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Narrow-band Ultraviolet B Exposures Improve Vitamin D Balance

Trials Involving Dermatological and Haemodialysis Patients and Healthy Subjects

ACADEMIC DISSERTATION
To be presented, with the permission of
the Board of the School of Medicine of the University of Tampere,
for public discussion in the Small Auditorium of Building M,
Pirkanmaa Hospital District, Teiskontie 35,
Tampere, on November 22nd, 2013, at 12 o'clock.

UNIVERSITY OF TAMPERE

ACADEMIC DISSERTATION
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Acta Universitatis Tamperensis 1870
ISBN 978-951-44-9260-0 (print)
ISSN-L 1455-1616
ISSN 1455-1616

Acta Electronica Universitatis Tamperensis 1351
ISBN 978-951-44-9261-7 (pdf)
ISSN 1456-954X
http://tampub.uta.fi

Suomen Yliopistopaino Oy – Juvenes Print
Tampere 2013

To all those who contribute vitamin D research

Contents

1 LIST OF ORIGINAL PUBLICATIONS

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

I Vähävihu K*, **Ala-Houhala M***, Peric M, Karisola P, Kautiainen H, Hasan T, Snellman E, Alenius H, Schauber J, Reunala T. Narrowband ultraviolet B 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.

II **Ala-Houhala MJ**, Vähävihu K, Hasan T, Kautiainen H, Ylianttila L, Viljakainen HT, Snellman E, Reunala T. Comparison of narrowband ultraviolet B exposure and oral vitamin D substitution on serum 25-hydroxyvitamin D concentration. *Br J Dermatol* 2012; 167:160–4.

III **Ala-Houhala MJ**, Vähävihu K, Hasan T, Kautiainen H, Snellman E, Karisola P, Dombrowski Y, Schauber J, Saha H, Reunala T. Narrow-band ultraviolet B exposure increases serum vitamin D levels in haemodialysis patients. *Nephrol Dial Transplant* 2012; 27:2435–40.

IV **Ala-Houhala MJ**, Vähävihu K, Snellman E, Hasan T, Kautiainen H, Karisola P, Dombrowski Y, Schauber J, Saha H, Reunala T. A narrow-band ultraviolet B course improves vitamin D balance and alters cutaneous CYP27A1 and CYP27B1 mRNA expression levels in haemodialysis patients supplemented with oral vitamin D. *Nephron Clin Pract* 2013; 124:17–22.

* Equal inputs from both authors.

2 ABBREVIATIONS

AMP	antimicrobial peptide
BB-UVB	broad-band ultraviolet B
BMI	body mass index
CIE	Commission Internationale de l'Eclairage
CKD	chronic kidney disease
CYP24A1	24-hydroxylase (25-hydroxyvitamin D-24-hydroxylase)
CYP27A1	25-hydroxylase (vitamin D-25-hydroxylase)
CYP27B1	1α-hydroxylase (25-hydroxyvitamin D-1α-hydroxylase)
GFR	glomerular filtration rate
HBD2	human β-defensin 2
MED	minimal erythema dose
mRNA	messenger ribonucleic acid
NB-UVB	narrow-band ultraviolet B
$1,25(OH)_2D$	1,25-dihydroxyvitamin D (calcitriol)
25(OH)D	25-hydroxyvitamin D (calcidiol)
PASI	psoriasis area and severity index
PCR	polymerase chain reaction
PTH	parathyroid hormone
SED	standard erythema dose
SCORAD	severity scoring of atopic dermatitis
UV	ultraviolet
VDBP	vitamin D binding protein
VDR	vitamin D receptor
Vitamin D_2	ergocalciferol
Vitamin D_3	cholecalciferol

3 ABSTRACT

Narrow-band ultraviolet B (NB-UVB) phototherapy is used to treat dermatological diseases such as psoriasis and atopic dermatitis. Some previous studies have suggested that it also increases serum 25-hydroxyvitamin D (25(OH)D) concentrations. On the other hand, most patients with chronic kidney disease (CKD) requiring dialysis are known to have insufficient vitamin D. We therefore conducted trials to assess how short NB-UVB courses could affect serum 25(OH)D concentrations in dermatological and haemodialysis patients in winter, when little UVB from the sun is available for vitamin D synthesis. In addition, we compared the effects of an NB-UVB course and oral vitamin D supplementation on serum 25(OH)D concentrations in healthy subjects.

In the first trial (I), 89% of the patients with psoriasis, 94% of those with atopic dermatitis and 53% of the healthy subjects were found to have baseline vitamin D insufficiency (serum 25(OH)D <50 nmol/L). A course of 15 whole body NB-UVB exposures significantly increased serum 25(OH)D ($p < 0.001$), by 59.9 nmol/L in the psoriasis patients, 68.2 nmol/L in the atopic dermatitis patients and 90.7 nmol/L in the healthy subjects. PASI (psoriasis area and severity index) and SCORAD (severity scoring of atopic dermatitis) improved significantly ($p < 0.001$), but no correlation with the increase in serum 25(OH)D was found. Expression of antimicrobial peptides (AMPs), cathelicidin and human β-defensin 2 (HBD2) was high in the psoriasis skin lesions. After 6 NB-UVB treatments cathelicidin had increased further, while HBD2 expression had decreased. NB-UVB caused a marked but non-significant decrease in the cytokines interleukin (IL)-1β and IL-17 in the psoriasis lesions. It was concluded that, in addition to a significant improvement of psoriasis and atopic dermatitis, NB-UVB treatment effectively corrects vitamin D insufficiency. It also increases cathelicidin and lowers HBD2 levels in healing psoriasis and atopic dermatitis skin lesions. This effect may be mediated by the improved vitamin D balance and the local cytokine network.

In the second trial (II) healthy adult hospital employees and medical students having serum 25(OH)D below 75 nmol/L were randomly given either a course of 12 whole body NB-UVB exposures or 20 μg of oral cholecalciferol daily for 4 weeks. The baseline serum 25(OH)D concentrations were similar in both groups: 52.9 nmol/L in the 33 NB-UVB-treated subjects and 53.5 nmol/L in the 30 treated with oral cholecalciferol. The mean increase in serum 25(OH)D was 41.0 nmol/L in the NB-UVB group and 20.2 nmol/L in the cholecalciferol group, the difference being significant at 2 weeks ($p = 0.033$) and at 4 weeks ($p < 0.001$). Two months after the treatments the 25(OH)D

concentrations had decreased in both groups but were still clearly higher than the baseline values. It was concluded that 12 NB-UVB exposures given over 4 weeks increase the serum 25(OH)D concentration significantly more than do daily doses of 20 μg oral cholecalciferol. A short, low-dose NB-UVB course is therefore an effective way of improving the vitamin D balance in winter, and the response is still evident 2 months after the course.

In the third trial (III) fifteen haemodialysis patients and twelve healthy subjects received nine upper body NB-UVB exposures. Mean serum 25(OH)D levels before NB-UVB were 32.5 nmol/L in the dialysis patients and 60.2 nmol/L in the healthy subjects ($p < 0.001$). After eight NB-UVB exposures serum 25(OH)D had increased by 13.8 nmol/L (43%; $p < 0.001$) in the dialysis patients and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) by 3.3 pmol/L (27%; $p = 0.002$). Serum 25(OH)D in the dialysis patients was still 10% higher two months after NB-UVB exposures than initially. The mRNA expression level of CYP27B1, an enzyme needed for the final hydroxylation of vitamin D to its active metabolite, was examined in skin biopsy specimens and was found to have increased after NB-UVB exposures relative to the level in non-treated healthy subjects ($p = 0.04$). It was concluded that a short course of NB-UVB exposures significantly increases serum 25(OH)D and $1,25(OH)_2D$ in dialysis patients, but the effect is short-lasting suggesting that patients need cyclic NB-UVB exposures to maintain their improved vitamin D concentrations.

In the fourth trial (IV) fourteen haemodialysis patients and fifteen healthy subjects receiving oral cholecalciferol supplements of 20 μg daily were given nine whole body NB-UVB exposures. Given baseline serum 25(OH)D concentrations of 57.6 nmol/L in the dialysis patients and 74.3 nmol/L in the healthy subjects the NB-UVB course increased serum 25(OH)D significantly ($p < 0.001$), by 14.0 nmol/L in the former and 17.0 nmol/L in the latter. The dialysis patients showed significantly increased baseline CYP27B1 levels and decreased CYP27A1 levels in the skin relative to the healthy subjects. It was concluded that a short NB-UVB course is an efficient way of improving the vitamin D balance in dialysis patients who are receiving oral vitamin D supplementation. The increased cutaneous CYP27B1 levels in the dialysis patients suggest that the loss of the renal activity of this enzyme is at least partially compensated for in the skin.

To conclude, NB-UVB exposures were shown to be an efficient way of increasing the serum 25(OH)D concentration in dermatological and dialysis patients and in healthy subjects in winter. A short NB-UVB course was shown to increase serum 25(OH)D in healthy subjects significantly more than did daily supplementation with 20 μg oral cholecalciferol. NB-UVB courses offer a new possibility for improving the vitamin D balance in dialysis patients who have an insufficiency of this vitamin.

