapy in the Canary Islands with spore film dosimeters. These were used always in the daytime. We estimated the personal UVB dose also alternatively using a Robertson Berger-type broadband meter (RB meter) and combining the results of irradiance with personal diary records, which included notes on clothing, sunbathing and use of sunscreens. The results of these two methods showed a close correlation, indicating that the dosimeters give reliable estimates of personal whole-day exposure. The dosimeters appeared to be easy and practical to use. They can be recommended for use in further studies, when estimations of personal solar UVB doses are needed.

The UVB dose received was 75 SED in January and 131 SED in March. This is in line with the results of Osmancevic et al. (Osmancevic et al. 2009b). They estimated the UVB dose to be 166 SED during 15 days of heliotherapy for PS. However, they divided the ambient sunbathing dose, recorded with a broadband UV meter, by two, because only half of the body is exposed at a time. Thus it can be assumed that with our method the dose would have been bigger, which would explain the more pronounced increase in serum calcidiol these patients experienced.

9.5. Effect of narrow-band UVB exposures on antimicrobial peptides in skin lesions of psoriasis and atopic dermatitis

Earlier it has been shown that the expression of two antimicrobial peptides, cathelicidin and HBD-2 (human β-defensin-2), is elevated in PS skin lesions and high enough to kill S*taphylococcus aureus* (Ong et al. 2002). In AD, the increase is less pronounced, which might explain the susceptibility of these patients to skin infections (Ong et al. 2002, Gambichler et al. 2006, Ballardini et al. 2009). We also found an increased expression of cathelicidin and HBD-2 in the skin lesions of PS and AD confirming the earlier results (Gambichler et al. 2006, Hollox et al. 2008). We found that a total of six NB-UVB exposures reduced HBD-2 expression in healing PS and AD skin lesions. Similar HBD-2 findings have been reported previously by Peric et al. (2009) and others (Gambichler et al. 2006, Ballardini et al. 2009,) in AD skin lesions treated by NB-UVB. Together with the decreasing HBD-2 levels, we saw a significant increase in serum calcidiol. It is likely that simultaneously also the local synthesis of calcidiol and calcitriol in the keratinocytes is triggered by NB-UVB (Lehmann et al. 2007). Calcitriol is known to exert potent anti-inflammatory actions through NfκB activation, and it inhibits IL-17A induced expression of HBD-2 in ceratinocytes *in vitro* (Peric et al. 2008). Therefore, we conclude that the accelerated vitamin D synthesis and calcitriol formation in the skin after NB-UVB exposure, might outweight the HBD-2 inducing effect of short-time UVB in our patient population.

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We found that expression of cathelicidin was significantly increased in PS lesions after six NB-UVB exposures. A minor increase was also seen in AD lesions. In contrast to HBD-2, UVB treatment has not been found to induce cathelicidin in keratinocytes *in vitro* (Schauber et al. 2006). However, in healthy skin *in vivo,* UVB radiation induces cathelicidin HCAP18 expression (Weber et al. 2005). Calcitriol is known to induce cathelicidin in skin epithelial cells (Schauber et al. 2007). In addition, application of vitamin D analogues on PS skin lesions is known to induce cathelicidin

(Peric et al. 2009). However, theoretically, increased cathelicidin should lead to more skin inflammation as cathelicidin LL-37 binds self-DNA and triggers an immune response in PS (Hollox et al. 2008). These contrasting observations need to be further investigated. Possible explanations include the anti-inflammatory activities of vitamin D, such as inhibition of T cell recruitment or inhibition of dendritic cell activation, which might be responsible for the immunosuppressive effects of NB-UVB (Cruz and Bergstresser 1988) observed in the present study.

10. CONCLUSIONS AND FUTURE ASPECTS

The present studies performed in winter showed that as many as 83 % of healthy subjects and patients with AD and PS had vitamin D insufficiency. In addition, in several subjects even vitamin D deficiency was detected. This result is in agreement with previous studies in Finland and elsewhere (Lamberg-Allardt et al. 2001, Brustad et al. 2004, Välimaki et al. 2004, Hyppönen and Power 2007, Bogh et al. 2010) implying that action to prevent vitamin D insufficiency especially in winter is important. Low levels were observed even in those subjects whose vitamin D intake met the current recommendations, 7.5 µg daily. Several studies have shown, that 15 – 20 µg of vitamin D is needed daily to achieve a concentration of 60 – 80 nmol/L, when no sunshine is available (Vieth et al. 2007, Viljakainen et al. 2009).

In the present study (I) we were able to show that a two-week course of heliotherapy (HT) in the Canary Islands in winter effectively improved vitamin D balance in patients with AD. Similar findings were recently reported in PS patients during HT (Osmancevic et al. 2009b). Apparently, this effect would be the same in healthy subjects. Thus, a holiday in a sunny resort for at least two weeks during winter could provide sufficient vitamin D levels for at least one to two months. In the present study the AD patients did not use sunscreens during their HT sunbaths. Sunscreens with SPF 15 or more are known to stop vitamin D synthesis, if properly applied (Matsuoka et al. 1987). Therefore, further studies should be carried out in healthy subjects during their wintertime holidays in sunny resorts to find out how the time spent sunbathing and use of sunscreen affects their vitamin D balance. Comparison of vitamin D response should be done between a group using sunscreens and groups taking sunbaths for short periods without sunscreens.

Studies III and IV concentrated on the effects of NB-UVB exposures on vitamin D balance. We were able to show that already very small doses which are clearly below the erythemal threshold of skin, such as two SED, induced vitamin D synthesis. In healthy subjects, seven NB-UVB exposures given only on the head and arms were as effective as exposures given on the whole body (III). The increase in serum calcidiol was about 11 nmol/L. When the NB-UVB exposures were given three times a week using increasing UV doses for a total of 15 times (IV), the increase in serum calcidiol was more pronounced. The mean increase was as high as 68.2 nmol/L in AD, 59.9 nmol/L in PS and 90.7 nmol/L in healthy subjects, and the effect persisted for at least one month. The clinical

improvement of PS and AD was statistically significant, but did not correlate with the increase in serum calcidiol.

Our results show that a short course of NB-UVB exposures using low UV doses could serve as an alternative to quickly and safely correct vitamin D deficiency or insufficiency. In future, NB-UVB exposures could also be used to prevent wintertime vitamin D insufficiency especially in certain patient groups. There is still need to test different UVB dosing and schedules, e.g. daily compared to one to three times a week in a larger group to find out which NB-UVB dosage gives the maximum response in serum calcidiol. Further comparisons between different body areas are needed to confirm the optimal skin sites for vitamin D synthesis. The duration of calcidiol response should also be studied further. The possible risk of skin cancers is a concern. NB-UVB treatment has, however, proved to be safe when treating patients with PS and AD (Hearn et al. 2008). NB-UVB treatment protocols aimed for dermatologic therapeutic goals involve high UV doses in comparison to the doses needed to induce vitamin D synthesis. Thus, it seems obvious that the low doses of UV radiation needed for vitamin D synthesis would hardly cause any harm.

Spore film UV dosimeters proved to be reliable and feasible during heliotherapy. The dosimeters were easy to use and can be recommended for further studies, when estimations of personal solar UVB doses are needed. The results showed a good correlation with the estimated doses based on Robertson Berger meter readings and diary records. Our patients wore the dosimeters from morning till evening on their upper arm or chest. It would be of interest to compare results of UV dosimeters worn all day to dosimeters worn only during sunbathing.

In our study, two antimicrobial peptides, cathelicidin and HBD-2 (human β-defensin-2), showed increased expression in PS and AD skin lesions. The same observation has been made in some other studies (Ong et al. 2002, Gambichler et al. 2006, Ballardini et al. 2009). Six NB-UVB exposures caused a decrease in HBD-2, as expected according to earlier studies. In contrast to earlier *in vitro* observations, expression of cathelicidin increased in PS lesions. However, the healing process of PS lesions was just beginning and this may have affected the results. Therefore, further NB-UVB studies measuring the expression of cathelicidin through the whole healing process of PS lesions would be of interest. Calcitriol is known to trigger expression of cathelicidin (Schauber et al. 2007). In future, it would also be of interest to study the effect of NB-UVB exposures in parallel with measurements of antimicrobial peptides, serum calcitriol and local calcitriol synthesis in keratinocytes.

To conclude, vitamin D insufficiency in winter is common in northern latitudes and its prevention clearly demands more powerful actions than education to promote a healthy diet. Our studies confirmed that NB-UVB exposures could be used to treat vitamin D insufficiency. Further study comparing the effects and costs of NB-UVB exposures to oral vitamin D substitution would be of importance. Much research is still needed to establish the most practical, safe and effective protocols to improve vitamin D balance in different populations. It would also be of interest to develop a lamp peaking at 300 nm, which is regarded as the most efficient wavelength *in vitro* for vitamin D synthesis in keratinocytes, and then to compare its efficacy with NB-UVB exposures given to healthy subjects and patients prone to vitamin D insufficiency.

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Heliotherapy improves vitamin D balance and atopic dermatitis

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Summary

Background Vitamin D insufficiency during winter is common in the Nordic countries. Heliotherapy (HT) may heal atopic dermatitis (AD) but its effect on vitamin D balance has not been examined.

Objectives To study the effect of HT on serum calcidiol (25-hydroxyvitamin D) concentration and on healing of AD.

Methods Twenty-three adult patients with AD received a 2-week course of HT in the Canary Islands in either January or March 2005. Daily solar ultraviolet (UV) radiation was measured and personal UV exposure calculated as standard erythema doses (SED). Blood samples were taken during HT and during a 1–2 month follow-up. Serum calcidiol concentration was measured by radioimmunoassay. Healing of AD was examined by SCORAD index.

Results Before HT 17 (74%) AD patients had vitamin D insufficiency (calcidiol < 50 nmol L^{-1}) and four patients high (> 80 nmol L^{-1}) serum calcidiol values. The median personal UV dose during the 2-week HT course was 60 SED in the January group and 109 SED in the March group. Serum calcidiol concentration increased significantly in both groups, by 13·4 and 24·0 nmol/L^{-1}, respectively, and after HT only four (17%) patients had vitamin D insufficiency. SCORAD improved from 34 to 9 in the January HT group and from 30 to 9 in the March group.

Conclusions A 2-week course of HT significantly improved vitamin D balance by increasing serum calcidiol concentration, and caused a marked healing of AD. These parallel positive responses should be taken into account when the benefits of HT are considered.

Vitamin D made in the skin after exposure to sunlight or ingested in the diet is essential for human health. Keratinocytes have the capacity for ultraviolet (UV) B-induced photochemical conversion of 7-dehydrocholesterol to vitamin D.[1] Thereafter, vitamin D is hydroxylated to form calcidiol (25-hydroxyvitamin D), which is the major circulating form of vitamin D and the best indicator of vitamin D status.[2] A second hydroxylation is needed to form calcitriol (1α,25-dihydroxyvitamin D), the active and hormone-like metabolite of vitamin D. Calcitriol is not only crucial in calcium metabolism and bone health, but also has a wide variety of other biological functions, such as regulation of cell growth, including keratinocyte growth, and modulation of the immune system.[3–5] The capacity to regulate keratinocyte growth is used in dermatology when treating psoriasis with calcipotriol, a vitamin D derivative, or with calcitriol.[6]

UVB phototherapy is effective in reducing symptoms of psoriasis and of atopic dermatitis (AD).[7,8] Heliotherapy (HT) in winter in the Canary Islands is an alternative chosen by many patients from Nordic countries because HT treatment results have been good both in psoriasis and in AD.[9,10] In winter very little, if any, vitamin D is produced in the skin of

Northern people.[11,12] At present, a large number of Finnish and Norwegian people is known to suffer from seasonal vitamin D insufficiency or even deficiency.[13–16]

In the present study we examined whether a 2-week course of HT in the Canary Islands in winter improves vitamin D balance and simultaneously heals AD.

Materials and methods

Patients and heliotherapy protocol

Twenty-five patients (20 women, five men; mean ± SD age 36 ± 12 years, range 21–57) with AD living in various parts of Finland (60–70°N) were included in the study. Inclusion criteria were skin types II or III according to Fitzpatrick,[17] and no phototherapy, solarium, sun holidays or vitamin D supplementation during the preceding 2 months. The ethics committee of Päijät-Häme Central Hospital approved the study protocol and all patients gave their informed consent to participate. At the beginning two women withdrew from the study for personal reasons. The severity of AD was scored by SCORAD index[18] at the beginning and at the end of HT.

HT was undertaken in Puerto Rico, Gran Canaria, Spain (30°N) in winter 2005. The first group of 11 patients (nine women, two men) received HT from 24 January to 4 February and the second group of 12 patients (nine women, three men) from 12 March to 26 March. On the first day the sunbathing time was 15 min for patients with skin type II or severe AD (eight patients) and 30 min for the rest. Patients sunbathed while lying on both sides of the body, without sunscreens. The time was increased daily by 15 min until a maximum duration of 2 h in the sun was reached. Thereafter, clothes and sunscreen with sun protection factor (SPF) 15 or higher were used to protect the skin from the sun.

Personal ultraviolet exposure and solar ultraviolet radiation measurements

The patients recorded each day in a diary the exact times of sunbathing, hours spent outdoors and use of clothes and sunscreens. The data were recorded in 10-min intervals and stored in a computer.