TIIVISTELMÄ

Kapeakaistaista ultravioletti B (narrow-band ultraviolet B, NB-UVB) -valohoitoa käytetään ihotautien, kuten psoriaasin ja atoppisen ihottuman hoitamiseen. Muutamat aikaisemmat tutkimukset ovat osoittaneet, että tämä valohoito saattaa nostaa veren 25-hydroksivitamiini D (25(OH)D) -pitoisuuksia. Toisaalta tiedetään, että useimmilla dialyysihoidossa olevilla munuaispotilailla on D-vitamiinivaje. Tässä työssä tutkimme, kuinka paljon NB-UVB-valotus nostaa ihottumaa sairastavien ja hemodialyysissä käyvien munuaispotilaiden veren 25(OH)D-pitoisuuksia. Lisäksi vertasimme NB-UVB-valotuksen ja suun kautta annettavan D-vitamiinilisän vaikutuksia terveiden henkilöiden D-vitamiinipitoisuuksiin.

Ensimmäisen työn (I) lähtötilanteessa 89 % psoriaasia ja 94 % atooppista ihottumaa sairastavilla potilailla sekä 53 % terveillä henkilöillä oli D-vitamiinivaje (seerumin 25(OH)D <50 nmol/L). Viisitoista NB-UVB-valotusta annettuna koko keholle nostivat merkitsevästi ($p < 0.001$) seerumin 25(OH)D pitoisuutta; nousu oli 59.9 nmol/L psoriaasia ja 68.2 nmol/L atooppista ihottumaa sairastavilla potilailla sekä 90.7 nmol/L terveillä henkilöillä. PASI (psoriasis area and severity index) ja SCORAD (severity scoring of atopic dermatitis) -asteikoilla mitattuina ihottumat parantuivat merkitsevästi ($p < 0.001$), mutta paranemisen ja 25(OH)D-pitoisuuden nousun välillä ei ollut yhteyttä. Antimikrobiaalisten peptidien (AMP), katelisidiinin ja human β-defensiini 2:n (HBD2) ekspressio oli runsasta psoriaasin ihomuutoksissa. Kuuden NB-UVB-valotuksen jälkeen katelisidiinin ekspressio lisääntyi ja HBD2:n ekspressio väheni. NB-UVB-valotus vähensi, joskaan ei merkitsevästi, psoriaasimuutosten sytokiinien, IL-1β:n ja IL-17:n pitoisuuksia. Johtopäätöksenä oli, että psoriaasin ja atooppisen ihottuman paranemisen lisäksi NB-UVB-hoito korjaa tehokkaasti D-vitamiinivajetta. Lisäksi se nostaa katelisidiini- ja HBD2-tasoja psoriaasin ja atooppisen ihottuman ihomuutoksissa. Tämä vaikutus voi välittyä parantuneen D-vitamiinitason ja paikallisen sytokiiniverkoston kautta.

Toisessa työssä (II) sairaalan henkilökuntaan kuuluville terveille aikuisille ja lääketieteen opiskelijoille, joiden seerumin 25(OH)D-pitoisuus oli alle 75 nmol/L, annettiin satunnaistetusti joko 12 NB-UVB-valotusta koko keholle tai suun kautta 20 μg kolekalsiferolia päivittäin neljän viikon ajan. Lähtötilanteessa 25(OH)D-pitoisuudet olivat samaa tasoa molemmilla ryhmillä: 52.9 nmol/L 33 NB-UVB-hoidetulla ja 53.5 nmol/L 30 suun kautta kolekalsiferolilla saaneella tutkittavalla. Seerumin 25(OH)D-pitoisuus nousi keskimäärin 41.0 nmol/L NB-UVB-

ryhmässä ja 20.2 nmol/L kolekalsiferoli-ryhmässä. Hoitojen välinen ero oli merkitsevä kahden viikon ($p = 0.033$) ja neljän viikon ($p < 0.001$) kuluttua hoitojen alusta. Kahden kuukauden kuluttua hoidoista 25(OH)D-pitoisuudet olivat laskeneet molemmissa ryhmissä, mutta ne olivat edelleen selvästi korkeammat kuin lähtötilanteessa. Tuloksista pääteltiin, että neljän viikon aikana saadut 12 NB-UVB-valotusta nostavat 25(OH)D-pitoisuutta merkitsevästi enemmän kuin suun kautta päivittäin annettu 20 μg kolekalsiferolia. Lyhyt, pieniannoksinen NB-UVB-valotusjakso on tehokas tapa lisätä talvista D-vitamiinitasoa ja tämä vaikutus säilyy vähintään kaksi kuukautta.

Kolmannessa työssä (III) 15 hemodialyysipotilaalle ja 12 terveelle henkilölle annettiin yhdeksän NB-UVB-valotusta ylävartalon alueelle. Lähtötilanteessa keskimääräinen seerumin 25(OH)D oli dialyysipotilailla 32.5 nmol/L ja terveillä henkilöillä 60.2 nmol/L. Ryhmien välinen ero oli merkitsevä ($p < 0.001$). Kahdeksan NB-UVB-valotuksen jälkeen dialyysipotilailla seerumin 25(OH)D nousi 13.8 nmol/L (43 %; $p < 0.001$) ja 1,25-dihydroksivitamiini D ($1,25(OH)_2D$) 3.3 pmol/L (27 %; $p = 0.002$). Kahden kuukauden kuluttua NB-UVB-jaksosta dialyysipotilaiden 25(OH)D oli yhä 10 % korkeampi kuin lähtötilanteessa. Ihonäytepaloista tutkittiin CYP24A1 ja CYP27B1 lähetti-RNA ekspression tasot. NB-UVB-valotusten jälkeen CYP27B1 lähetti-RNA lisääntyi ($p = 0.04$) verrattuna valotusta saamattomiin terveisiin henkilöihin. Johtopäätöksenä oli, että lyhyt NB-UVB-jakso nostaa merkitsevästi dialyysipotilaiden 25(OH)D- ja $1,25(OH)_2D$-tasoja. Vaikutus on kuitenkin lyhytkestoinen, joten potilaat tarvitsevat jaksottaisia NB-UVB-valotuksia säilyttääkseen kohonneet D-vitamiinipitoisuutensa.

Neljännessä työssä (IV) 14 hemodialyysipotilaalle ja 15 terveelle henkilölle, jotka käyttivät päivittäin suun kautta D-vitamiinilisänä 20 μg kolekalsiferolia, annettiin yhdeksän NB-UVB-valotusta koko keholle. Lähtötilanteessa seerumin 25(OH)D-pitoisuus oli dialyysipotilailla 57.6 nmol/L ja terveillä henkilöillä 74.3 nmol/L. NB-UVB-jakso nosti 25(OH)D-pitoisuutta dialyysipotilailla 14.0 nmol/L ja terveillä henkilöillä 17.0 nmol/L. Nämä nousut olivat merkitseviä ($p < 0.001$). Lähtötilanteessa dialyysipotilailla oli ihossa merkitsevästi korkeammat CYP27B1- ja matalammat CYP27A1-tasot kuin terveillä henkilöillä. Johtopäätösenä oli, että lyhyt NB-UVB-jakso on tehokas tapa parantaa D-vitamiinitasoa myös niillä dialyysipotilailla, joilla on käytössä D-vitamiinilisä suun kautta. Dialyysipotilaiden kohonneet ihon CYP27B1-tasot viittaavat siihen, että kyseisen entsyymin aktiivisuuden väheneminen munuaisissa on ainakin osittain korvattu ihossa.

Johtopäätöksenä näistä tutkimuksista voidaan todeta, että NB-UVB-valotus nostaa tehokkaasti seerumin 25(OH)D-pitoisuutta ihotauti- ja dialyysipotilailla sekä terveillä henkilöillä. Lyhyt NB-UVB-jakso nostaa terveillä henkilöillä 25(OH)D-tasoa merkitsevästi enemmän kuin päivittäinen suun kautta otettu 20 μg kolekalsiferolilisä. D-vitamiinivajeisilla dialyysipotilailla NB-UVB-valotukset on uusi keino parantaa alentunutta D-vitamiinitasoa.

4 INTRODUCTION

Vitamin D insufficiency is common worldwide (Holick 2007, Holick & Chen 2008). In the Nordic countries and Britain this condition affects people especially during the winter when vitamin D synthesis induced by the sun is negligible (Burgaz et al. 2007, Hyppöen & Power 2007, Grant et al. 2011). The desirable concentration of serum 25-hydroxyvitamin D (25(OH)D), which is the best indicator of vitamin D status, is still under debate (Holick & Chen 2008, Pludowski et al. 2013), but a concentration below 75 nmol/L is considered to be insufficient for bone fracture prevention (Dawson-Hughes et al. 2005, Bischoff-Ferrari et al. 2006). In addition to osteoporosis, low serum 25(OH)D concentrations have recently been associated with a risk of colorectal cancer and cardiovascular disease (Gandini et al. 2011, Pittas et al. 2010). Vitamin D is also known to affect skin inflammation and innate or adaptive cutaneous immune responses (Schauber & Gallo 2008, Pludowski 2013).