The solar UV radiation was measured continuously with a Robertson Berger type broadband UV meter (Solar Light Model 501 UV-meter s/n 4845; Solar Light Co. Inc., Glenside, PA, U.S.A.) which also gives the results in 10-min intervals. The UV meter is calibrated annually at the Radiation and Nuclear Safety Authority, Helsinki, Finland. The calibration uncertainty (2σ) of the broadband UV meter is 8% and the calibration is traceable to the National Institute of Standards and Technology (Gaithersburg, MD, U.S.A.). The UV meter was placed on a high roof near the place the patients were sunbathing. The UV doses are given as biologically weighted standard erythema doses (SED). One SED is equivalent to 10 mJ cm^{-2} CIE erythema-weighted irradiance.[19]

The personal UV exposure was calculated from the UV meter and diary data. The protective effect of clothes was calculated using coverage factors based on the rule of nines (e.g. 0·44 for trousers, 0·22 for shorts, 0·42 for a pullover, 0·30 for a T-shirt, 0·28 for a woman's swimsuit, 0·10 for a man's swimming trunks or for a bikini). The protective factor of sunscreens (SPF > 15) applied after sunbathing was divided by four[20] and one application was assumed to give UV protection for 3 h.

Calcidiol measurements

The dietary intake of vitamin D was determined by a semiquantitative food frequency questionnaire[21] completed 1 month before and 1 month after HT. Blood samples were taken 1 day before HT and in the evenings of days 1, 2, 6 and 13. Follow-up samples were taken 1 month and 2 months (January group only) after HT. The samples were protected from light, centrifuged, and serum was frozen (−20 °C) and transported to Finland.

Serum calcidiol concentration was measured in duplicate by radioimmunoassay (Immunodiagnostic Systems, Boldon, U.K.). At calcidiol levels of 26·5, 58·4 and 151 nmol L^{-1}, the intra-assay variation was 5·3%, 5·0% and 6·1%, respectively. At calcidiol levels of 19·6, 56·7 and 136 nmol L^{-1}, the interassay variation was 8·2%, 8·1% and 7·3%, respectively. A calcidiol concentration below 50 nmol L^{-1} was regarded as vitamin D insufficiency and below 25 nmol L^{-1} as deficiency.[22]

Statistical methods

Because of the non-normal distributions of the variables involved, the data are described as medians and lower and upper quartiles unless otherwise specified, and nonparametric methods were used. The significance of the changes in serum calcidiol concentrations and in SCORAD was analysed by Wilcoxon signed rank test. For the correlations between the personal UV dose received and changes in serum calcidiol concentrations as well as changes in SCORAD, Spearman's rho was used. The analyses were accomplished by SPSS for Windows, version 11.5 (SPSS, Chicago, IL, U.S.A.).

Results

Calcidiol concentration before heliotherapy

Before HT 17 patients with AD (74%) had mild or moderate vitamin D insufficiency, showing serum calcidiol concentrations < 50 nmol L^{-1}. The lowest value was 27·4 nmol L^{-1}. In four patients, three of whom had received UV phototherapy 2–3 months earlier, the calcidiol concentration exceeded 80 nmol L^{-1}, and their results were assessed separately. Before HT the median dietary vitamin D intake of the 23 patients was 4·7 μg daily (range 1·3–10·8) and it was the same 1 month after HT; there were no statistically significant differences between the January and the March group. The intake of

vitamin D before HT was not associated with the initial calcidiol concentrations in the January group (r = 0·28), while there was a moderate correlation in the March group (r = 0·50).

Effect of heliotherapy on calcidiol concentration

In the January group (n = 8) the median personal UV dose received during the 2-week course of HT was 60 SED (Table 1). In the March group (n = 11) the UV dose was almost double this value, at 109 SED, although the duration of sunbathing was equal in both groups. Also the median increase in serum calcidiol concentration was almost double in the March group compared with the January group, at 24·0 nmol L^{-1} and 13·4 nmol L^{-1}, respectively. The increase was statistically significant in both groups ($P < 0·001$, $P = 0·008$, Table 1). Already at day 6 the increase in calcidiol concentration was almost significant in both the January and the March groups, at 4·8 nmol L^{-1} ($P = 0·055$) and 13·3 nmol L^{-1} ($P = 0·067$), respectively (Fig. 1). Two days of sunbathing in January and 1 day in March induced a small drop in the calcidiol concentration, but the decrease was statistically nonsignificant in both groups. Similarly, in the four patients with initial calcidiol concentrations > 80 nmol L^{-1} the concentration decreased on the first 2 days while thereafter it increased markedly in two of them (Fig. 2). There was no correlation between the initial calcidiol concentration and its increase either in the January group (r = −0·24) or in the March group (r = 0·16). Four patients (21%) were still vitamin D insufficient at the end of HT, showing serum calcidiol concentrations < 50 nmol L^{-1}. After HT the calcidiol concentration slightly decreased in the January group, while in the March group it continued to increase further (Fig. 1); both changes were statistically nonsignificant.

Effect of heliotherapy on atopic dermatitis

At the onset of HT the median SCORAD was 34 (range 15–41) in the January group and 30 (range 6–57) in the March

Table 1 Personal ultraviolet (UV) dose received during a 2-week heliotherapy course, and the effect of heliotherapy on serum calcidiol concentration and on SCORAD in patients with atopic dermatitis

		Median calcidiol (nmol L^{-1})				SCORAD, median (range)		
Patient group	Personal UV dose (SED), median (IQR)	At start	At end	Change in calcidiol (nmol L^{-1}), median (IQR)	P-value	At start	At end	P-value
January (n = 8)	60·2 (48·0–75·1)	42·9	56·4	13·4 (8·5–18·5)	0·008	34 (15–41)	9 (0–16)	0·008
March (n = 11)	109·3 (84·7–150·7)	42·4	62·3	24·0 (12·0–39·5)	< 0·001	30 (6–57)	9 (4–32)	0·002

SED, standard erythema doses; IQR, interquartile range.

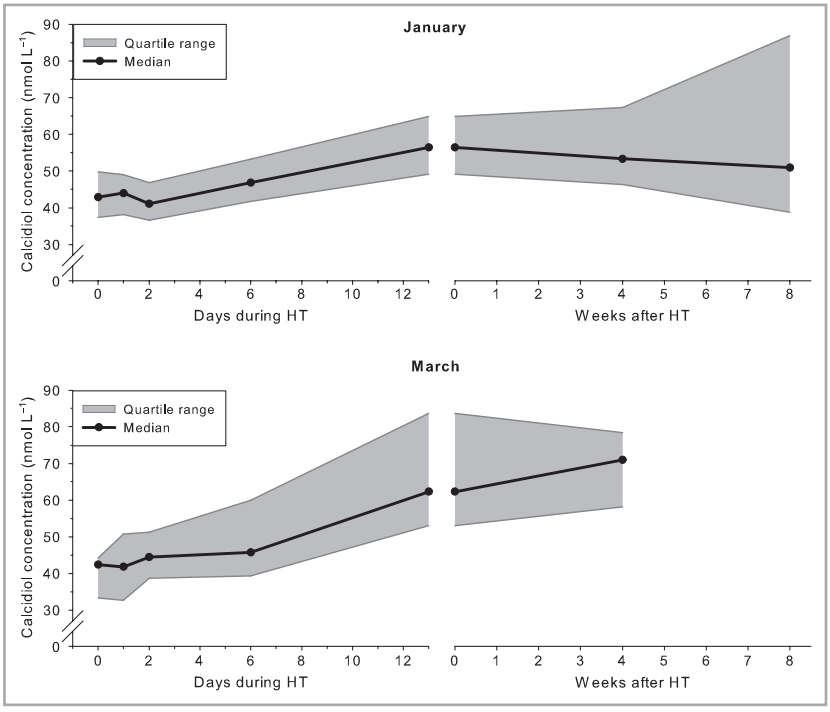

Fig 1. Calcidiol concentrations during and after a 2-week course of heliotherapy (HT) in patients with atopic dermatitis in January (n = 8) and March (n = 11).

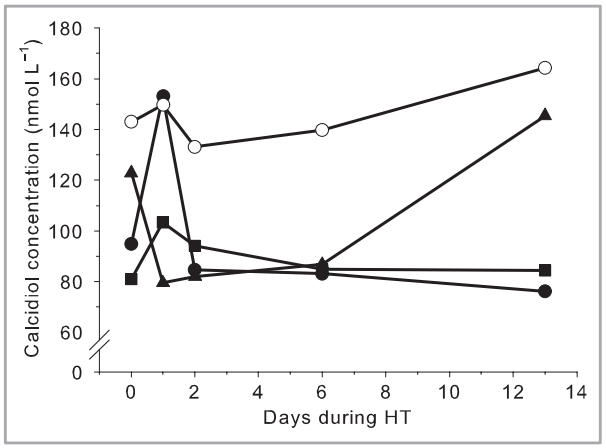

Fig 2. Calcidiol concentrations during a 2-week course of heliotherapy (HT) in four patients with atopic dermatitis with high initial calcidiol concentrations (> 80 nmol L^{-1}). One of the patients (open circles) received HT in March, the others in January.

group and at the end of HT it was 9 in both groups (Table 1). Accordingly, the net improvement of AD in the SCORAD was 74% ($P = 0{\cdot}008$) in the January group and 57% ($P = 0{\cdot}002$) in the March group. The initial SCORAD of the four patients with high calcidiol concentrations ranged from 22 to 48, and their scores also decreased markedly, ranging from 0 to 7 at the end of HT.

Personal ultraviolet dose, calcidiol concentration and SCORAD

In the March HT group, the personal UV dose received and the increase in serum calcidiol concentration showed a positive correlation ($r = 0{\cdot}63$, Fig. 3a) during the 2-week course of HT. No such correlation was found in the January group ($r = 0{\cdot}07$) although in both groups a UV exposure of 10 SED was equivalent to a median increase of 2·2 nmol L^{-1} in serum calcidiol. In the March group, but not in the January group, there was also a positive correlation between the personal UV dose and the improvement of the AD assessed using SCORAD ($r = 0{\cdot}67$, Fig. 3b). Similarly, there was a positive correlation between the increase of calcidiol concentration and improvement of SCORAD in the March ($r = 0{\cdot}50$) but not in the January group ($r = -0{\cdot}48$).

Discussion

Solar UV exposure is crucial for vitamin D synthesis and as much as 90% of all requisite vitamin D has to be formed in the skin. Calcitriol, the active form of vitamin D produced in the liver and kidney, but also in other tissues such as the skin or prostate, is considered to be an autocrine or paracrine hormone, which regulates various cellular functions including cell growth.[2] Due to this, vitamin D insufficiency seems to have much more extensive consequences than previously thought, ranging from well-known bone disease to prostate and other

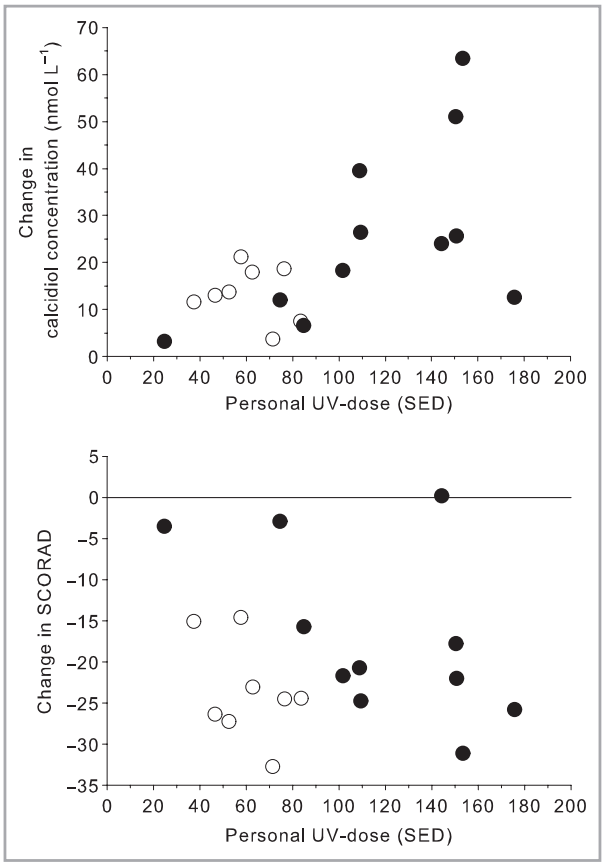

Fig 3. (a) Correlation between the personal ultraviolet (UV) dose in standard erythema doses (SED) and the change in calcidiol concentration during a 2-week course of heliotherapy in patients with atopic dermatitis. There was a marked correlation in the March group (closed circles; $r = 0{\cdot}63$) but not in the January group (open circles; $r = 0{\cdot}07$). (b) Correlation between the personal UV dose and the change in SCORAD. There was a marked correlation is in the March group ($r = 0{\cdot}67$) but not in the January group ($r = 0{\cdot}17$).

cancers and even to autoimmune diseases.[3,4] Knowledge of this has led to the on-going debate on the balance between the positive (vitamin D) and the negative (skin cancer) effects of sunlight.[23,24] In summer at latitude 42°N in Boston (MA, U.S.A.), 5–10 min of solar exposure three times a week on the hands, arms and face is sufficient to maintain vitamin D balance, but in winter very little, if any, vitamin D is produced in the skin.[1,11,12] In line with this, many people living in Northern Europe, like in Finland and Norway,[13–16] and possibly also in other northern European countries, have been found to have mild to moderate seasonal vitamin D insufficiency.

In the present study 17 of 23 (74%) patients with AD showed vitamin D insufficiency, i.e. their serum calcidiol concentration was < 50 nmol L^{-1} at the onset of the study. The 2-week course of HT in the Canary Islands proved to have a significant effect on serum calcidiol concentration which increased to an optimal level in 13 of 17 (76%) patients. The median increase was 13 nmol L^{-1} in the January group and

24 nmol L^{-1} in the March group. Interestingly, a 2-week HT course had a similar increasing effect on serum calcidiol concentration as fortifying milk products and margarine with vitamin D. This nationwide dietary intervention occurred in Finland in 2003 and in young men it increased the serum calcidiol concentration by a mean of 17 nmol L^{-1}.[16]

In the first 2 days of HT serum calcidiol concentration seemed to decrease a little (Fig. 1). This tendency was more apparent in the four patients with high initial calcidiol concentrations. Perhaps UV exposure is capable of destroying vitamin D in the skin, or alternatively of producing inert isomers when the vitamin D status is well saturated.[25] It has also been shown that when calcidiol concentration exeeds 100 nmol L^{-1} the synthesis of 24-hydroxylase increases which leads to inactivation of calcidiol.[26] An important finding was also that in the January group, after HT, the increased calcidiol concentration persisted at nearly the same level for the next 2 months. In the March group the calcidiol concentration continued to increase after HT. It is not known whether this could be due to outdoor exposure to sun in April or due to some other factors.

The improvement of AD was significant during the 2-week course of HT. This is in agreement with an earlier study.[10] The median decrease in SCORAD was 70%, i.e. about the same as that achieved with UVB phototherapy.[7,27] In the March group the personal UV dose received was double compared with the January group. The reason is mainly due to the difference in the zenith angle of the sun but the weather also was unfavourably cloudy in January. This might explain why in the March, but not in the January HT group, there was a clear correlation between the personal UV dose received and the improvement of SCORAD. Despite the lack of this correlation the patients healed well in the January group, implying that also factors other than UV dose alone seem to have an impact on improvement of AD during HT.

The present study was performed in the Canary Islands where a Finnish patient association has organized HT courses for many years. During HT specially educated nurses guide patients how to sunbathe according to an individual plan made by a dermatologist. Despite this, the personal cumulative UVB doses showed large variation because of different personal behaviour after the programmed sunbathing time. This indicates that it is much more difficult to standardize HT than the UVB treatments given in outpatient clinics at home. No major sunburns occurred during the present courses but the possible long-term effects of HT should be considered. A retrospective, nationwide cohort study of Danish patients with psoriasis who had received climatotherapy at the Dead Sea during 1972–93 showed an increased risk for basal cell and squamous cell carcinomas.[28] In contrast, a controlled cross-sectional study of 460 patients with psoriasis from the same treatment place did not find any increase in nonmelanoma skin cancers but it documented significantly more actinic damage in the patients with psoriasis than in controls.[29] We are not aware of any similar skin cancer studies in HT-treated patients with AD. Such studies are, however, warranted even though epidemiological or long-term follow-up studies of hospitalized patients with AD have not shown any evidence for increased nonmelanoma or other cancer risks.[30,31]

A recent study in mice showed that calcitriol is able, via signalling in keratinocytes, to induce an AD-like syndrome.[32] Whether this mechanism could somehow be implicated in the pathogenesis of AD should be examined, although it seems unlikely in humans. AD is known to be exacerbated in adults and also in children during winter,[33] when serum calcidiol concentration is at its lowest. Moreover, in the present study the March HT group showed a positive and the January group a negative correlation between the increase in calcidiol concentration and healing of AD. The question whether UV-induced calcidiol synthesis could have any influence on the healing of AD remains to be elucidated in further studies.

In conclusion, the present study showed that a 2-week course of HT is capable of significantly correcting serum calcidiol insufficiency and AD simultaneously. The positive effect of HT on vitamin D balance should be taken into account when considering the benefits and risks of HT for patients with AD.

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Spore Film Dosimeters Are Feasible for UV Dose Monitoring During Heliotherapy

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ABSTRACT

The objective of the study was to compare *Bacillus subtilis* spore film dosimeters with a Robertson Berger UV meter (RB meter) and diary records for assessing personal UV-B doses during a 13-day heliotherapy (HT) for atopic dermatitis (AD). In addition, the relationship between the personal UV-B dose and change in serum 25-hydroxyvitamin D (25(OH)D) was studied. Altogether 21 adult patients with AD completed the study arranged in the Canary Islands, either in January or March 2005. The spore film dosimeters were used throughout the day during the HT. Serum 25(OH)D was analyzed using radioimmunoassay. The mean personal UV-B dose measured with the dosimeters was 75 SED in January and 131 SED in March. The respective results gained from the RB meter combined with diary records were 63 SED and 119 SED showing a close correlation with the dosimeter results. Serum 25(OH)D concentration increased by 9.7 nmol L^{-1} in January and by 26.0 7 nmol L^{-1} in March. The increase in serum 25(OH)D correlated with the UV-B dose received. The patients complied well to use the dosimeters. We conclude *s*pore films to be a feasible and reliable personal UV dosimeter *in vivo* in field conditions.

INTRODUCTION

Since the 1970s various personal UV dosimeters have been used to monitor UV doses received from the sun. Such meters may take advantage of a chemical (1) or a biological (2) reaction, or they are based on an electronic (3) or a digital method. Also vitamin D has been introduced as a biologic UV dosimeter (4,5). Several dosimeter studies in healthy subjects have been conducted (6–10). Only few studies have assessed UV-B doses received during treatment of skin diseases, such as heliotherapy (HT) for psoriasis (PS), and these studies have mostly used broadband UV-B equipment (11–14). UV-B doses received during HT for atopic dermatitis (AD) have not been studied before. This might be important, as we have earlier shown that HT in the Canary Islands efficiently improves AD by decreasing the mean SCORAD index from 34 to 9 in January ($P = 0.008$) and from 30 to 9 in March ($P = 0.002$) (15).

The personal, solar UV-B dose is always an estimate, because no device is able to take into account all variables affecting skin exposure, such as clothing, sunscreen, motion, shadows, scattering and so on. Nilsen *et al.* (11) measured ambient UV-B doses during sunbathing hours using in parallel two broadband instruments and dividing the result by two. In another study the personal UV-B dose was defined as the ambient UV dose measured using a broadband UV meter during sunbathing hours (12). This dose was compared with the dose received in personal polysulphone dosimeters (12). Thieden *et al.* (16) showed that the wrist receives about half of the UV-B dose compared with the top of the head and concluded that the dose received in the wrist area could depict the average UV-B dose received in the skin.

In the present study we compared two methods for assessing personal UV-B doses received by patients with AD during a 2-week HT in the Canary Islands. *Bacillus subtilis* spore films were used as personal dosimeters and the ambient UV-B recordings of a Robertson-Berger type UV meter (RB meter) were combined with sun exposure data gained from personal diaries. The diary records included sunbathing hours, other hours outdoors, clothing and use of sunscreen. The RB meter results were also weighted for vitamin D production. Both the erythemally weighted and vitamin D weighted UV-B doses were compared with changes in serum 25(OH)D concentration during HT.

MATERIALS AND METHODS

Subjects. Altogether 25 patients with AD participated in the study either from 24 January to 4 February 2005 or from 12 March to 26 March 2005. The 2-week HT courses were implemented in Puerto Rico, Gran Canaria, Spain (30°N, 15°W). Patients with skin phototypes II or III (Fitzpatrick) were included. We excluded patients having had phototherapy, solarium, solar exposure or vitamin D supplementation in the preceding 2 months. Two females withdrew from the study for personal reasons, and one male and one female were

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excluded for losing their UV dosimeters. Accordingly, 21 patients (15 females and 6 males; mean age 37 years, range 21–57 years) were included in the analysis. The Ethics Committee of Päijät-Häme Central Hospital approved the study protocol and all volunteers gave their informed consent to participate.

Heliotherapy protocol. On the first day, the patients displaying skin Type II or severe AD (eight patients) sunbathed for 15 min and those with skin Type III for 30 min. On subsequent days the time to sunbathe was increased with 15 min steps up to 2 h, which was the maximum time to sunbathe on 1 day. The sunbaths were scheduled to be taken without sunscreens while all the other times strict sun protection was recommended.

Personal UV dosimeters. To measure the personal UV-B dose each patient was provided with one Type III *B. subtilis* spore film dosimeter (VioSpor blue line Type III; BioSense, Bornheim, Germany). The meter was attached either on the chest or on the upper arm, and it was worn from the morning to the evening for altogether 13 days. During sunbaths the dosimeter was placed beside the patient. Additionally, one dosimeter of Type II (VioSpor Blue line Type II) was worn on day 1 and another on day 2. The dosimeters of Type II are more photosensitive than those of Type III, the detectable minimum and maximum dose they measure are 1.0–55.0 SED and 1.5–90 SED, respectively. The dosimeters are 32 mm in diameter and function by UV-induced DNA damage to a film of dried bacterial spores (*B. subtilis*) (17,18). The bacterial spore together with an optical filter mimics the erythemal spectral sensitivity of human skin (18,19). The UV doses measured by the spore film dosimeters are given in ''biologically weighted'' MED, 1 MED being equivalent to 25 mJ cm^{-2} and 2.5 SED according to CIE 1987 (20–22). We used the SED, the internationally recognized unit to express the UV dose. For comparison, 10 spore film dosimeters (five of Type III and five of Type II) were placed beside the RB meter for varying exposure times.

Robertson Berger meter and weighting for vitamin D production. We recorded all available ambient solar UV-B radiation during the HT period using a Robertson Berger type broadband UV meter (RB meter) (Solar Light Model 501 UV-meter s/n 4845, Glenside). This was placed on the roof of a terrace close to the place where the patients took sunbaths as described in our previous work (15). The meter gives the ambient UV-B exposure as standard erythema doses (SED) at 10 min intervals. The meter is calibrated annually at the Radiation and Nuclear Safety Authority, Helsinki, Finland. The calibration uncertainty (2σ) of the broadband UV meter for erythemally weighted irradiance is 8% and the calibration is traceable to the National Institute of Standards and Technology, USA. In addition to the total ambient solar UV-B dose, we report the ambient solar UV-B dose recorded by the RB meter during sunbathing hours and during all outdoor hours of the patients.

The RB meter was additionally calibrated to measure the vitamin D production-weighted irradiance (23). The calibration factor has strong dependence on total ozone and solar zenith angle. Therefore, a calibration factor which depends on total ozone and solar zenith angle was used. The calibration method is similar to the calibration method suggested for erythemally weighted irradiance by working group 4 of COST 726 (24). Earth Probe TOMS overpass values for Izana (28°17′N, 16°30′W) were used for the total ozone (25). During the January HT the total ozone varied between 343 and 285 DU; the average value was 313 DU. During the March HT the total ozone varied between 266 and 321 DU; the average value was 302 DU. The UV spectra needed for the calculation of calibration factors were calculated with the FastRT UV simulating tool (26). The ambient UV-B dose recorded by the RB meter during sunbathing hours was weighted for vitamin D production.

Estimate of personal UV-B dose using the ambient solar UV-B dose and the diaries. The patients kept a diary of their exact time of sunbathing, hours spent outdoors and application of sunscreen as well as clothing used. The estimated personal UV-B dose to skin was calculated using this computerized diary data as described earlier (15). This dose gives an estimate of the cumulative UV-B dose received in the skin during the whole study period.

25(OH)D measurements. Blood samples were taken 1 day before HT and in the evenings of days 6 and 13. The samples were processed and serum 25(OH)D concentration measured by radioimmunoassay (Immunodiagnostic Systems, Boldon, UK) as described in our previous study (15).

Statistical methods. The agreement between measurements was calculated by using the concordance correlation coefficient (CCC). Scatter plots as well as Bland and Altman plots for each pair of assessments were provided to visualise the level of agreement in relation to the measurement scale. Confidence intervals for the concordance correlations were obtained by bias-corrected and accelerated bootstrapping (5000 replications). Correlation coefficients (r) were calculated by the Pearson method.

RESULTS

All available ambient solar UV-B radiation during the 13-day HT period using a RB meter was 203 SED in January and 493 SED in March. The daily solar exposure time increased gradually throughout the HT course and varied considerably among the patients. The mean cumulative time to sunbathe was 24.3 h in January and 21.6 h in March (range 7.7–43.5 h), and the mean respective ambient solar UV-B dose recorded was 65 SED (range 31–102 SED) in January and 112 SED (range 38–239 SED) in March. The mean cumulative time spent outdoors with strict sun protection of skin was 64.4 h in January and 62.4 h in March. The mean ambient solar UV-B dose during all outdoor hours including sunbathing was 135 SED in January and 270 SED in March.

The mean personal UV-B dose during 13 days of HT measured with Type III *B. subtilis* spore film dosimeters was 75 SED in January and 131 SED in March (Table 1). The mean estimated personal UV-B dose received in the skin and calculated using RB meter readings and diary records was 63 SED in January and 119 SED in March (Table 1). These personal UV-B doses compiled one-third to one-fourth of all available UV-B radiation during the study periods. When the results of January and March were combined, the Type II dosimeters worn only for 1 day showed a mean dose of 5.0 (0.6–10.6) SED on day 1 and 5.2 (1.3–13.0) SED on day 2. The respective doses calculated from RB meter and diary data were 2.8 (0.4–6.2) SED on day 1 and 4.8 (0.2–12.3) SED on day 2.