Narrow-band ultraviolet B (NB-UVB) phototherapy is used to treat dermatological diseases such as psoriasis and atopic dermatitis (Bandow & Koo 2004, Patel et al. 2009) and both broad-band ultraviolet B (BB-UVB) and NB-UVB treatments seem to increase serum 25(OH)D concentrations (Guilhou et al. 1990, Vähävihu et al. 2010). On the other hand, most chronic kidney disease (CKD) patients on dialysis are known to suffer from vitamin D insufficiency (LaClair et al. 2005, Bhan et al. 2010, Nigwekar et al. 2012) and their response to supplementation, as evaluated in terms of increased 25(OH)D, seems to be rather slow (Quinibi et al. 2010, Kandula et al. 2011). The effect of NB-UVB on vitamin D balance in dialysis patients has not been studied previously.

The main topics of the present work were to examine the effect of NB-UVB phototherapy on the vitamin D balance of dermatological patients in winter, and to assess in healthy subjects whether NB-UVB exposures improve vitamin D balance better than does oral vitamin D supplementation. Further topics were to study the effect of NB-UVB exposures on CKD patients treated with haemodialysis and to investigate the effects of NB-UVB on the enzymes hydroxylating vitamin D, antimicrobial peptides and cytokines in the skin.

5 REVIEW OF THE LITERATURE

5.1 Vitamin D

5.1.1 Historical background

The first clinical descriptions of the devastating bone-deforming disease rickets was produced in the 17th century by Daniel Whistler (1645) and Francis Glisson (1650), as reviewed by Rajakumar et al. (2007). The lack of sunlight and its association with rickets in children was first recognized by Jerdzej Sniadecki in 1822, but one hundred years passed before Kurt Huldschinsky (1919) observed that exposure to ultraviolet B (UVB) radiation from a mercury arc lamp or sunlight was a means of preventing and treating rickets. In the early 1930s the US government set up an agency to provide recommendations for parents concerning the beneficial effect of sensible exposure to sunlight for the prevention of rickets (Holick & Chen 2008).

It was common practice in the 19th century to give children cod liver oil to prevent and cure rickets. The first scientific report to recognise rickets as a nutritional deficiency disease was published by Edward Mellanby in 1918 (Rajakumar et al. 2007) and 7-dehydrocholesterol was identified as being the precursor of vitamin D in the 1920s through co-operation between Alfred Hess, Adolf Windaus and Otto Rosenheim (Wolf 2004). Hess and Harry Steenbock discovered that UV-irradiated food, particularly whole milk containing butterfat, had an antirachitic potency, and thereafter the chemical structure of vitamin D (ergocalciferol) was subsequently identified in 1931, as cited by Nigewekar et al. (2012).

The fortification of milk with vitamin D was effective in eradicating rickets in the United States and Europe in the 1930s, but then an unfortunate outbreak of hypercalcaemia in infants occurred in Great Britain in the 1950s that was blamed on the overfortification of milk with vitamin D. This resulted in a ban on the fortification of dairy products with vitamin D in Europe (Holick & Chen 2008). In Finland, vitamin D supplements have been systematically given to children since the 1930s, initially in the form of cod liver oil. The Ministry of Social Affairs and Health recommended the fortification of liquid milk products, margarines and cheese spreads with vitamin D in 2003 (Lehtonen-Veromaa et al. 2008).

5.1.2 Photosynthesis of vitamin D

Humans receive vitamin D from exposure to sunlight, from their diet and from dietary supplements. The major source, however, is the skin when exposed to sunlight (UVB radiation), which under normal circumstances contributes more than 90% of the serum concentration of vitamin D (Reiracht 2006). Solar UVB radiation (wavelength 280 to 315 nm) penetrates the skin and converts 7-dehydrocholesterol to previtamin D3 in basal and suprabasal layers (Fig. 1; Holick 2007) by means of a photochemical reaction, which activates its maximum spectral effectiveness at about 297 nm (Lehmann & Meurer 2010).

This previtamin D_3 then undergoes heat isomerization to form 25-hydroxyvitamin D (25(OH)D). This takes several hours (Webb 2006, Holick 2007) and the circulating concentration of 25(OH)D reaches its maximum level 12–24 hours after UVB exposure (Lehmann & Meurer 2010). Alternatively, the previtamin D_3 can be photoisomerised further into one of two inert isomers, or back to 7-dehydrocholesterol (Webb 2006). Since any excess previtamin D_3 or 25(OH)D is destroyed by sunlight, further exposure to sunlight does not cause 25(OH)D intoxication (Holick 2007).

There are several factors that limit cutaneous 25(OH)D synthesis and it is in any case difficult to say how much solar radiation is needed to achieve and maintain an adequate vitamin D status as it depends on the solar zenith angle, latitude, time of year and day, weather, person's age, skin type, clothing and activity (Webb 2006, Holick & Chen 2008, Lehmann & Meurer 2010). There is a seasonal variation in circulating 25(OH)D (Hyppönen & Power 2007, Holick & Chen 2008) and the angle at which the sun strikes the earth has a dramatic effect on the numbers of UVB photons that reach the surface. Consequently, little if any 25(OH)D synthesis occurs when the zenith angle is increased during the wintertime and in the early morning and late afternoon (Holick & Chen 2008). A radiative transfer model has been used to calculate that poleward of 51° latitude there is at least some period of the year when no vitamin D synthesis can occur, but no limit has been placed on exposure times (Webb 2006).

Sensible sun exposure can provide an adequate amount of 25(OH)D and this can be stored in body fat and released during the winter, when 25(OH)D cannot be produced. Exposure of the arms and legs for 5 to 30 minutes between the hours of 10 a.m. and 3 p.m. twice a week is often adequate (Holick 2007). Exposure to one minimal erythema dose while wearing only a bathing suit is equivalent to the ingestion of approximately 500 μg of ergocalciferol (Holick 2007) and people with abundant exposure to sunlight can easily exhibit a serum 25(OH)D >150 nmol/L (Vieth 2007). Ageing is associated with decreased concentrations of 7-dehydrocholesterol, the precursor of 25(OH)D in the skin (Holick & Chen 2008).

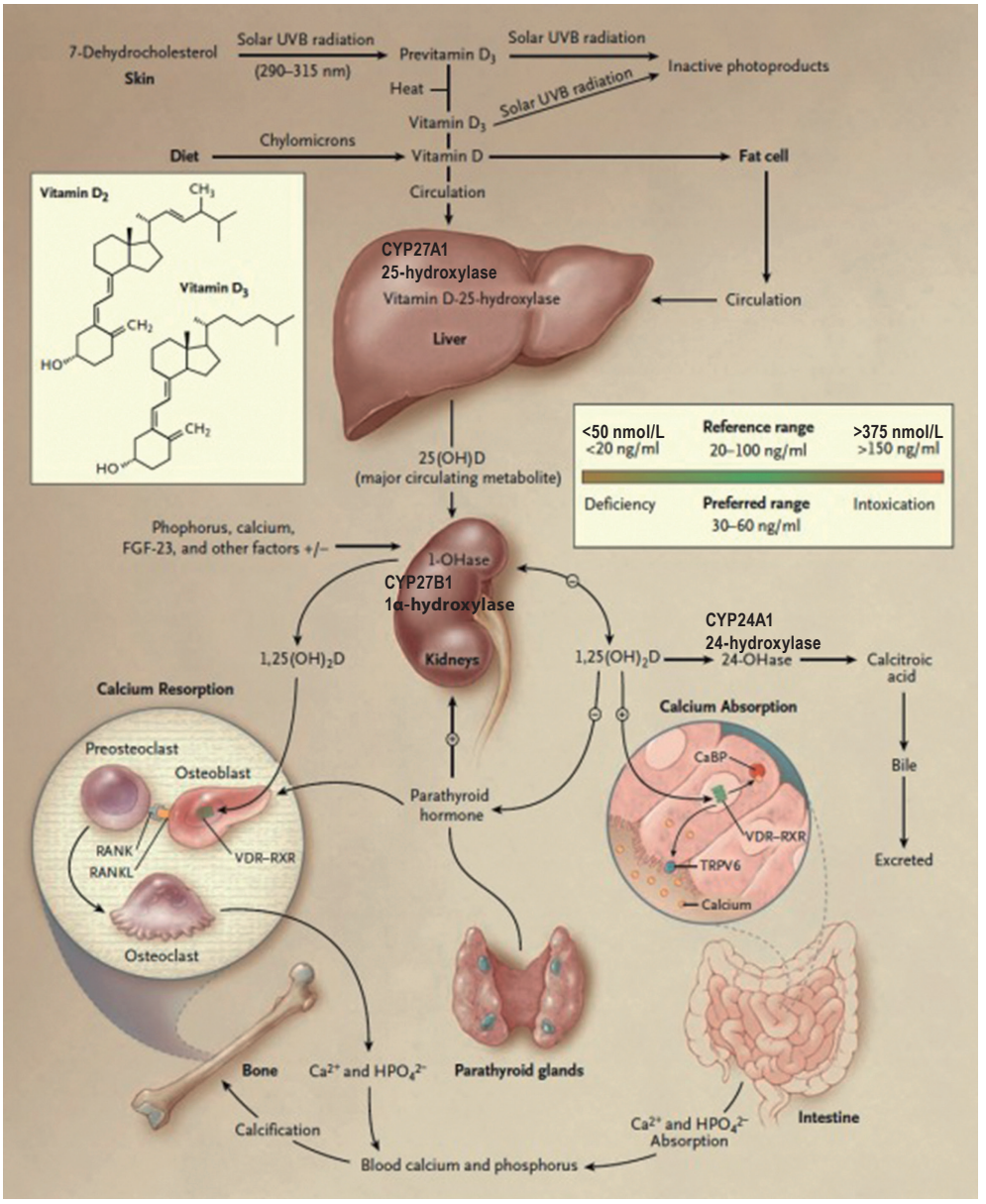

Figure 1. Synthesis of vitamin D starts in the skin and is completed in the kidneys. CYP27A1 in the liver and CYP27B1 in the kidneys are the major hydroxylating enzymes in the cascade. Adapted from Holick 2007.