The mean vitamin D production-weighted UV-B dose derived from ambient recordings during sunbathing hours was 10.4 kJ m^{-2} (SD 3.1, range 4.9–16.5) in January and 17.6 kJ m^{-2} (SD 10.3, range 6.7–36.6) in March. During HT, the mean serum 25(OH)D increased by 9.7 nmol L^{-1} (SD 12.0 nmol L^{-1}) in January and by 26.0 nmol L^{-1} (SD 18.7 nmol L^{-1}) in March.

The concordance correlation between the personal UV-B dose measured by Type III *B. subtilis* spore film dosimeters and the estimated UV-B dose to skin, derived from the RB meter readings and diary data, was strong (CCC = 0.63, Fig. 1). The ambient solar UV-B doses recorded by the RB

Table 1. Mean personal UV-B doses received during a 13-day heliotherapy course for atopic dermatitis. Assessments made using *Bacillus subtilis* spore film dosimeters and a Robertson Berger meter combined with diary data showed close concordance correlation (CCC = 0.63).

Heliotherapy	Dosimeter, SED mean (SD, range)	RB meter and diaries, SED mean (SD, range)
January	75 (14, 48–94)	63 (16, 37–84)
March	131 (69, 44–225)	119 (44, 25–176)

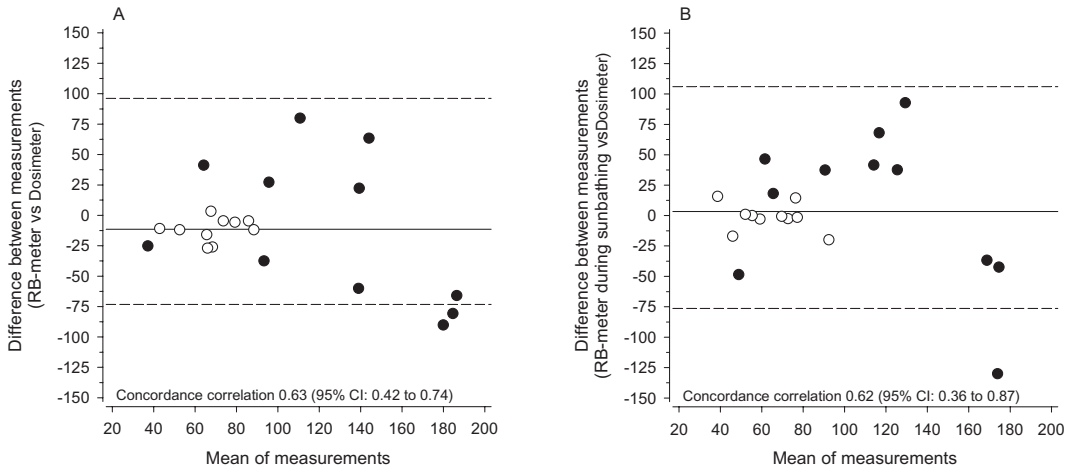

Figure 1. Bland–Altman plots for personal UV-B dose assessments using the RB meter combined with diary records *versus* dosimeter results (A) and using RB meter recordings during sunbathing hours *versus* dosimeter results (B). Dotted lines show 95% limits of agreement. Patients received heliotherapy either in January (empty circles) or in March (black circles).

meter during sunbathing hours were very similar to the all-day RB meter doses combined with diary data, indicating that after intentional sunbathing the UV protection was successful. The single day exposure readings for days 1 and 2 showed a moderate concordance correlation between the personal dosimeter and RB meter plus diary results (CCC = 0.42 and 0.38, respectively). In January and March, the ratio of outdoor hours with strict sun protection of skin *versus* sunbathing hours was 4.4 on day 1, 3.3 on day 2 and on average 2.8 during days 1–13.

The increase in serum 25(OH)D concentration clearly correlated with the personal UV-B doses measured with dosimeters ($r = 0.55$, Fig. 2A) and with the dose estimated using the RB meter and diary data ($r = 0.56$, Fig. 2B). A mean UV-B exposure of 7.6 SED in January, and 7.0 SED in March, measured by the dosimeter, was needed to increase serum 25(OH)D by 1 nmol L^{-1}. When the increase in serum 25(OH)D concentration and the vitamin D production-weighted UV-B dose during sunbathing hours was compared, the correlation was even better ($r = 0.72$, Fig. 2C).

All five Type II dosimeters placed beside the RB meter showed a good concordance with the RB meter readings. The respective dosimeter–RB meter ratios were 1.06, 1.01, 0.89, 1.07 and 0.77. Three Type III dosimeters were overexposed, as the ambient UV-B dose exceeded 200 SED causing saturation of the dosimeters. The remaining two Type III dosimeters gave slightly higher results than the RB meter showing a dosimeter–RB meter ratio of 1.46 and 1.21. These two meters expressed a dose close to 100 SED, exceeding the maximum optimal dose for the Type III dosimeters as informed by the manufacturer.

DISCUSSION

To our knowledge, our study is one of the first to monitor UV-B exposure and simultaneous vitamin D formation in field conditions as a part of regular HT for atopic patients. The close correlation between the personal UV-B dose measurements studied using *B. subtilis* spore film dosimeters and a RB meter combined with diary records during 13 days of HT surprised us. Both methods have various measurement errors differing from each other. The dosimeters measure the dose received on a specific skin site and take into account the shadows and motion, but not the effect of clothing and sunscreens. In contrast, the method using RB meter readings combined with diary data estimates the average UV-B dose received on the whole skin and takes into account the effect of clothing and sunscreens but ignores the effect of shadows and motion. However, depending on the garment, some exposure can also be received through the clothes (27,28), and this protectiveness of different fabrics against UV radiation was not taken into account in our estimated UV-B doses.

The personal UV-B dose measured with spore film dosimeters was 63 SED in January and 122 SED in March. This is in line with the results of climate therapy for PS in the Canary Islands (11,12) and at the Dead Sea (13). A 15-day climate therapy for PS in March led to a cumulative dose of 166 SED (11) and 1 month of climate therapy to 330 SED (12). However, the treatment protocol for PS included more sunbathing than that in the present study for AD.

As regards the use of personal dosimeters, the compliance of patients to wear them was high. The use of personal dosimeters was easy and practical as compared to the very time-consuming method of combining the RB meter and diary data. Only two dosimeters were lost during the study because the attachment system of the dosimeters (taped safety pins) in some cases tended to slip off. An arm strap might have worked out better. A prerequisite for the analysis of the personal UV dosimeters was the information on hours spent outdoors decreasing to some extent the feasibility of the meters. To our knowledge, the manufacturer needed the data to be able to choose the method of analysis. The single-day dosimeter results from day 1 and day 2 were clearly higher than those obtained from RB meter readings and diaries. This is because the dosimeters were carried throughout the day while on the first 2 days the time to actually sunbathe was very short. Thus, when measuring UV-B doses of short, intentional sunbathing periods, the dosimeters should be used only during the respective time period.

Vitamin D has also been introduced as a biodosimeter and *in vitro* models have been developed (4,5). However, *in vivo*, the

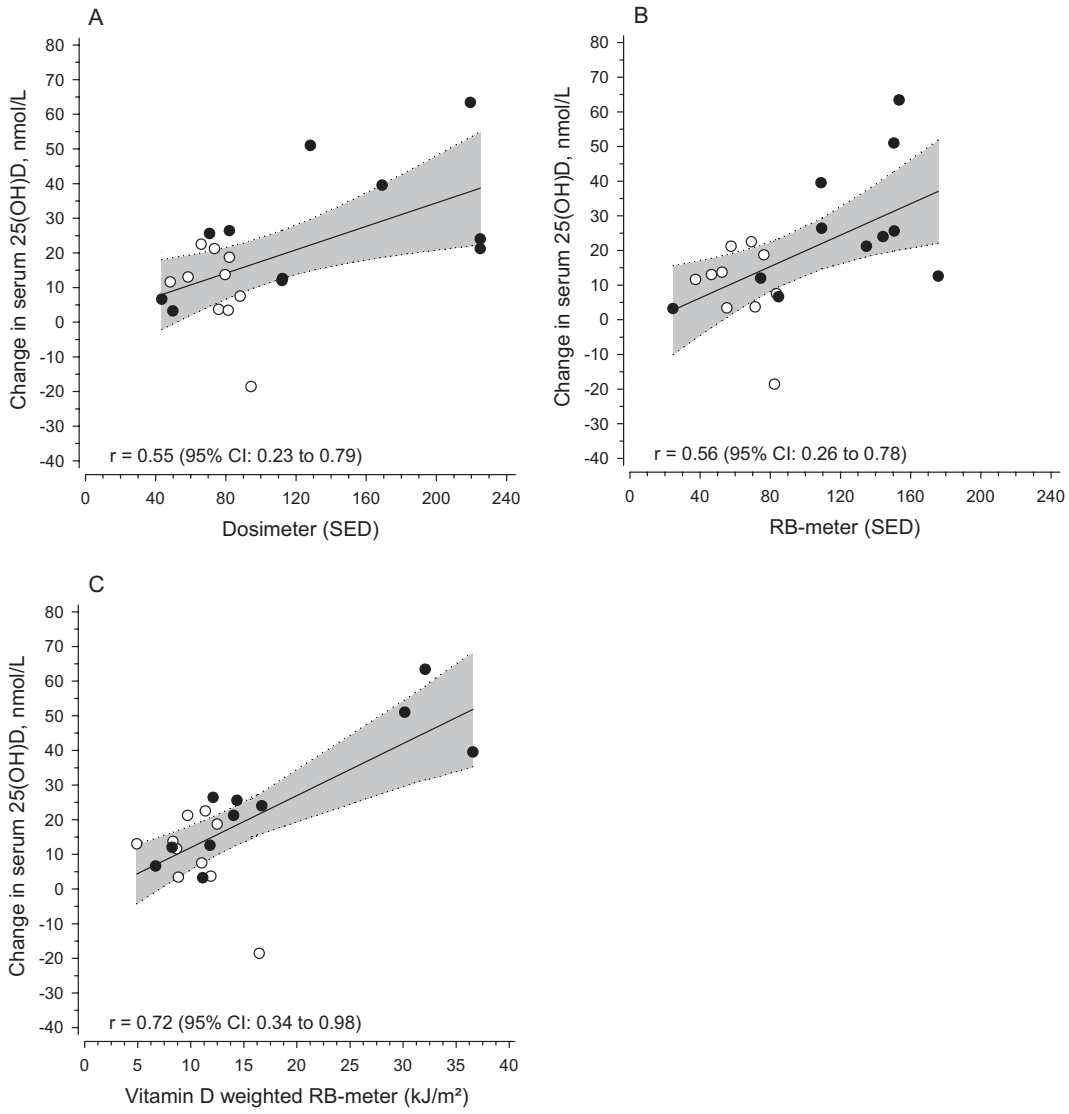

Figure 2. Relationship of serum 25(OH)D concentration against: (A) personal UV-B dose measured with spore film dosimeters; (B) personal UV-B dose estimated with RB meter combined with diary records; (C) vitamin D production-weighted UV-B dose derived from ambient RB meter recordings during sunbathing hours (kJ m^{-2}). The line shows the estimated linear regression. The gray bands are 95% confidence intervals.

photoproduction of vitamin D and its hydroxylation to 25(OH)D and other photoproducts is such a complex and carefully regulated process that estimation of UV-B dose on the basis of the increase in serum 25(OH)D concentration is prone to biases. Excess sunshine can lead to degradation of the newly formed vitamin D or alternatively to production of inert isomers (29). It has been shown that when the concentration of 25(OH)D exceeds 100 nmol L^{-1} the synthesis of 24-hydroxylase increases, which leads to inactivation of 25(OH)D (30). However, in the present study, there was a clear correlation between the increase in serum 25(OH)D concentration and the personal UV-B dose received. When comparing the increase in serum 25(OH)D concentration and the vitamin D-weighted UV-B dose, the correlation was even better. An increase in 25(OH)D was seen also in subjects with high initial 25(OH)D levels or high personal UV-B doses. Roughly each 7 SED increased serum 25(OH)D by 1 nmol L^{-1}. In previous narrow-band UV-B studies of ours only 13 SED during 1 week on the head and arms led to an increase of 11 nmol L^{-1} in serum 25(OH)D in healthy subjects (31) and 71.5 SED during 5 weeks increased serum 25(OH)D by 68.2 nmol L^{-1} in patients with AD (32). These findings suggest that, spectrally, narrow-band UV-B is a better light source than sunlight in inducing vitamin D synthesis. Another explanation could be that the patients received too much of sunlight during HT as regards the synthesis of vitamin D and this could have led to degradation of newly formed vitamin D.

In conclusion, the results of personal UVB doses achieved from *B. subtilis* spore film dosimeters and from RB meter and diary records showed a close correlation. The increase in serum 25(OH)D correlated with the UV-B dose received but because of the complex regulatory system of vitamin D synthesis, serum 25(OH)D seems not to be suitable in estimating UV-B doses. We regard that the spore film dosimeters are feasible both for laboratory studies as well as clinical work when an estimate of UV-B dose received is needed.

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Narrowband ultraviolet B course improves vitamin D balance in women in winter

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Summary

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Conflicts of interest
None declared.

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Background Vitamin D insufficiency is common in winter in the Nordic countries.
Objectives To examine whether a short course of narrowband ultraviolet B (NB-UVB) improves vitamin D balance.
Methods Fifty-six healthy, white women (mean age 41 years) volunteered and 53 completed the study. NB-UVB exposures were given on seven consecutive days either on the whole body (n = 19), on the head and arms (n = 9) or on the abdomen (n = 14). Similarly, seven solar simulator exposures were given on the face and arms (n = 11). The cumulative UVB dose was 13 standard erythema doses in all regimens. Serum calcidiol (25-hydroxyvitamin D) concentration was measured by radioimmunoassay before and after the NB-UVB exposures. Follow-up samples were taken from the whole-body NB-UVB group at 2 months.
Results At onset 41 women (77%) had vitamin D insufficiency (calcidiol < 50 nmol L^{-1}) and six (11%) had vitamin D deficiency (calcidiol < 25 nmol L^{-1}). Calcidiol concentration increased significantly, by a mean of 11·4 nmol L^{-1} when NB-UVB was given on the whole body, by 11·0 nmol L^{-1} when given on the head and arms and by 4·0 nmol L^{-1} when given on the abdomen. Solar simulator exposures given on the face and arms increased calcidiol by 3·8 nmol L^{-1}. After 2 months serum calcidiol was still higher than initially in the group who received NB-UVB exposures on the whole body.
Conclusions NB-UVB exposures given on seven consecutive days on different skin areas of healthy women significantly improved serum calcidiol concentration. A short low-dose NB-UVB course can improve vitamin D balance in winter.

An adequate vitamin D supply is crucial for bone health and it is hypothesized to be important in the prevention of certain cancers and autoimmune diseases.[1–3] Solar ultraviolet (UV) exposure is the major source of vitamin D and as much as 90% of all requisite vitamin D has to be formed in the skin.[4,5] The desirable circulating concentration of calcidiol (25-hydroxyvitamin D), which is the best indicator of vitamin D status,[1] is still under debate, but a concentration of 50–80 nmol L^{-1} is considered to be optimal for the skeleton.[6] At present vitamin D insufficiency or deficiency is common worldwide.[7] In the Nordic countries and Britain this condition frequently affects people especially in winter when vitamin D synthesis induced by the sun is zero.[8–11] In Finland fortification of milk and margarine with vitamin D has only partially improved vitamin D balance.[12] Recently, we found that 74% of patients with atopic dermatitis had vitamin D insufficiency in winter, i.e. their serum calcidiol was below 50 nmol L^{-1}.[13] A 2-week heliotherapy course in the Canary Islands healed atopic dermatitis and at the same time significantly improved serum calcidiol concentration.[13] Artificial UVB irradiation is also able to

induce vitamin D synthesis. Broadband UVB exposures have earlier shown to increase serum calcidiol in healthy subjects and in postmenopausal women with psoriasis.[14,15]

Narrowband UVB (NB-UVB) cabins equipped with TL01 tubes are now widely used in the treatment of psoriasis, atopic dermatitis and various other inflammatory skin diseases.[16,17] The output of NB-UVB is predominantly within wavelengths 311–13 nm, i.e. near the optimal wavelength, 297 nm, for vitamin D synthesis.[4] In the present study we examined whether a short course of NB-UVB would improve vitamin D balance in winter in healthy women. To study the response of various skin sites, NB-UVB exposures were given either on the whole body, on the head and arms or on the abdomen. For comparison, one group received exposures with a solar simulator on the face and arms.

Materials and methods

Subjects

Fifty-six healthy, white, female healthcare workers (nurses or doctors) or students (mean age 41 years, range 21–61; Table 1) with skin type II–III[18] volunteered to take part in the study. The study was performed in the Central Hospitals of Päijät-Häme and Kanta-Häme (location 67°N and 34 or 25°E) in winters (December–March) 2004–2006. The ethics committees of the hospitals approved the study protocol and all subjects gave informed consent to participate. Inclusion criteria were no phototherapy, solarium, sun holidays or vitamin D supplementation during the two preceding months. Three women withdrew from the study, one for personal reasons and two due to mild erythema after one or two NB-UVB exposures and they were excluded from the study.

Narrowband ultraviolet B and solar simulator exposures

NB-UVB or solar simulator exposures were given on seven consecutive days. The UVB dose was 1 standard erythema dose (SED) on the first day and thereafter 2 SED on each of the following 6 days. The daily dose was below 1 minimal erythema dose (MED), as 2 SED corresponds to 1/3–1 MED in white-skinned people depending on their skin phototype.[19] One SED is equivalent to 10 mJ cm^{-2} CIE erythema-weighted irradiance.[20]

Group I ($n = 19$) received NB-UVB exposures on the whole body with a round Waldmann UV 7001 cabin (Waldmann, Villingen-Schwenningen, Germany) equipped with 20 TL01 tubes located 30 cm away from the subject (Table 1). Group II ($n = 9$) received NB-UVB exposures on the head and arms, i.e. the T-shirt-free area, with a similar Waldmann UV 7001 cabin equipped with 40 TL01 tubes. Group III ($n = 14$) received NB-UVB on the abdomen with a Waldmann UV 801KL panel equipped with four TL01 tubes. The exposed area was 25×40 cm and the distance from the tubes 20 cm. Group IV ($n = 11$) received solar simulator exposures on the face and arms from a Philips HB411 panel (Philips, Eindhoven, the Netherlands) equipped with a broadband UVB lamp (HPA 400). The subjects sat in front of the panel and held their forearms and upper arms beside their face. The distance from the panel was 30 cm. The nonexposed skin areas were carefully protected from radiation using a thick, white T-shirt and trousers made of 65% polyamide and 35% cotton. The penetration of UVB rays through this clothing measured by a spectroradiometer (Ocean Optics S2000; Ocean Optics, Dunedin, FL, U.S.A.) was negligible, 0·15%.

The spectral irradiances of the lamps measured with an Ocean Optics S2000 single-monochromator spectroradiometer are shown in Figure 1. The spectroradiometer was placed into the round cabin at the same distance, 30 cm, from the tubes as is the skin when the exposed subject is standing in the cabin. The height of the measurement point was 110 cm. Similarly, the spectroradiometer was placed in front of the flat irradiators at the same distance from the tubes as the exposed skin. The exact time to receive a dose of 2 SED was determined in all regimens. When the stray light and other systematic errors are corrected, the estimated measurement uncertainty (2σ) is 14%[21] and the measurements are traceable to National Institute of Standards and Technology, U.S.A.

Calcidiol measurements

Blood samples for serum calcidiol measurements were taken before the first NB-UVB or solar simulator exposure and there-

Table 1 Characteristics of the study groups receiving narrowband ultraviolet B (NB-UVB) or solar simulator exposures on seven consecutive days

	Group I NB-UVB whole body	Group II NB-UVB head and arms	Group III NB-UVB abdomen	Group IV solar simulator face and arms
Number of women	19	9	14	11
Age (years), mean (range)	42 (21–57)	44 (22–58)	35 (21–58)	47 (28–61)
Skin phototype[a] II/III	9/10	3/6	11/3	6/5
Dietary vitamin D intake at onset (μg daily), mean ± SD	5·9 ± 3·3	7·2 ± 5·5	7·7 ± 3·5	6·4 ± 2·8

[a]According to Fitzpatrick.[18]

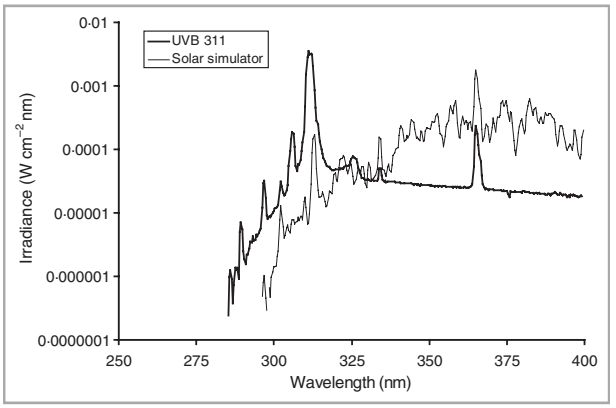

Fig 1. Spectral irradiances of the narrowband ultraviolet B (UVB) equipment (UVB 311; Waldmann UV 7001 cabin or 801KL panel with TL01 tubes; Waldmann) and solar simulator (Philips) used in the study. The optimal wavelength for vitamin D synthesis in the skin is 297 nm.

after 24 h after the first, third and seventh exposure. In addition, 14 subjects from group I gave follow-up samples at 2-week intervals up to 2 months.

The serum samples were protected from light, centrifuged and then stored at −20 °C. Calcidiol concentration was analysed in duplicate by using radioimmunoassay (Immunodiagnostic Systems, Boldon, U.K.) as in our previous study.[13] A calcidiol concentration < 50 nmol L^{-1} was regarded as vitamin D insufficiency and < 25 nmol L^{-1} as deficiency.[6]

The dietary intake of vitamin D was determined by a semiquantitative food frequency questionnaire[22] before and 1 month after the study.

Statistical methods

The changes in serum calcidiol concentration in the four study groups were analysed by using a permutation test. The changes in serum calcidiol concentration between the study groups were analysed by a bootstrap type analysis of covariance with the baseline values as covariables. The 95% confidence intervals were obtained by bias-corrected and accelerated bootstrapping (5000 replications), because of the skewed distribution of the variables.

Results

Serum calcidiol and dietary intake of vitamin D at onset of the study

At onset of the study the mean dietary intake of vitamin D was 6·6 μg daily (range 0·2–18·5; Table 1) and the mean serum calcidiol concentration was 39·1 nmol L^{-1} (range 16·4–81·6; Table 2). Forty-one subjects (77%) had vitamin D insufficiency (calcidiol < 50 nmol L^{-1}) and six (11%) had vitamin D deficiency (calcidiol < 25 nmol L^{-1}). The serum calcidiol concentration correlated positively with the dietary intake of vitamin D ($r = 0·30$, $P = 0·011$).

Effect of narrowband ultraviolet B or solar simulator exposures on serum calcidiol

Seven NB-UVB or solar simulator exposures caused a statistically significant increase in serum calcidiol concentration in all four study groups (Table 2, Fig. 2). Exposing the whole body to NB-UVB caused the highest increase, 11·4 nmol L^{-1}. Nearly the same increase, 11·0 nmol L^{-1}, was seen when NB-UVB was given only on the head and arms. Already after three NB-UVB exposures the increase of serum calcidiol was significant in both of these groups, i.e. 7·3 nmol L^{-1} and 6·8 nmol L^{-1}. Seven NB-UVB exposures given on the abdomen increased serum calcidiol by 4·0 nmol L^{-1}. Seven solar simulator exposures given on the face and arms caused an increase of 3·8 nmol L^{-1} (Table 2, Fig. 2). The increase in serum calcidiol in the four groups was not due to changes in dietary intake of vitamin D as it remained the same 1 month after the NB-UVB course as before it ($P = 0·66$).

Fourteen women in the group receiving NB-UVB on the whole body were followed up to 2 months. The highest mean serum calcidiol (65·2 nmol L^{-1}) was seen 2 weeks after the NB-UVB course (Fig. 3). Thereafter, serum calcidiol slowly decreased but still, 2 months after the NB-UVB course, it was

Table 2 Serum calcidiol response to seven narrowband ultraviolet B (NB-UVB) or solar simulator exposures. The cumulative UVB dose was 13 standard erythema doses in all four groups

	Mean serum calcidiol (nmol L^{-1})			
Group	At onset, mean (range)	After seven exposures, mean (range)	Change in serum, mean (95% CI[a])	P-value[b]
I. NB-UVB whole body (n = 19)	44·3 (24·5–81·6)	55·8 (34·5–85·4)	11·4 (8·9–14·1)	< 0·001
II. NB-UVB head and arms (n = 9)	37·8 (21·8–71·4)	48·8 (32·0–92·9)	11·0 (6·2–16·7)	0·0046
III. NB-UVB abdomen (n = 14)	35·1 (16·4–49·1)	39·2 (23·6–55·6)	4·0 (1·4–6·3)	0·011
IV. Solar simulator face and arms (n = 11)	38·1 (22·7–58·2)	41·9 (27·2–59·1)	3·8 (0·8–7·6)	0·038

[a]CI, confidence interval. CIs for the means were obtained by bias-corrected and accelerated bootstrapping (5000 replications). [b]Permutation test.

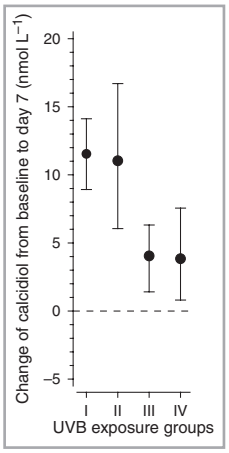

Fig 2. Change in serum calcidiol concentration after seven narrowband ultraviolet B (NB-UVB) or solar simulator exposures. NB-UVB exposures were given either on the whole body (group I, n = 19), on the head and arms (group II, n = 9) or on the abdomen (group III, n = 14) and solar simulator exposures on the face and arms (group IV, n = 11). Mean values are marked with dots and 95% confidence intervals by bars.