5.1.3 Dietary sources of vitamin D

Vitamin D comprises two closely related substances of nutritional importance: vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). Vitamin D_3 is formed from its precursor 7-dehydrocholesterol, which is found in ample amounts in the skin of humans and animals. Vitamin D_2 is formed by UV radiation from its precursor ergosterol, which is present in plants, yeast and fungi (Lehmann & Meurer 2010). Plants are a poor source of vitamin D_2, however.

There are a few foods that naturally contain vitamin D including fish (e.g. salmon, mackerel and herring), fish oils (including cod liver oil), mushrooms and egg yolk (Holick 2007, Hyppönen & Power 2007, Lehmann & Meurer 2010). Farmed fish have a lower vitamin D content than wild fish, so that 100 g of farmed salmon contains 2.5–6.3 μg of vitamin D while 100 g of wild salmon has 15–25 μg (Holick 2007).

The range of vitamin D intake required to ensure maintenance of wintertime vitamin D status in the vast majority of 20–40-year-old adults, considering a variety of sun exposure preferences, is between 7.2 and 41.1 μg daily (Cashman et al. 2008). The dietary intake of vitamin D in Finland, evaluated using a validated food frequency questionnaire, is approximately 10 μg daily in elderly people (Viljakainen et al. 2006) and 2–6 μg daily in adolecents (Tylavsky et al. 2006).

5.1.4 Synthesis of vitamin D and calcium homeostasis

Vitamin D (D_2 or D_3) from the skin and diet is metabolized in the liver to 25-hydroxyvitamin D (25(OH)D, calcidiol), which is used to determine a patient's vitamin D status (Holick 2007, Pludowski et al. 2013). 25(OH)D is then metabolized in the kidneys to its active form, 1α,25-dihydroxyvitamin D (1,25$(OH)_2$D, calcitriol). These hydroxylations are enabled by the enzymes vitamin D-25-hydroxylase (25-hydroxylase, CYP27A1) and 25-hydroxyvitamin D-1α-hydroxylase (1α-hydroxylase, CYP27B1), respectively (Fig. 1; Holick 2007, Lehmann & Meurer 2010). The serum 25(OH)D concentration regulates the levels of these enzymes by a negative feedback system, which implies that the baseline concentration of 25(OH)D is an important factor for how a person responds to an oral dose of vitamin D (Holick & Chen 2008).

Vitamin D synthesized in the skin or received from the diet can be stored in fat cells and later released from them. Obesity is associated with vitamin D deficiency, which is believed to be due to sequestration of vitamin D by the large body fat pool (Hyppönen & Power 2007, Holick & Chen 2008). In the circulation vitamin D is bound to carrier proteins, in particular, vitamin D-binding protein (VDBP). 25(OH)D quickly enters the circulation, where it has a half-life of about 15 days (Holick 2007, Lehmann & Meurer 2010). A negative relationship exists between serum 25(OH)D and serum

parathyroid hormone (PTH). The threshold for serum 25(OH)D, at which serum PTH starts to rise is reported in most surveys to be about 75 nmol/L (Lips 2006).

25(OH)D bound to VDBP is transported to the kidneys and hydroxylated to hormonally active $1,25(OH)_2D$. This 1α-hydroxylation of 25(OH)D to $1,25(OH)_2D$ is tightly regulated by PTH and also to some extent by calcium, phosphate, calcitonin, fibroblast growth factor 23 and $1,25(OH)_2D$ itself. $1,25(OH)_2D$ has biological effects in the kidneys, but it also is transported by VDBP to other vitamin D receptor (VDR)-positive target tissues, mainly the bones, intestines, and parathyroid gland. The main effect of $1,25(OH)_2D$ is to increase the absorption of calcium from the small intestine (Lips 2006, Lehmann & Meurer 2010), although it also mobilizes osteoclastic activity (Holick & Chen 2008). It has a serum half-life of 10–24 hours (Lips 2006, Lehmann & Meurer 2010). $1,25(OH)_2D$ induces the expression of the enzyme 25-hydroxyvitamin D-24-hydroxylase (CYP24A1), which catabolizes both 25(OH)D and $1,25(OH)_2D$ to biologically inactive, water-soluble calcitroic acid (Fig. 1; Holick 2007, Nigwekar et al. 2012), which is then excreted in the bile.

It is recognized that many tissues in the body, including the macrophages, brain, colon, prostate, breast and others, have the enzymatic machinery to produce $1,25(OH)_2D$ locally (Holick & Chen 2008) and it has been discovered that human keratinocytes exhibit an autonomous vitamin D pathway also *in vivo* (Lehmann & Meurer 2010). Keratinocytes also possess vitamin D catabolic pathways. A five-step inactivation pathway from $1,25(OH)_2D$ to calcitroic acid in epidermal keratinocytes is attributed to the multifunctional enzyme 25-hydroxyvitamin D-24-hydroxylase (CYP24A1), which is induced by $1,25(OH)_2D$. The hydroxylation of 25(OH)D to $1,25(OH)_2D$ occurs in the kidneys under the influence of PTH, or in extrarenal cells and tissues under the influence of cytokines (Lips 2006).

5.2 Vitamin D insufficiency

5.2.1 Vitamin D insufficiency in general population

The best method for determining a person's vitamin D status is to measure the circulating 25(OH)D concentration, which is regarded as remaining fairy stable (Holick & Chen 2008, Pludowski et al. 2013). Most laboratories report the normal range of 25(OH)D to be 50 to 250 nmol/L (20 to 100 ng/mL) (Holick 2007). Circulating levels of $1,25(OH)_2D$ are approximately 1/1000th that of 25(OH)D (K-DIGO 2009). The serum levels of $1,25(OH)_2D$ range from 75 to 200 pmol/L (29 to 77 pg/mL) (Lehmann & Meurer 2010).

The desirable concentration of serum 25(OH)D is still under debate, but vitamin D deficiency can be diagnosed if its serum levels of 25(OH)D are below 50 nmol/L (Holick

2007, Querfeld & Mak 2010) and some authors have used the term severe deficiency for levels below 25 nmol/L. Relative vitamin D deficiency (vitamin D insufficiency) is considered at serum levels of 25 to 75 nmol/L. Substitution with vitamin D preparations should aim at serum levels of at least 75 nmol/L or preferably 100 to 200 nmol/L. Highly elevated levels, above 200 nmol/L, or more typically above 150 nmol/L, may indicate vitamin D toxicity (Querfeld & Mak 2010).

Vitamin D deficiency is recognized as a pandemic and it has been estimated that 1 billion people worldwide have vitamin D deficiency or insufficiency (<75 nmol/L; Holick 2007). The prevalence of vitamin D deficiency in the general population has been described as ranging from 20% to 50%, with race, age, sunlight exposure, and comorbid conditions such as diabetes mellitus and obesity accounting for its wide variations (Nigwekar et al. 2012).

In one large-scale evaluation of trends in vitamin D insufficiency in the US population (Ginde et al. 2009) in which the serum 25(OH)D levels of 18 883 participants in the Third National Health and Nutrition Examination Survey (NHANES III) of 1988–1994 were compared with those of 13 369 participants in NHANES 2001–2004 the mean serum level had decreased markedly, from 75 nmol/L to 60 nmol/L between the two periods. Correspondingly, the prevalence of 25(OH)D levels of less than 25 nmol/L increased from 2% to 6%, and that levels of 75 nmol/L or more decreased from 45% to 23%.

In a European study, researchers measured 25(OH)D in 7437 whites from the 1958 British birth cohort when they were 45 years old (Hyppönen & Power 2007) and found the prevalence of hypovitaminosis D to be highest during the winter and spring, when 25(OH)D concentrations <25, <40 and <75 nmol/L were found in 15.5%, 46.6% and 87.1% of participants, respectively. The corresponding proportions during the summer and autumn were 3.2%, 15.4%, and 60.9%, respectively.