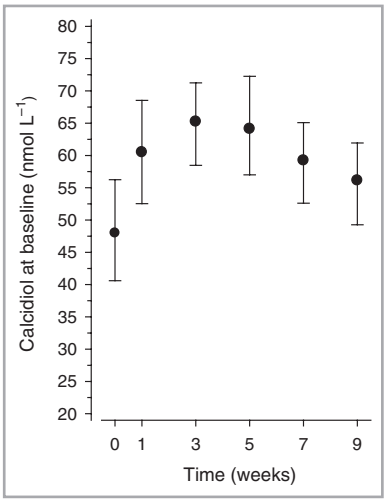

Fig 3. Serum calcidiol concentration at onset and up to 2 months after seven narrowband ultraviolet B (NB-UVB) exposures on the whole body in 14 women. Mean values are marked with dots and 95% confidence intervals by bars. The highest calcidiol concentration was measured at week 3, i.e. 2 weeks after the NB-UVB course.

markedly higher (56·1 nmol L^{-1}) than at onset of the study (48·0 nmol L^{-1}). Seven, four and only one of the 14 women had vitamin D insufficiency at onset, at the end of the NB-UVB course and 2 weeks after the NB-UVB exposures, respectively.

Discussion

In the present study we showed that a 7-day course of NB-UVB significantly increased serum calcidiol concentration, i.e. markedly improved vitamin D balance in healthy, Finnish women in winter. The increase in serum calcidiol was 11·4 nmol L^{-1} when NB-UVB was given on the whole body and 11·0 nmol L^{-1} when given on the head and arms only. We are not aware of similar studies with NB-UVB in healthy subjects. Recently, Czarnecki[23] treated seven patients with psoriasis with 18 NB-UVB exposures and, in agreement with the present results, found markedly increased serum calcidiol in all patients. Similarly, a mean of over 20 broadband UVB exposures both in postmenopausal women with psoriasis and other psoriatic patients showed a significant increase in serum calcidiol.[15,24] In the light of our present study with healthy subjects and the study of Armas *et al.*[14] it is, however, apparent that psoriatic patients could also obtain a significant increase in serum calcidiol with markedly fewer than 20 exposures. When making a comparison with sun exposure, it is of interest that in the present study the increase in serum calcidiol after seven NB-UVB exposures was about the same as that achieved by patients with atopic dermatitis during a 2-week heliotherapy course in winter in the Canary Islands.[13]

In the present study the response in serum calcidiol was almost the same whether NB-UVB was given on the whole body or only on the head and arms. This was surprising, as the latter consists of only one-fourth of the total body area. Both groups received NB-UVB with a Waldmann 7001 cabin, and the cumulative UVB dose was the same, i.e. 13 SED. One explanation could be that the skin areas most often exposed to the sun, i.e. the face and arms, might have a more rapidly activated and effective system for vitamin D synthesis than other skin areas. The exposed groups were, however, rather small and differed both in size and in initial serum calcidiol concentration. Due to this the result should be confirmed in a further study. Also direct comparisons with calcidiol responses after exposure of the abdomen with NB-UVB or solar simulator exposure of the face and arms should be avoided, because the exposures in these regimens were given with a panel of lamps and not in a round cabin as in the two other regimens. However, if one estimates that the exposed abdominal area was 6–10% and the T-shirt-free area 20–25% of the total body area, a comparison of the results of these groups suggests that the abdominal skin is nearly as effective in producing vitamin D as is the skin of the head and arms. There arises a question whether there is a certain saturation point in the capacity to hydroxylate vitamin D to calcidiol. The important result is, however, that all four regimens with a cumulative dose of 13 SED significantly increased serum calcidiol.

After the NB-UVB course given on the whole body, serum calcidiol concentration was followed up in 14 women up to 2 months. Interestingly, serum calcidiol continued to increase and it was at its highest 2 weeks after the last NB-UVB exposure. The study was performed in the middle of winter, so sun exposure could not affect this result. Unfortunately – for practical reasons – the other groups were not followed up. Apparently, despite the minimal UVB doses, the UVB exposures caused reversible photoisomerization of both previtamin D_3 and vitamin D_3 to biologically inert sterols, which afterwards were mobilized and further converted to calcidiol.[25,26]

This self-regulating system inhibits overdosing of vitamin D by UVB. One month after the NB-UVB course serum calcidiol had decreased somewhat but at 2 months it was still markedly higher than initially. A similar long-lasting increase in serum calcidiol was also seen in our previous heliotherapy study.[13]

Vitamin D insufficiency or deficiency is common in winter in healthy adults in the Nordic countries and in Britain.[8–11,13] In agreement with this, as many as 88% of the women in the present study had serum calcidiol < 50 nmol L^{-1} before the NB-UVB exposures. The women were doctors, nurses and other healthcare workers who have better knowledge than most people about healthy food and the need for vitamin D in winter. Their dietary vitamin D intake was somewhat lower than the recommended intake, 7·5 μg daily,[27] indicating that even after fortification of milk and margarine people do not get enough vitamin D from food in winter in Finland.[12] Similarly, in Britain, the prevalence of hypovitaminosis D was recently found to be alarmingly high during winter and spring in a cohort of 45-year-old adults and the authors recommended actions both at population level and in at-risk groups.[11] Vitamin D supplements, such as cod liver oil and multivitamin products, are used to improve vitamin D balance. To obtain recently recommended calcidiol concentrations of > 75 nmol L^{-1} a dietary intake of vitamin D of 17–20 μg daily is required.[28,29] Thus the current dietary recommendation is too low. The present study shows that a short course of NB-UVB is one possible way to improve vitamin D balance in women and presumably also in men in winter. Seven suberythemal NB-UVB exposures rapidly increased serum calcidiol and the response was long-lasting, i.e. it persisted partly up to 2 months. The time to receive 2 SED of NB-UVB in a Waldmann 7001 cabin is short, below 1 min. Theoretically, tens of subjects could be handled during a day with one NB-UVB cabin for the treatment or prophylaxis of vitamin D insufficiency. To find out the optimal NB-UVB protocol to improve vitamin D insufficiency or deficiency clearly warrants a further study. Different NB-UVB doses and schedules, e.g. daily compared with one to three times a week, should be investigated to find out which dosage gives the maximum response in serum calcidiol. A study comparing NB-UVB course vs. oral vitamin D replacement would also be of importance.

The safety aspect of NB-UVB includes the risk for skin cancer.[30] Several authors consider NB-UVB less risky than broadband UVB.[16] In agreement with this, a recent British study of nearly 4000 patients found no significant association between NB-UVB treatment and squamous or basal cell carcinoma, or malignant melanoma.[30] In regard to cancer risk, the treatment of psoriasis or atopic dermatitis with NB-UVB usually needs at least 20 exposures with a cumulative dose of 80–100 SED. These doses are markedly higher than the 13 SED used in the present study, suggesting that vitamin D balance can be improved with a short NB-UVB course unlikely to increase skin cancer risks greatly. Moreover, the dose of 2 SED, which is usually below 1 MED,[19] was well tolerated and only two women got mild erythema in the present study.

To conclude, the present study shows that a short course of NB-UVB is an effective way to improve vitamin D balance in healthy women in winter. To find the optimal NB-UVB schedule and to show whether a NB-UVB course is effective also in at-risk groups such as postmenopausal women and old people having risk for fractures warrants further studies.

What's already known about this topic?

- An adequate vitamin D supply is crucial for bone health and it is hypothesized to be important in the prevention of certain cancers and autoimmune diseases.
- Vitamin D insufficiency is common in winter in the Nordic countries and in Britain.

What does this study add?

- Narrowband ultraviolet B exposures given on seven consecutive days on different skin areas of healthy women significantly improved serum calcidiol (25-hydroxyvitamin D) concentration.
- A short low-dose narrowband ultraviolet B course can improve vitamin D balance in winter.

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Narrowband ultraviolet B treatment improves vitamin D balance and alters antimicrobial peptide expression in skin lesions of psoriasis and atopic dermatitis

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Conflicts of interest
None declared.

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Summary

Background Narrowband ultraviolet B (NB-UVB) is a routine treatment for psoriasis and atopic dermatitis (AD) but its effect on vitamin D balance is not well studied.

Objectives To examine whether NB-UVB treatment in winter improves vitamin D balance in psoriasis and AD, and to study the effects of NB-UVB on antimicrobial peptide and cytokine expression in the skin.

Methods Eighteen adult patients with psoriasis, 18 with AD and 15 healthy subjects received a total of 15 NB-UVB exposures on the whole body, given three times a week. Serum calcidiol (25-hydroxyvitamin D) was measured by radioimmunoassay. Antimicrobial peptide and cytokine expression in skin lesions was examined by real-time quantitative polymerase chain reaction.

Results At onset 16 (89%) patients with psoriasis, 17 (94%) patients with AD and eight (53%) healthy subjects had vitamin D insufficiency (calcidiol < 50 nmol L^{-1}). NB-UVB treatment significantly increased ($P < 0{\cdot}001$) serum calcidiol. The increase was 59·9 nmol L^{-1} (95% confidence interval, CI 53·5–66·9) in psoriasis, 68·2 nmol L^{-1} (95% CI 55·4–80·1) in AD and 90·7 nmol L^{-1} (95% CI 63·8–123·4) in healthy subjects. Psoriasis Area and Severity Index and SCORAD improved significantly ($P < 0{\cdot}001$) but no correlation to the increase of serum calcidiol was found. Cathelicidin and human β-defensin 2 (HBD2) expression was high in skin lesions of psoriasis. After six NB-UVB treatments cathelicidin increased further while HBD2 expression decreased. A similar trend was observed in AD lesions. NB-UVB caused a marked but nonsignificant decrease of interleukin (IL)-1β and IL-17 in psoriasis lesions.

Conclusions The present study shows that in addition to a significant improvement of psoriasis and AD, NB-UVB treatment effectively corrects vitamin D insufficiency. It also increases cathelicidin and decreases HBD2 levels in healing skin lesions of psoriasis and AD. This effect might be mediated by improved vitamin D balance and the local cytokine network.

At present vitamin D insufficiency is common worldwide.[1] In the Nordic countries and Britain this condition frequently affects people especially during winter when vitamin D synthesis induced by the sun is zero.[2–5] The desirable concentration of serum calcidiol (25-hydroxyvitamin D), which is the best indicator of vitamin D status, is still under debate but a concentration of 50–80 nmol L^{-1} is considered to be optimal for the skeleton.[6,7] Recently, it was shown that heliotherapy in the Canary Islands in winter significantly improved serum calcidiol both in Finnish patients with atopic dermatitis (AD) and in Swedish patients with psoriasis (PS).[8,9] Also artificial broadband ultraviolet (UV) B irradiation has been shown to

increase serum calcidiol in postmenopausal women with PS.[10] We recently showed that a short low-dose narrowband UVB (NB-UVB) course is an alternative to improve vitamin D balance in healthy women in winter.[11]

Vitamin D is important not only for the health of the bones but also an association between vitamin D deficiency and the incidence or unfavourable prognosis of a broad variety of diseases, such as various types of cancer (e.g. colon, prostate and breast cancer), autoimmune diseases, infectious diseases and cardiovascular diseases, has been described in a number of studies.[1,12,13] However, these associations need further studies to be confirmed. Vitamin D has long been known to affect skin inflammation and cutaneous innate or adaptive immune responses.[14] In particular, calcitriol (1,25-dihydroxyvitamin D) is a major factor involved in the regulation of the antimicrobial peptide cathelidicin.[15] This and another inducible cutaneous antimicrobial peptide, human β defensin 2 (HBD2), can act as proinflammatory mediators or 'alarmins' and link adaptive and innate immune responses.[16] Recent studies suggest that defensins and cathelicidin have a role in the pathogenesis of skin inflammation in PS. In particular, increased gene copy numbers of the β-defensins correlate with the risk of developing this disease.[17] In addition, cathelicidin peptide LL-37, which is increased in psoriatic skin, induces an autoinflammatory cascade leading to skin inflammation.[18,19] Thus, vitamin D seems to have different functions in addition to maintaining skeletal health. However, even if the only function of vitamin D is shown to be regulation of calcium homeostasis, it should be mandatory to prevent vitamin D insufficiency worldwide.

The aim of the present study was to examine whether NB-UVB treatment for patients with PS or AD improves their vitamin D balance. As antimicrobial peptides are directly involved in the pathogenesis of inflammatory skin diseases, we also studied whether NB-UVB treatment has an effect on the expression of cathelicidin, HBD2 and various cytokines in psoriatic and atopic skin lesions.

Materials and methods

Subjects, narrowband ultraviolet B exposures and calcidiol measurements

Altogether 56 adult subjects representing Fitzpatrick skin types II and III volunteered and 51 completed the study. The ethics committee of the Tampere University Hospital approved the study protocol and all subjects gave informed consent to participate. Inclusion criteria were no phototherapy, solarium, sun holidays or vitamin D supplementation during the preceding 2 months. The study was conducted during January–March in 2008 and 2009. Eighteen patients with PS (eight women and 10 men; mean age 48 years), 18 with AD (nine women and nine men; mean age 33 years) and 15 healthy doctors, nurses or other healthcare workers (15 women; mean age 42 years) received a total of 15 NB-UVB exposures, given three times a week, on the whole body with a Waldmann UV 7001 cabin (Waldmann, Villingen-Schwenningen, Germany) equipped with 20 TL-01 tubes. The initial NB-UVB dose for all participants was 0·13 J cm^{-2} corresponding to one standard erythema dose (SED). Thereafter the dose was gradually increased up to 1·19 J cm^{-2} (9·5 SED). The mean cumulative UVB dose received after 15 NB-UVB exposures was 8·88 J cm^{-2} which corresponds to 71·5 SED. The clinical improvement was measured in PS by Psoriasis Area and Severity Index (PASI) and in AD by SCORAD. Two subjects withdrew from the study for personal reasons, two due to skin reactions (erythema or photosensitivity) and one because of pregnancy.