Culture and habits affect serum 25(OH)D levels (Holick 2007). A Finnish study examining two groups of immigrant women (Bangladeshi and Somali) in Helsinki in winter pointed to a high prevalence of vitamin D insufficiency (25(OH)D <50 nmol/L) in the Somali group, 89.6% (Islam et al. 2012).

5.2.2 Health effects of vitamin D insufficiency

Serum levels of about 75 nmol/L are currently considered by many investigators to be optimal for health (Holick 2007, Holick & Chen 2008, Lehmann & Meurer 2010) while a concentration below 75 nmol/L is considered to be insufficient for bone fracture prevention (Dawson-Hughes et al. 2005, Bischoff-Ferrari et al. 2006). Cross-sectional studies have shown serum 25(OH)D concentrations to be related to serum PTH concentration and bone marrow density in adolescents (Lamberg-Allardt

& Viljakainen 2008). When given in appropriate doses, calcium and vitamin D have been shown to be pharmacologically active, safe and effective for the prevention and treatment of osteoporotic fractures (Boonen et al. 2004) and a recent review states that the anti-fall and anti-fracture actions of vitamin D administration at a dose of at least 20 μg daily, achieving serum 25(OH)D levels of at least 60 nmol/L, appear to be effective and beneficial for the musculoskeletal machinery (Pludowski et al. 2013).

In addition to osteoporosis, low serum 25(OH)D concentrations have been associated with a risk of developing and dying from cancer (Holick & Chen 2008, Gandini et al. 2011, Pludowski et al. 2013). It has been suggested that adults with 25(OH)D below 50 nmol/L have a 30–50% increase in their risk of developing colorectal, breast and prostate cancers (Holick 2007). A meta-analysis showed that increasing the intake of vitamin D to 25 μg cholecalciferol daily is associated with a decrease of as much as 50% in the risk of colorectal and breast cancer (Holick & Chen 2008).

Although skin cancers are known to be associated with sun exposure, sunlight may provide protection from many cancers through the production of vitamin D. In a large cohort of 416 134 skin cancer cases and 3 776 501 non-skin cancer cases as the subject's first cancer extracted from 13 cancer registries (Tuohimaa et al. 2007) 10 886 of the melanoma and 35 620 of the non-melanoma skin cancer patients developed second cancers. The risk of a second primary cancer after a non-melanoma skin cancer was lower in sunny countries for most of the cancers except for the lip, mouth and non-Hodgkin lymphoma types. Vitamin D production in the skin seems to reduce the risk of many solid cancers, especially stomach, colorectal, liver and gallbladder, pancreas, lung, female breast, prostate, bladder and kidney cancers.

Vitamin D deficiency has also been linked to an increased incidence of many chronic illnesses, most notably type 1 and type 2 diabetes mellitus, hypertension, multiple sclerosis, Crohn's disease, rheumatoid arthritis, osteoarthritis, neurocognitive dysfunction, mental illness, infertility, adverse pregnancy and birth outcomes (Holick 2007, Holick & Chen 2008, Pludowski et al. 2013). It has also been thought to be associated with an increased risk of cardiovascular events and mortality (de Boer et al. 2009), although it is maintained in one review that the association between vitamin D status and cardiometabolic outcomes is uncertain (Pittas et al. 2010) as trials have shown no clinically significant effect of vitamin D supplementation at the dosages given. Lower vitamin D status seems to be associated with an increased risk of hypertension and cardiovascular disease, but we do not yet know whether vitamin D supplementation will affect the clinical outcomes (Nigwekar et al. 2012). However, one large test of the association of low 25(OH)D levels with all-cause, cancer and cardiovascular disease mortality in 13 331 nationally representative adults aged 20 years or older as recorded in the Third National Health and Nutrition Examination Survey mortality files (Melamed et al. 2008) in which the participants' vitamin D levels were collected from 1988 to 1994 and the individuals were followed up passively for mortality until

2000 found the lowest quartile in terms of the 25(OH)D level (<45 nmol/L) to be independently associated with all-cause mortality in the general population. According to a more recent review, the all-cause mortality risk in the general population seems to be lowest at 25(OH)D levels ranging from 75 to 113 nmol/L (Pludowski et al. 2013).

5.3 Vitamin D and antimicrobial peptides in innate immunity

$1,25(OH)_2D$ has been shown to inhibit cancer cell growth, induce cancer cell maturation, induce apoptosis and reduce angiogenesis and to have an immunomodulatory activity on monocytes and activated T and B lymphocytes (Holick & Chen 2008, Pludowski et al. 2013). It also has an antiproliferative effect and downregulates inflammatory markers. Extrarenal synthesis of $1,25(OH)_2D$, occuring under the influence of cytokines, is important for the paracrine regulation of cell differentiation and function and this may explain why vitamin D deficiency can play a role in the pathogenesis of autoimmune diseases and cancer (Lips 2006).

Our skin is constantly being challenged by microbes, but is rarely infected. Epidermal production of antimicrobial peptides (AMPs) is the primary protective system, and the expression of some AMPs increases further in response to microbial invasion (Schauber & Gallo 2008). AMPs act to form a chemical shield on the surface of the skin, and they are also thought to trigger and coordinate multiple components of the innate and adaptive immune systems. Many cell types that permanently reside in the skin produce AMPs, including keratinocytes, sebocytes, eccrine glands and mast cells while circulating cells recruited to the skin, such as neutrophils and natural killer cells, are also significant contributors to the total amount of AMPs present. Cathelicidins and β-defensins are the best characterized of the AMPs found in the skin, but a list of the known cutaneous AMPs will identify more than 20 individual proteins that have shown antimicrobial activity (Schauber & Gallo 2008).

Cathelicidins are unique AMPs that protect the skin through both direct antimicrobial activity and the initiation of a host response resulting in cytokine release, inflammation, angiogenesis and re-epithelialization (Schauber & Gallo 2008). Human cathelicidin is often referred to by one of its peptide forms (LL-37) or by the nomenclature assigned to its precursor protein (hCAP18). Cathelicidin dysfunction emerges as a central factor in the pathogenesis of several cutaneous diseases, including atopic dermatitis, in which cathelicidin is suppressed, rosacea, in which cathelicidin peptides are abnormally processed to forms that induce inflammation, and psoriasis, in which a cathelicidin peptide converts self-DNA to a potent stimulus in an autoinflammatory cascade (Schauber & Gallo 2008).

Cathelicidin expression in the skin is known to be induced by $1,25(OH)_2D$, which is a potent immunomodulator (Pludowski et al. 2013). When a macrophage or

monocyte is stimulated by an infectious agent through its toll-like receptor, the signal up-regulates the expression of the vitamin D receptor and 1α-hydroxylase (Holick 2007). A 25(OH)D level of 75 nmol/L or higher provides an adequate substrate for 1α-hydroxylase to convert 25(OH)D to $1,25(OH)_2D$ which then travels to the nucleus, where it increases the expression of cathelicidin, a peptide capable of promoting innate immunity and inducing the destruction of infectious agents (Holick 2007).

UVB irradiation of human keratinocytes supplemented with 7-dehydrocholesterol has been shown to trigger the synthesis of hormonally active $1,25(OH)_2D$, which then affects expression of the AMPs cathelicidin and human β-defensin 2 (Schauber & Gallo 2008, Peric et al. 2010). Recent work has demonstrated direct upregulation of AMPs in healthy skin by cutaneous UV exposure (Felton et al. 2013). The induction of antimicrobial peptides by UV irradiation appears to have been one of the first indications that UV irradiation may foster the innate immune response (Gläser et al. 2009). Since UV exposure can result in a disruption of the epidermal barrier, and may thus increase the risk of bacterial infections, the induction of antimicrobial peptides as a counterregulatory phenomenon may be beneficial. Suppression of the adaptive immune system by UV irradiation plays an important role in photocarcinogenesis and inhibits the induction of contact hypersensitivity (Schwartz 2010). Thus UV radiation exerts diverse effects on the immune system, suppressing the adaptive immune response but inducing the innate immune response. This seems to explain why T-cell–mediated immune reactions are suppressed upon UV exposure but host defence reactions to bacterial attacks are not (Gläser et al. 2009). Schwartz et al. (2012), in their recent investigation into whether $1,25(OH)_2D$ might be involved in UV-induced immunosuppression, were able to show that Langerhans cells are required for the induction of regulatory T-cells by $1,25(OH)_2D$. This indicates that $1,25(OH)_2D$ exerts similar immunosuppressive effects to UV but is dispensable for local UV-induced immunosuppression, as vitamin D receptor knockout mice were equally as susceptible to UV-induced immunosupppression as wild-type controls.