Blood samples for serum calcidiol measurements were taken before the first NB-UVB treatment and then 2 days after the sixth and 15th NB-UVB exposures. In addition, a follow-up sample was taken 1 month after the NB-UVB course. The serum samples were protected from light, centrifuged and then stored at −20 °C. Serum calcidiol concentration was analysed in duplicate by using radioimmunoassay (Immunodiagnostic Systems, Boldon, U.K.) as in our previous study.[8] Calcidiol concentration < 50 nmol L^{-1} was regarded as vitamin D insufficiency and < 25 nmol L^{-1} as deficiency.[7]

Skin biopsies and real-time quantitative polymerase chain reaction

Punch biopsies were taken from the same representative skin lesions from patients with PS ($n = 7$) or AD ($n = 8$) immediately before the first and seventh NB-UVB exposures. Skin biopsies from healthy subjects ($n = 7$) served as controls. The biopsies were immediately frozen and stored at −70 °C before being examined.

Total RNA from biopsies was isolated using Eurozol Reagent (EuroClone, Milan, Italy). One microgram of RNA was reverse transcribed with High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, U.S.A.) to cDNA as previously described.[20] The expression of cathelicidin and HBD2 was evaluated using a LightCycler® 2.0 system and the corresponding human Universal Probe Library Set (Roche, Basel, Switzerland). The primers were designed by an algorithm on http://www.universalprobelibrary.com and hydroxymethylbilane synthase (*HMBS*; previously known as porphobilinogen deaminase) was used as housekeeping gene in a duplex real-time quantitative polymerase chain reaction (qPCR). *HMBS* was chosen because it, cathelicidin and HBD2 belong to a low-abundance class of mRNAs. Analysed genes and corresponding primers are listed in Table 1. Fold induction relative to the healthy volunteers was calculated as previously described.[21] Results were considered significant when at least a twofold difference in expression levels was detected and statistical analysis revealed $P < 0·05$.

Real-time qPCR for various cytokines and chemokines was performed with PerfeCTa qPCR FastMix (Quanta Biosciences, Gaithersburg, MD, U.S.A.) in the ABI Prism 7700 Sequence Detector System (Applied Biosystems). PCR primers and probes for cytokines and chemokines were obtained as

Table 1 Target genes and corresponding primers for cathelicidin and human β-defensin 2 (HBD2) in real-time quantitative polymerase chain reaction

Target gene	Forward	Reverse
Cathelicidin	5′-TCGGATGCTAACCTCTACCG-3′	5′-ACAGGCTTTGGCGTGTCT-3′
HBD2	5′-TCAGCCATGAGGGTCTTGTA-3′	5′-GGATCGCCTATACCACCAAA-3′

predeveloped assay reagents [interleukin (IL)-1β, IL-4, IL-10, IL-17A, interferon (IFN)-γ, transforming growth factor (TGF)-β, tumour necrosis factor (TNF)-α, CCL20 (macrophage inflammatory protein-3a, MIP-3a)] or they were self-designed [β-actin, CCL17 (thymus and activation regulated chemokine, TARC)] and ordered from Applied Biosystems.

Statistics

The changes in serum calcidiol concentration, PASI and SCORAD were analysed by a permutation test with Monte-Carlo *P*-value. Confidence intervals (CIs) for the changes were obtained by bootstrapping (5000 replications). Cathelicidin and HBD2 expression in PS and AD skin lesions before and after NB-UVB treatment was compared with that in healthy controls using the Mann–Whitney test. Comparison of cathelicidin and HBD2 expression in the untreated and NB-UVB-treated skin lesions was performed with the Wilcoxon matched pairs test. Statistical analysis of cytokine expression in PS skin vs. AD skin was performed with the nonparametric Mann–Whitney test.

Results

Narrowband ultraviolet B treatment and serum calcidiol

At onset the mean ± SD serum calcidiol concentration was 36·8 ± 12·45 nmol L^{-1} in patients with PS, 32·2 ± 12·2 nmol L^{-1} in patients with AD and 60·5 ± 21·8 nmol L^{-1} in healthy subjects (Fig. 1). Sixteen (89%) patients with PS, 17 (94%) with AD and eight (53%) healthy controls had vitamin D insufficiency (calcidiol < 50 nmol L^{-1}). Of these subjects five with PS and seven with AD had vitamin D deficiency (calcidiol < 25 nmol L^{-1}).

The NB-UVB course significantly ($P < 0·001$) increased serum calcidiol in PS, AD and healthy subjects (Fig. 1). After 15 NB-UVB exposures serum calcidiol increased by a mean of 59·9 nmol L^{-1} (95% CI 53·5–66·9) in PS, by 68·2 nmol L^{-1} (95% CI 55·4–80·1) in AD and by 90·7 nmol L^{-1} (95% CI 63·8–123·4) in healthy subjects. There was no difference in the increase of serum calcidiol between these three groups ($P = 0·21$). One month after the NB-UVB course calcidiol was nearly at the same elevated level in PS and AD but showed some decrease in the healthy subjects (Fig. 1).

The NB-UVB course improved PASI from 8·0 (range 3·5–16·1) to 3·6 (range 0·7–9·0) in PS ($P < 0·001$). The SCORAD improved from 37·1 (range 12·9–74·0) to 14·2 (range 4·8–41·2) in AD ($P < 0·001$). The clinical improvement (mean PASI reduction −4·1, mean SCORAD reduction −22·9) did not correlate to the increase of serum calcidiol either in PS [$P = 0·43$, $r = −0·20$ (95% CI −0·65 to 0·29)] or in AD [$P = 0·08$, $r = 0·42$ (95% CI −0·13 to 0·80)].

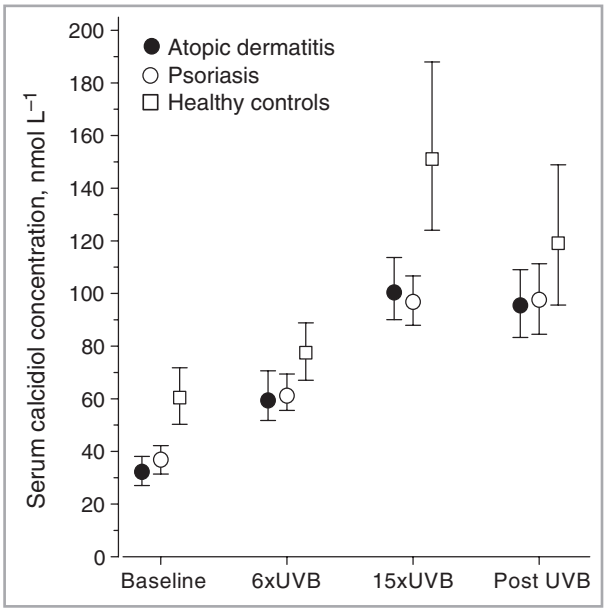

Fig 1. Effect of narrowband ultraviolet B (NB-UVB) treatment on serum calcidiol concentration. Fifteen NB-UVB exposures significantly increased serum calcidiol concentration (mean; 95% confidence intervals) in 18 patients with psoriasis, 18 patients with atopic dermatitis and 15 healthy subjects ($P < 0·001$). The serum calcidiol level was still elevated in patients and healthy subjects 1 month after the last NB-UVB exposure.

Expression of antimicrobial peptides cathelicidin and human β-defensin 2

Analysis of mRNA expression levels of cathelicidin before NB-UVB treatment revealed significantly elevated levels of cathelicidin in lesional PS skin compared with the skin of healthy subjects (Fig. 2a). In AD skin lesions cathelicidin was also expressed but the increase was not significant (Fig. 3a). After six NB-UVB treatments cathelicidin expression increased markedly in PS and slightly in AD (Figs 2a and 3a). However, neither observation reached statistical significance.

Before NB-UVB treatment HBD2 mRNA expression levels were also significantly upregulated in skin lesions of PS and AD compared with healthy controls (Figs 2b and 3b). NB-UVB treatment significantly reduced HBD2 expression in PS lesions (Fig. 2b) and also in AD lesions (Fig. 3b) but this change did not reach statistical significance.

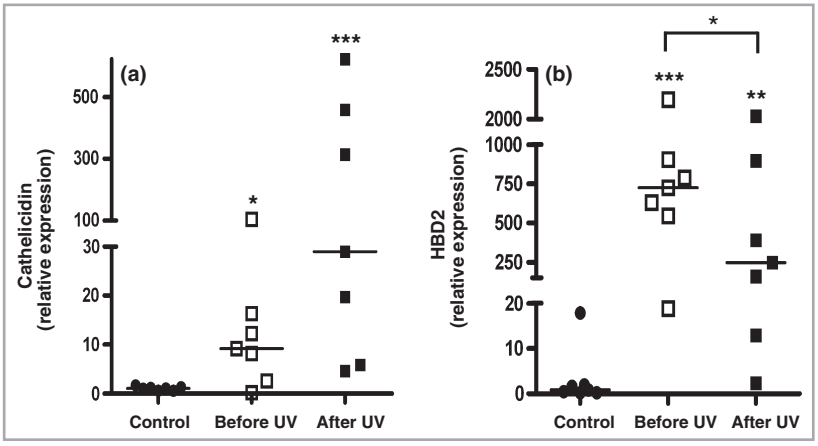

Fig 2. Expression of cathelicidin and human β-defensin 2 (HBD2) in psoriasis. Biopsies of a marker psoriatic plaque (n = 7) were taken before and after six narrowband ultraviolet (UV) B treatments. Total mRNA was extracted and transcript levels of cathelicidin (a) and HBD2 (b) analysed by real-time quantitative polymerase chain reaction. Statistical analysis of psoriatic skin before and after UV treatment vs. healthy (nonpsoriatic) controls (n = 7) was performed with the Mann–Whitney test. Comparison of treated and untreated biopsies was performed using the Wilcoxon matched pairs test. *P < 0·05, **P < 0·01, ***P < 0·001.

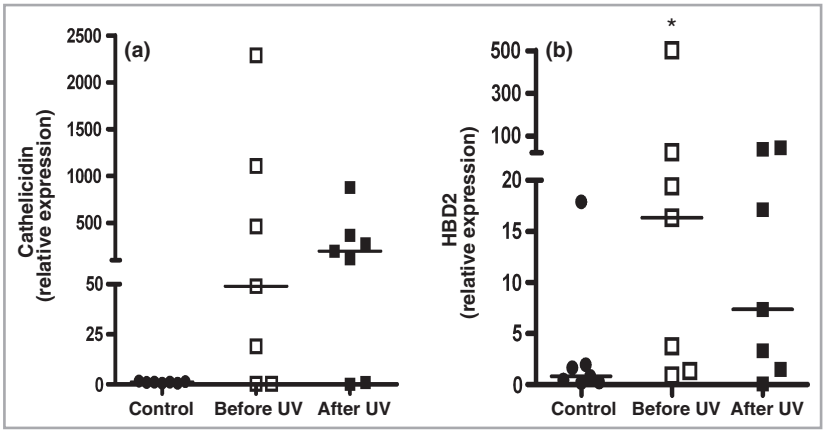

Fig 3. Expression of cathelicidin and human β-defensin 2 (HBD2) in atopic dermatitis. Biopsies of atopic dermatitis skin lesions (n = 7) were taken before and after six narrowband ultraviolet (UV) B treatments. Total mRNA was extracted and transcript levels of cathelicidin (a) and HBD2 (b) analysed by real-time quantitative polymerase chain reaction. Statistical analysis of atopic dermatitis skin before and after UV treatment vs. healthy controls (n = 7) was performed with the Mann–Whitney test. Comparison of treated and untreated biopsies was performed using the Wilcoxon matched pairs test. *P < 0·05.

Cytokine and chemokine expression

Before NB-UVB treatment PS lesions expressed significantly higher amounts of IL-1β (P < 0·01), IL-17A (P < 0·01) and IFN-γ (P < 0·05) compared with AD lesions (Fig. 4). CCL17 (TARC) was significantly (P < 0·05) higher in AD lesions whereas IL-10, TGF-β1, TNF-α and CCL20 (MIP-3a) were expressed similarly in both diseases. IL-4 expression was basically negative in PS and AD lesions.

After six NB-UVB treatments expression of IL-1β and IL-17A was markedly reduced in PS lesions (Fig. 4). However, these changes were not significant. NB-UVB treatment did not change the expression of IL-4, IL-10, IFN-γ, TGF-β1, TNF-α, CCL17 (TARC) or CCL20 (MIP-3a) (Fig. 4).

Discussion

The present study performed in winter showed low concentrations of serum calcidiol in patients with PS and AD. The same observation was made in our earlier heliotherapy study for AD.[8] In the present study the subjects were consecutive patients referred for NB-UVB treatment in our department. Almost all patients had vitamin D insufficiency and some of them even had vitamin D deficiency (calcidiol < 25 nmol L^{-1}). Vitamin D insufficiency was not as pronounced in the healthy subjects. They were educated doctors, nurses and other personnel from our department who have better knowledge of healthy food and vitamin D sources than people on average. However, 53% of them also had vitamin D insufficiency. These results clearly

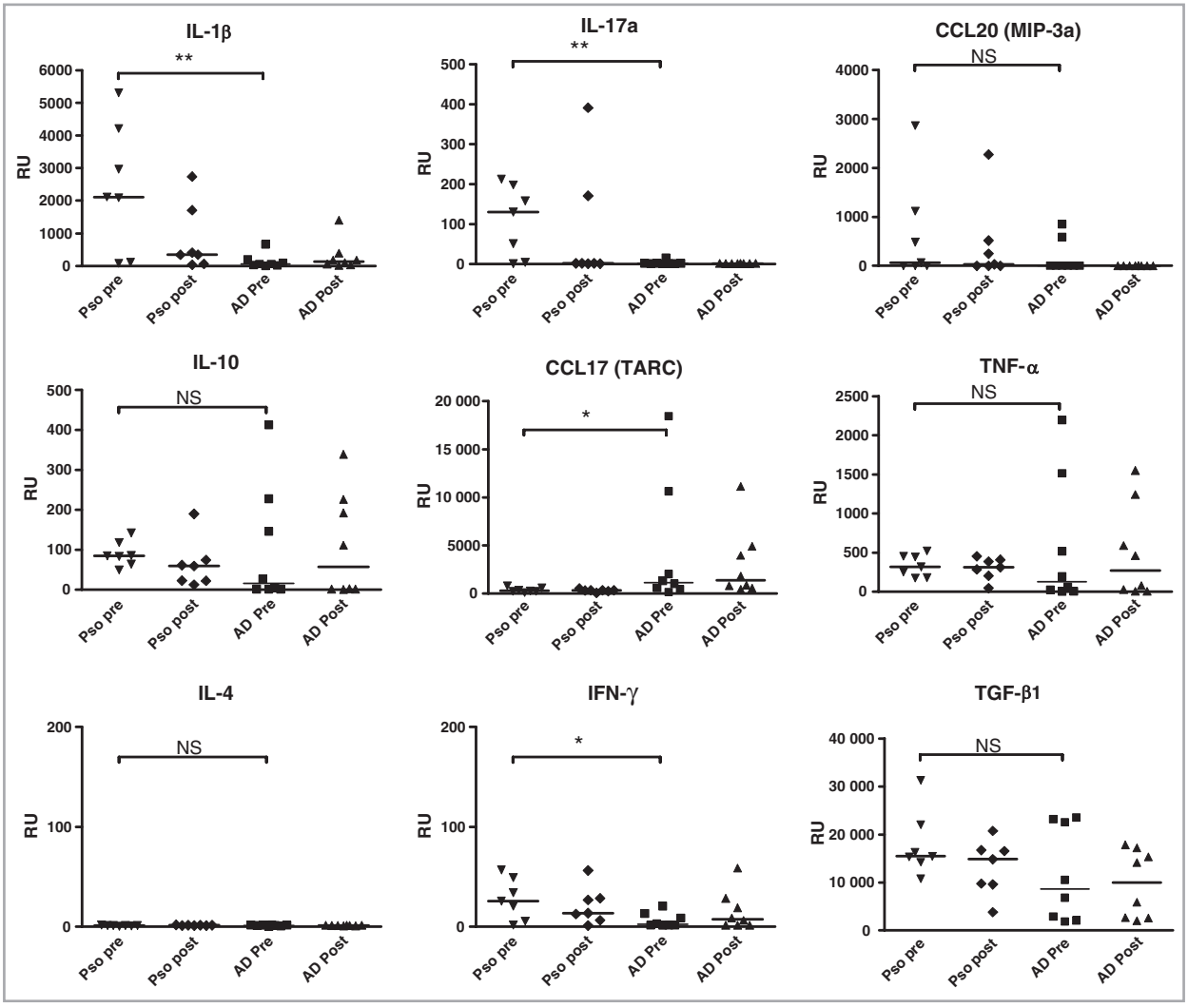

Fig 4. Expression of cytokines and chemokines in the skin lesions of psoriasis (Pso) and atopic dermatitis (AD) before and after narrowband ultraviolet B (NB-UVB) treatment. Real-time quantitative polymerase chain reaction was used to analyse the mRNA expression levels of different cytokines and chemokines in Pso ($n = 7$) and AD ($n = 8$) skin lesions before and after six NB-UVB treatments. Relative units (RU) are expressed as fold differences relative to the calibrator (β-actin). IL, interleukin; MIP, macrophage inflammatory protein; TARC, thymus and regulation activated chemokine; TNF, tumour necrosis factor; IFN, interferon; TGF, transforming growth factor. Statistical analysis of Pso skin vs. AD skin was performed with the nonparametric Mann–Whitney U-test. NS, not significant, *$P < 0{\cdot}05$, **$P < 0{\cdot}01$.

show that seasonal vitamin D insufficiency or deficiency continues to be very common in Finland and that this condition also affects patients with PS and AD.[3,22]

Fifteen NB-UVB treatments given with a similar protocol that we usually use to treat AD significantly increased serum calcidiol in all three study groups. In agreement with this, two recent studies in patients with PS have shown that NB-UVB exposures markedly increase serum calcidiol.[23,24] In the study of Osmancevic *et al.*[24] the patients received a mean of 28 NB-UVB treatments. However, the calcidiol response was not as high as in the present study. The reasons for this could be differences in the patient material, rather high initial concentration of serum calcidiol, previous UVB treatments and the variable length of the treatment period. In the present study 15 NB-UVB treatments significantly improved PASI in PS and SCORAD in AD. The clinical improvement did not, however, correlate to the increase of serum calcidiol.

The NB-UVB course increased serum calcidiol in the healthy subjects similarly to the patients with PS and AD. This confirms our previous observation that a short NB-UVB course is an efficient way to increase low serum calcidiol in winter.[11] In that study healthy subjects received NB-UVB exposures on seven consecutive days. However, seven exposures given on consecutive days did not increase serum calcidiol as much as six exposures in the present study although the cumulative UVB dose was nearly the same. This suggests that calcidiol synthesis is more effective when NB-UVB exposures are given every second day compared with daily exposures. More studies should be performed to confirm the most effective NB-UVB protocol.

After the NB-UVB course serum calcidiol was followed up for 1 month. At this time point, serum calcidiol was still elevated in the patients with PS and AD, as in our previous study with healthy subjects.[11] These results show that a short course of NB-UVB is a possible and easy way to improve vitamin D balance in winter. Fifteen suberythemal NB-UVB exposures every second or third day rapidly increase serum calcidiol, and this effect is long lasting. The time to receive two SED of NB-UVB in a Waldmann 7001 cabin is short, < 1 min. Theoretically, tens of subjects could be handled during a day with one NB-UVB cabin to treat low serum calcidiol effectively during winter.

In wintertime, if no UVB exposure is available, vitamin D supplements should be used to improve vitamin D balance. In a recent Swedish study vitamin D supplements increased serum calcidiol by 11·0 nmol L^{-1} and a sun vacation by 14·5 nmol L^{-1}.[4] In previous Finnish studies the daily dietary vitamin D intake was about 7·5 μg in winter.[11,22] This meets the current Finnish recommendation for vitamin D intake, but it seems not to be enough to maintain a sufficient vitamin D balance. To obtain recently recommended calcidiol concentrations of > 75 nmol L^{-1} a dietary intake of vitamin D of 17–20 μg daily is required.[25,26] This is hard to receive without supplements in winter as for only 7·5 μg per day one needs to consume, for example, fish two or three times a week together with having 6 dL of vitamin D-fortified milk daily and five or six sandwiches with vitamin D-fortified margarine. A study comparing the effects and costs of oral vitamin D substitution with a short NB-UVB course in the treatment of vitamin D insufficiency would be of importance.

Antimicrobial peptides such as cathelicidin and HBD2 can act as proinflammatory mediators or 'alarmins' in the skin and link adaptive and innate immune responses.[15] Recent studies suggest a role for the defensins and cathelicidin in the pathogenesis of skin inflammation in PS and AD.[17,27] Indeed, we found increased expression of these antimicrobial peptides in the skin lesions of PS and AD, confirming earlier results.[17,28] We also showed that repeated treatment with NB-UVB reduced HBD2 expression in healing PS and AD skin lesions. Similar HBD2 findings have previously been reported in AD skin lesions treated by NB-UVB.[28–30] Gläser *et al.*[31] recently showed an increase of HBD2 after a short UV exposure to healthy skin whereas we found decreasing HBD2 levels in the NB-UVB-treated healing PS and AD lesions. At the same time we saw a significant increase in serum calcidiol, the best indicator of the hormonally active form of vitamin D (calcitriol, i.e. 1,25-dihydroxyvitamin D) which exerts potent anti-inflammatory actions through inhibition of NFκB activation, and inhibits IL-17A-induced HBD2 in keratinocytes *in vitro*.[32] NB-UVB is known to induce local synthesis of calcidiol and calcitriol in keratinocytes.[33] Both local and systemic vitamin D metabolism are triggered by the same stimulus. We propose that the effects of NB-UVB-triggered vitamin D production might outweigh the HBD2-inducing effect of short-term UVB in our study population.

In contrast to HBD2, UVB treatment does not induce cathelicidin in keratinocytes *in vitro*[21] but induces cathelicidin hCAP18 expression in healthy skin *in vivo*.[34] Calcitriol is the only factor known to date which induces cathelicidin in skin epithelial cells.[15] Treatment of healthy skin with active vitamin D or the application of vitamin D analogues to PS skin induces cathelicidin.[30] Cathelicidin expression thus correlates with local vitamin D concentrations. Cathelicidin expression, however, did not correlate with the disease activity in the present study as NB-UVB decreased skin inflammation but increased cathelicidin transcript abundance. In particular, increased cathelicidin in PS should lead to more skin inflammation as cathelicidin LL-37 has been suggested to serve as a proinflammatory signal in PS.[18,19] LL-37 binds self-DNA and the LL-37/self-DNA complexes trigger the activation of plasmacytoid dendritic cells in the skin and an immune response in PS.[18] These contrasting observations need to be further investigated in future studies. Possible explanations include anti-inflammatory activities of vitamin D, such as inhibition of T-cell recruitment or inhibition of dendritic cell activation, which might be responsible for the immunosuppressive effects of NB-UVB observed in this study.[35]

PS and AD both cause local inflammation but their cellular mechanism and effects on the cytokine milieu are different in the skin lesions of these patients. PS consists of chronic inflammation including activation of Th1 but also Th17 cytokines whereas, depending on the stage of the disease, Th1 cytokines, Th2 cytokines and CCL17 (TARC) dominate in AD.[36–38] Supporting the previous results, we found significantly higher amounts of IL-1β, IFN-γ and IL-17A in psoriatic skin lesions, whereas the hallmark cytokine CCL17 (TARC) was highly expressed in AD.

This is the first study in which NB-UVB treatment has been shown to reduce expression of IL-1β and IL-17A in psoriatic skin lesions. IL-1β is a proinflammatory cytokine that is processed from its inactive form (pro-IL-1β) to its active form by microbial products and metabolic stress, leading to enhanced lymphocyte activation and destruction of local tissues. In addition to the proinflammatory function, IL-1β is suggested to be a critical mediator of the differentiation of human T cells producing IL-17.[39,40] In turn, IL-17 is shown to enhance vitamin D_3-induced expression of the antimicrobial peptide cathelicidin.[32] Although the expression of cathelicidin did not immediately decrease along with IL-17A in this study, lowered IL-17 levels may later decrease inflammation and neutrophil activation in lesional skin sites.[41,42] Our results suggest that the interplay between cytokines and vitamin D in the regulation of antimicrobial peptides in keratinocytes of cutaneous inflammatory diseases is complex and only partly understood. As these molecules exert cytokine-like functions the analysis of their regulatory pathways in inflammatory skin disease deserves further study.

In conclusion, the present study showed that, in addition to clinical response, a short NB-UVB course effectively corrects vitamin D insufficiency in patients with PS and AD and in healthy subjects. NB-UVB treatment altered HBD2 and cathe-

lidicin expression in healing skin lesions, which might be mediated by improved vitamin D balance and cytokine network.

What's already known about this topic?

- Two studies in patients with psoriasis (PS) have shown that narrowband ultraviolet B (NB-UVB) exposures markedly increase serum calcidiol. We recently showed that a short low-dose NB-UVB course is an alternative to improve vitamin D balance in healthy women in winter.
- Recent studies suggest a role of the defensins and cathelicidin in the pathogenesis of skin inflammation in PS and atopic dermatitis (AD). Calcitriol is involved in the regulation of cathelicidin.

What does this study add?

- Fifteen NB-UVB treatments significantly increased serum calcidiol in patients with AD or PS and in healthy subjects. The effect persisted for at least 1 month.
- Repeated treatment with NB-UVB reduced the initially increased expression of the antimicrobial peptide human β-defensin 2 in healing PS and AD lesions. Simultaneously serum calcidiol increased significantly.
- NB-UVB treatment reduced the expression of interleukin (IL)-1β and IL-17A in psoriatic skin lesions.

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