5.4 Ultraviolet B treatment for dermatological diseases

It has been known for more than 2000 years that several skin diseases improve upon exposure to the sun (heliotherapy). Systematic investigations into phototherapeutic modalities started at the beginning of the twentieth century (Berneburg et al. 2005) and in the 1920s Goeckerman showed the beneficial effect of natural sunlight in combination with tar for treating psoriasis. Broadband ultraviolet B (BB-UVB) therapy involves a wide spectrum of UVB wavelengths (280–320 nm) and has been available for more than 80 years for the treatment of psoriasis (Patel et al. 2009). In 1953, Ingram initiated a combination of UVB radiation, dithranol and tar bathing for

psoriasis and later, Fischer and Alsins (1974) and Parrish and Jaenicke (1981) showed that wavelengths around 311 nm provoke the least erythema while being most effective for clearing psoriasis, as reviewed by Berneburg et al. (2005). As a consequence, a fluorescent bulb was developed (TL-01) that emitted a major peak at 311 (± 2 nm) and a minor peak at 305 nm (Karvonen et al. 1989) and this treatment, which later came to be called narrowband ultraviolet B (NB-UVB) was shown to be superior to broadband phototherapy for treating a number of dermatoses. Furthermore, NB-UVB was found to be 5 to 10 times less potent than BB-UVB in inducing erythema, hyperplasia, oedema and Langerhans cell depletion in the skin (Patel et al. 2009).

At present NB-UVB is a standard dermatological therapy, especially for psoriasis (Bandow & Koo 2004). It is mostly given three times a week (Dawe et al. 1998), usually for a total of approximately 20–25 exposures (Green et al. 1988, Bandow & Koo 2004). NB-UVB is also effective in the treatment of atopic eczema, polymorphic light eruption, early stage mycosis fungoides, pruritus, graft-versus-host disease and several other inflammatory dermatoses (Berneburg et al. 2005, Hearn et al. 2008). It suppresses the IFN-γ and IL-17 signalling pathways in order to resolve psoriatic inflammation (Johnson-Huang et al. 2010, Rácz et al. 2011) and its antipsoriatic effect may also, at least in part, be based on the antiproliferative and prodifferentiative action of newly synthesized $1,25(OH)_2D$ on epidermal keratinocytes (Lehmann et al. 2007). NB-UVB is known to reduce systemic immune responsiveness via the induction of regulatory T cells (Milliken et al. 2012).

The UVB doses given to patients in phototherapy cabins are calculated in physical units J/m^2 or J/cm^2, but the physical dose can be weighted for the presence of erythema when measuring the UVB dose received by the skin. The minimal erythema dose (MED), which is an individual measure, is the smallest dose to cause erythema with well-defined borders in the phototest area 24 hours after irradiation of the skin while Diffey et al. (1997) proposed the term standard erythema dose (SED) when referring to erythema as a specific biological response. 1 SED is equivalent to an effective erythemal radiant exposure of 100 J/m^2 and 10 mJ/cm^2 (Commission Internationale de l'Eclairage (CIE) 1999). The MED in subjects with skin types I to IV lies between erythemal effective radiant exposures of 150–600 J/m^2 CIE, which is equivalent to 1.5–6 SED. The ambient diurnal exposure on a clear summer's day in Europe is approximately 30–40 SED (Diffey et al. 1997). An action spectrum for vitamin D production in the skin has also been defined (Commission Internationale de l'Eclairage (CIE) 2006), but this has been criticised.

The early side effects of NB-UVB include erythema and dryness of the skin. Erythema reaches its maximum 8–24 hours after irradiation (Berneburg et al. 2005). One notable risk after chronic UVB exposure is that of skin tumour induction, but phototherapy with NB-UVB seems to be a relatively safe treatment modality (Black & Gavin 2006). A German study of 195 psoriasis patients did not provide evidence for any increased

skin cancer risk in patients treated with either BB-UVB or NB-UVB (Weischer et al. 2004). On the other hand, a small but significant increase in basal cell carcinomas was detected in 1908 Scottish patients treated with NB-UVB (Man et al. 2005). A larger British retrospective study that included 3867 NB-UVB treated patients (Hearn et al. 2008) failed to find any significant association between NB-UVB treatment and basal cell carcinomas, squamous cell carcinomas or melanomas, but one should be cautious about interpreting these results as the cohort contained relatively few patients who had a high number of treatments and there were also some patients who were followed up for only six months. A longer follow-up would be essential in order to determine the true carcinogenic risk attached to NB-UVB phototherapy (Hearn et al. 2008, Patel et al. 2009, Archier et al. 2012).

5.5 Dialysis treatment and vitamin D insufficiency in chronic kidney diseases

Chronic kidney disease (CKD) is divided into five different stages according to the glomerular filtration rate (GFR) (Table 1; k/DOQI 2003). According to Report 2011 of the Finnish Registry for Kidney Diseases, the prevalence of renal replacement therapy, a term that includes both dialysis and kidney transplantation, is increasing, so that there were 2552 kidney transplantation patients and 1774 dialysis patients in Finland by the end of the year in question. Approximately 75% of renal replacement therapy patients recieve in-centre haemodialysis as their first modality, while 25% have peritoneal dialysis and 2% home haemodialysis (Finnish Registry of Kidney Diseases 2011).

Table 1. The five stages of chronic kidney disease (CKD) (k/DOQI 2003).

Stage	Description	GFR, mL/min/1.73m^2
1	Kidney damage with normal GFR	>90
2	Kidney damage with mildly decreased GFR	60–89
3	Kidney damage with moderately decreased GFR	30–59
4	Kidney damage with severely decreased GFR	15–29
5	Kidney failure	<15 or dialysis

GFR=glomerular filtration rate

Uraemic patients may suffer from fatigue, nausea, loss of appetite and/or itching, but nowadays dialysis is most often started before the patient develops uraemic symptoms, at the latest when the GFR has decreased to 5–10 mL/min/1.73m^2 (Korevaar et al. 2003). Conventional haemodialysis usually consists of a session of four to five hours

three times a week. The most common cause of dialysis in Finland is type 2 diabetes (Finnish Registry of Kidney Diseases 2011), while the incidence of dialysis due to type 1 diabetes has not increased in recent years. Other common causes of dialysis are age-related ischaemic kidney disease, chronic glomerulonephritis, polycystic kidney disease and chronic interstitial nephritis.

Vitamin D insufficiency (25(OH)D <75nmol/L) is very common in CKD patients, affecting approximately 50 to 80%, in both pre-dialysis and dialysis populations (LaClair et al. 2005, Blair et al. 2008, Mehrotra et al. 2008, Clayton & Singer 2009, Bhan et al. 2010, Nigwekar et al. 2012). GFR is associated with vitamin D metabolism (Karhapää et al. 2012). In advanced kidney disease the kidney is unable to produce $1,25(OH)_2D$ from 25(OH)D due to the loss of renal 1α-hydroxylase (CYP27B1) activity and hyperphosphataemia (Pitts et al. 1988, Holick 2007, Nigwekar et al. 2012). The Kidney Disease Outcomes Quality Initiative guidelines published by the National Kidney Foundation have recommended that CKD patients should have a serum 25(OH)D concentration above 75 nmol/L (k/DOQI 2003). There is an ongoing debate about the definition of a sufficient vitamin D level for such this patients, however (K-DIGO 2009).

Many patients taking an active vitamin D analogue still do not have adequate vitamin D levels (Holick 2007). Levels of 25(OH)D are inversely associated with PTH levels regardless of the degree of chronic renal failure. Patients with Stage 4 or 5 CKD and those requiring dialysis are unable to produce sufficient $1,25(OH)_2D$, and administration of $1,25(OH)_2D$ or one of its less calcaemic analogues is often recommended to maintain calcium metabolism and to reduce PTH levels and the risk of renal bone disease. 25(OH)D levels below 37 nmol/L are associated with a greater severity of secondary hyperparathyroidism even in CKD patients on dialysis (k/DOQI 2003), while secondary hyperparathyreoidism and changes in mineral metabolism develop early in the course of CKD and worsen with its progression. The major factors responsible for stimulating parathyroid gland function are hypocalcaemia, hyperphosphataemia, increased fibroblast growth factor 23 and diminished $1,25(OH)_2D$ levels (Holick 2007, Levin et al. 2007).

Vitamin D deficiency is associated with cardiovascular disease (de Boer et al. 2009), the most common cause of mortality in haemodialysis patients (Wolf et al. 2007). When the relationship between a low serum 25(OH)D level and death was determined among CKD patients in a large cohort of 3011 patients from the Third National Health and Nutrition Examination Survey who had CKD but were not on dialysis (Mehrotra et al. 2009), individuals with serum 25(OH)D levels less than 37 nmol/L had a higher risk of all-cause mortality than those with levels over 75 nmol/L. These results indicate there is a graded relationship between serum 25(OH)D and the risk of death among subjects with CKD. A meta-analysis of all-cause mortality in patients with CKD had

similar results (Pilz et al. 2011), with higher 25(OH)D levels in patients with CKD being associated with significantly improved survival.

Both vitamin D deficiency and vitamin D toxicity are associated with cardiovascular complications of CKD. These associations are best illustrated by means of a biphasic, or U-shaped, curve (Querfeld & Mak 2010), but in clinical practice the therapeutic window is rather small, so that the avoidance of both vitamin D deficiency and toxicity presents a therapeutic challenge.

Quinibi et al. (2010) found that the recommended 25(OH)D level for CKD patients (>75 nmol/L; k/DOQI 2003) is difficult to achieve with a 6-month course of oral ergocalciferol supplementation. Only 57% of their haemodialysis patients achieved this with a supplementation of 500 μg cholecalciferol weekly for 9 months (Tomak et al. 2008), although ergocalciferol supplementation of 1250 μg weekly for 6 months was associated with significant improvements in serum 25(OH)D in CKD patients, from a baseline of 46 nmol/L to 105 nmol/L (Blair et al. 2008).

Treatment with vitamin D (ergocalciferol or cholecalciferol) to raise 25(OH)D levels was shown to increase not only serum 25(OH)D but also $1,25(OH)_2D$ in both Stage 3 and Stage 4 CKD patients (Martin & González 2001, Zisman et al. 2007). Thus, ergocalciferol or cholecalciferol should be used to correct vitamin D levels in patients with CKD either before or during active vitamin D therapy (Gal-Moscovici & Sprague 2010). In addition to the endocrine effects of the vitamin D axis on bone and mineral metabolism, there is also a certain amount of extrarenal conversion of 25(OH)D to $1,25(OH)_2D$ in multiple cells leading to autocrine effects. Such local conversion has led to the speculation that CKD patients may also need ergocalciferol or cholecalciferol supplementation (Moorthi et al. 2011). Nutritional vitamin D may even be needed by patients with Stage 5 CKD (Melamed & Thadhani 2012).

Several papers have focussed attention on vitamin D supplementation in CKD patients (Kalantar-Zadeh & Kovesdy 2009, Gal-Moscovici & Sprague 2010, Matias et al. 2010), but although the available evidence shows that this supplementation improves biochemical end points, it has yet to be determined whether such improvements translate into clinically significant outcomes such as reduced cardiovascular mortality (Wolf et al. 2007, Kandula et al. 2011).

5.6 Effect of food fortification and oral supplementation on vitamin D balance

The occurrence of suboptimal vitamin D intake and vitamin D insufficiency in the general population led the authorities in many countries to take action to add vitamin D to various foods. Milk, some juice products, some breads, yogurts and cheeses are fortified with vitamin D in the United States (Holick & Chen 2008) and in Finland

an expert meeting recommended the enhancement of milk, sour milk and yogurt with 0.5 μg/100 g of vitamin D and an increase in the fortification of margarine and cheese spreads from 7.5 μg to 10 μg/100 g. Thus one teaspoonful of margarine would yield 0.5 μg of vitamin D (Tylavsky et al. 2006). The Ministry of Social Affairs and Health agreed to these recommendations, and the fortifications took effect in February 2003. Even this fortification and the recommended daily dietary vitamin D intake of 7.5 μg, however, has proved inadequate to prevent vitamin D insufficiency among adolescent Finnish girls (Lehtonen-Veromaa et al. 2008) and two other Finnish studies have shown that the vitamin D in milk products brought only a slight and inadequate improvement in the poor vitamin D status of young Finnish men in winter (Laaksi et al. 2006, Välimäki et al. 2007).

To achieve the recently recommended serum 25(OH)D concentrations of above 75 nmol/L, a dietary intake of 17 to 20 μg vitamin D daily would be required (Bischoff-Ferrari et al. 2006, Holick 2007), and as much as 20 to 25 μg daily for older people (Dawson-Hughes et al. 2005, Viljakainen et al. 2006). This target is hard to reach without supplements, since for a mean daily intake of only 7.5 μg one needs to consume fish 2 to 3 times a week, for instance, together with drinking 6 dl of vitamin D fortified milk daily and eating 5 to 6 sandwiches containing vitaminized margarine.

Over-the-counter vitamin D supplements take the form of either vitamin D_2, ergocalciferol, or D_3, cholecalciferol (Holick 2007). Vitamin D_2 is manufactured by the ultraviolet irradiation of ergosterol from yeast, and vitamin D_3 by the ultraviolet irradiation of 7-dehydrocholesterol from lanolin. There has been much discussion about vitamin D_2 being only 30–50% as effective as vitamin D_3 in maintaining serum concentrations of 25(OH)D implying that vitamin D_2 may need to be given in higher doses to raise the blood concentrations of 25(OH)D (Holick & Chen 2008).

At present the Finnish National Institute for Health and Welfare (National Institute for Health and Welfare 2011) recommends vitamin D supplementation as follows: 10 μg (400 international units, IU) daily for children below 2 years of age and for pregnant and breastfeeding women, 7.5 μg daily for children aged 2–18 years, 20 μg daily for over 60 years old and 10 μg daily from October to March for people aged 18–60 years if they do not regularly consume vitamin D fortified milk and eat fish.

It is well established that oral vitamin D supplementation markedly increases one's serum 25(OH)D concentration and two Finnish studies have shown this in elderly people. Viljakainen et al. (2006) observed that daily supplementation with 5, 10 and 20 μg of cholecalciferol increased serum 25(OH)D concentrations significantly and that an equilibrium was reached after 6 weeks while Kilpinen-Loisa et al. (2009) gave adults living in nursing homes 20 μg of oral vitamin D supplement for 6 months, and noted that 42% of them had a 25(OH)D concentration above 80 nmol/L. Previously Vieth et al. (2001) in Canada had given healthy adults 25 μg of oral cholecalciferol in winter and found that their serum 25(OH)D increased by 28.0 nmol/L while in

Sweden Burgaz et al. (2007) observed that a vitamin D supplement intake in winter increased women's serum 25(OH)D concentration by 11.0 nmol/L and Bischoff-Ferrari et al. (2006) found that 100 μg vitamin D daily increased 25(OH)D concentrations in young men and women by 56 nmol/L to a mean of 125 nmol/L.

Acute vitamin D intoxication on account of ergocalciferol or cholecalciferol supplements is a rare event (Querfeld & Mak 2010). It is characterized by hypercalcaemia, hypercalciuria and nephrocalcinosis, and can be caused by inadvertent or intentional ingestion of excessively high doses. Doses of more than 1250 μg of vitamin D daily raise serum 25(OH)D over 375 nmol/L (Holick 2007). The actual serum 25(OH)D concentration that marks the threshold for vitamin D toxicity has not been established, however, although it is known that the lowest dose of vitamin D causing hypercalcaemia in some healthy adults is 1000 μg of ergocalciferol daily for many months (Vieth 2007). The Institute of Medicine has set the tolerated upper vitamin D intake level at 100 μg daily, defining this as "the highest level of daily nutrient intake that is likely to pose no risks of adverse health effects to almost all individuals in the general population" (Vieth 2007, Pludowski et al. 2013).

5.7 Effect of heliotherapy and UVB treatment on vitamin D balance

It has been shown during last years that heliotherapy and artificial UVB treatment have a positive effect on serum 25(OH)D levels. 25(OH)D is produced in the epidermis by UV radiation at wavelengths of 280–315 nm, and BB-UVB phototherapy in a similar range of 280–320 nm has been used successfully for decades to treat psoriasis. Now, in addition to standard BB-UVB, monochromatic UV 311 nm, i.e. NB-UVB, has become an important mode of treatment for psoriasis.

An experiment as conducted in Finland in which 23 patients with atopic dermatitis, 74% of whom had prior vitamin D insufficiency (25(OH)D <50 nmol/L), received a 2-week course of heliotherapy in the Canary Islands (Vähävihu et al. 2008). The median personal UV dose received during the course was 60 SED in a group sent in January and 109 SED in a group sent in March. Serum 25(OH)D increased significantly in both groups, by 13.4 and 24.0 nmol/L, respectively. Thus only 17% of patients had vitamin D insufficiency after this 2-week course of heliotherapy, which significantly increased serum 25(OH)D concentration and caused a marked healing of the initial atopic dermatitis.

In a Swedish study 24 postmenopausal women with psoriasis were treated with BB-UVB two to three times per week for 8–12 weeks (Osmancevic et al. 2007), whereupon their levels of 25(OH)D increased from 92.0 to 149.0 nmol/L, serum PTH decreased, but the serum levels of $1,25(OH)_2D$, calcium, osteocalcin, thyroid hormones and creatinine were unaltered. The same team then compared the effect of BB-UVB

on vitamin D synthesis in patients with psoriasis with that of NB-UVB therapy (Osmancevic et al. 2009) treating 26 patients with BB-UVB and 42 with NB-UVB two to three times per week for 8–12 weeks. Here 25(OH)D increased from 94.8 to 173.5 nmol/L and in the BB-UVB group and from 87.0 to 138.3 nmol/L in the NB-UVB group and PTH decreased in the BB-UVB group, but serum concentrations of $1,25(OH)_2D$, calcium and creatinine remained unaltered.

Most tanning beds emit 2 to 6% UVB radiation, and a group of tanners are reported to have had 25(OH)D levels of approximately 112 nmol/L at the end of the winter and higher bone density as compared with non-tanners with levels of approximately 45 nmol/L (Holick 2007). A Danish group investigated whether the use of tanning beds with sunlamps emitting mainly UVA and only 0.5% or 1.4% UVB would increase the level of serum 25(OH)D, giving healthy females sunbed radiation on eight days within a couple of weeks, and reported an average increases in serum 25(OH)D of 12 nmol/L in the 0.5% UVB group and 27 nmol/L in the 1.4% UVB group after four exposures (Thieden et al. 2008). In another sunbed study, repeated exposures to small doses from a commercial sunbed over five weeks raised the subjects' 25(OH)D levels from typical winter values to typical summer values, i.e. to 80 nmol/L. A mean increase of 15 nmol/L was seen after 2–4 weeks, followed by a decrease back to the pre-exposure level (Moan et al. 2009).

It has also been determined how different body sites respond to NB-UVB and affect serum 25(OH)D (Vähävihu et al. 2010). NB-UVB exposures were given on seven consecutive days either to the whole body, to the head and arms or to the abdomen. Similarly, seven solar simulator exposures were given to the face and arms. The cumulative UVB dose was 13 SED in all these regimens. Where 77% of the patients had baseline vitamin D insufficiency (25(OH)D <50 nmol/L) and 11% vitamin D deficiency (25(OH)D <25 nmol/L), their 25(OH)D concentration increased significantly, by a mean of 11.4 nmol/L, when the whole body was exposed to NB-UVB, by 11.0 nmol/L when only the head and arms were exposed and by 4.0 nmol/L when only the abdomen was exposed. After two months the serum 25(OH)D of the group who had received NB-UVB to the whole body was still higher than initially.

According to a Danish BB-UVB study, the increase in serum 25(OH)D is dependent mainly on the BB-UVB dose (Bogh et al. 2011b), although the area of body surface irradiated is also important at small UVB doses. When analysing each body surface area separately for any 25(OH)D increase, the team found a significant UVB response correlation for a 6% or 12% body surface area, but not for 24%. They also found a significant correlation of the body surface area response for a dose of 0.75 SED, but not for 1.5 or 3.0 SED. Factors other than the BB-UVB dose also affected the serum 25(OH)D concentration during UVB exposure. The increase in 25(OH)D level after UVB correlated negatively with the baseline 25(OH)D level and positively with the baseline total cholesterol level, but there were no significant

correlations with constitutive or facultative skin pigmentation (Bogh et al. 2010). The increase in 25(OH)D after BB-UVB exposure is dependent on the dose but not on the timing of the dose (Bogh et al. 2011a), i.e. similar levels of 25(OH)D were achieved if the same total dose of BB-UVB was given over 1 minute or 20 minutes. It is also the case, however, that a significant increase in 25(OH)D can be achieved with a very low BB-UVB dose (four exposures of 0.375 SED). Sunscreens absorb UVB radiation, and vitamin D production is known to increase exponentially when thinner sunscreen layers than recommended (<2 mg/cm^2) are applied (Faurschou et al. 2012).

6 AIMS OF THE RESEARCH

Vitamin D insufficiency is common, and this condition affects people in Finland especially during the winter. The main aims of the present thesis were to study the effect of NB-UVB phototherapy on vitamin D balance in dermatological patients in winter and to assess whether NB-UVB exposures improve the vitamin D balance of healthy subjects better than does oral vitamin D supplementation. Further aims were to study the effect of NB-UVB exposures on CKD patients receiving haemodialysis, since they are especially prone to vitamin D insufficiency, and to investigate the effects of NB-UVB on the enzymes hydroxylating vitamin D, antimicrobial peptides and cytokines in the skin.

The specific aims were:

1. to study the effects of NB-UVB phototherapy on the vitamin D balance of patients with psoriasis and atopic dermatitis (I),
2. to compare the effects of NB-UVB exposures and oral vitamin D supplementation on the vitamin D balance of healthy subjects in winter (II),
3. to study whether NB-UVB exposures improve the vitamin D balance of CKD patients receiving haemodialysis without (III) or with continuous oral cholecalciferol supplementation (IV), and
4. to examine whether NB-UVB exposures alter the levels of antimicrobial peptide and cytokine expression in the skin lesions of psoriasis and atopic dermatitis patients (I), and of the expression the enzymes hydroxylating vitamin D (CYP27A1, CYP27B1) and antimicrobial peptides in the skin of CKD patients receiving haemodialysis (III, IV).

7 MATERIALS AND METHODS

7.1 Patients and healthy subjects (I–IV)

Altogether 36 dermatological patients, 29 dialysis patients and 105 healthy subjects voluntarily participated in the four trials (I–IV) included in the present thesis (Table 2). The first (I) included 18 patients with psoriasis (mean age 46.9 years), 18 patients with atopic dermatitis (mean age 32.1 years) and 15 healthy hospital employees (mean age 41.8 years; Table 2).

In trial II, 99 healthy adult hospital employees and medical students were screened for 25(OH)D concentrations below 75 nmol/L and were randomly allocated to receive either NB-UVB or oral cholecalciferol (Table 2). The NB-UVB group consisted of 33 subjects (mean age 43.8, range 23–59 years) and the 20 μg oral cholecalciferol group of 30 subjects (mean age 40.2, range 20–62 years).

Trial III involved 15 Stage 5 CKD patients (mean age 48.3, range 33–65 years) receiving haemodialysis (Table 2), three of whom were taking synthetic vitamin D analogues (alphacalcidol in two cases and paricalcitol in one). Twelve healthy hospital employees (mean age 43.6, range 31–60 years) served as controls.

Fourteen Stage 5 CKD patients (mean age 53.6, range 34–69 years) receiving haemodialysis (Table 2) were enrolled for trial IV, in which seven of them received active vitamin D analogues (six received 5 μg intravenous paricalcitol twice weekly and one took 0.5 μg oral alphacalcidol daily). The patients had all been taking 20 μg oral cholecalciferol daily for a mean of 5.3 (range 1–16) months before the NB-UVB course. The 15 healthy hospital employees (mean age 46.1, range 19–62 years) who served as controls had similarly been taking 20 μg oral cholecalciferol daily before the NB-UVB course, for a mean of 3.4 (range 1–24) months.

Table 2. Demographic data on the dermatological and dialysis patients and healthy subjects participating in the four (I–IV) narrow-band UVB (NB-UVB) trials. Numbers of NB-UVB exposures, mean cumulative NB-UVB doses and oral vitamin D supplementation details are also given.

Trial	NB-UVB time	Subjects (male / female)	Mean age, years (range)	NB-UVB total exposures	NB-UVB cumulative mean dose, SED (range)	Oral vitamin D supplementation, cholecalciferol 20 μg daily
I						
NB-UVB source Waldmann UV 7001 cabin	Jan–Mar 2008 and 2009	N=51 (19/32)				
• Psoriasis patients		N=18 (10/8)	46.9 (18–66)	15	65.5 (57.2–69.0)	none
• Atopic dermatitis patients		N=18 (9/9)	32.1 (19–48)	15	62.7 (43.4–70.4)	none
• Healthy subjects		N=15 (0/15)	41.8 (23–55)	15	64.5 (54.6–69.0)	none
II						
NB-UVB source Waldmann UV 7001 cabin	Dec 2010–Mar 2011	N=63 (9/54)				
Healthy subjects:						
• NB-UVB group#		N=33 (4/29)	43.8 (23–59)	12	48.4 (36.1–54.9)	none
• Cholecalciferol group#		N=30 (5/25)	40.2 (20–62)	none	none	4 weeks
III						
NB-UVB source Medisun 700 UVB-311	Dec 2009–Mar 2010	N=27 (12/15)				
• Dialysis patients		N=15 (10/5)	48.3 (33–65)	9	15.0 (15.0–15.0)	none
• Healthy subjects		N=12 (2/10)	43.6 (31–60)	9	15.0 (15.0–15.0)	none
IV						
NB-UVB source Waldmann UV 7001 cabin	Dec 2011–Mar 2012	N=29 (7/22)				
• Dialysis patients		N=14 (6/8)	53.6 (34–69)	9	26.6 (17.5–27.9)	continuous*
• Healthy subjects		N=15 (1/14)	46.1 (19–62)	9	25.7 (18.9–27.9)	continuous**

at baseline serum 25(OH)D <75.0 nmol/L

* for a mean of 5.3 months before NB-UVB, ** for a mean of 3.5 months before NB-UVB

Inclusion criteria for the trials were Fitzpatrick skin type II–IV, indicating that the skin does not burn easily in the sun, no pregnancy, no history of skin cancer and no phototherapy, solarium visits, sun tanning or vitamin D supplementation within the

two preceding months. The only exception to this was trial IV, where daily use of 20 μg oral cholecalciferol for at least one month were obligatory. The inclusion criteria also included age: over 18 years for trial I, 18–65 years for trials II and III and 18–70 years for trial IV.

Clinical improvement of dermatitis was measured using the psoriasis area and severity index (PASI; Fredriksson and Pettersson 1978) in the patients with psoriasis and by Severity Scoring of Atopic Dermatitis (SCORAD 1993) in those with atopic dermatitis. The indices were determined before and after the NB-UVB course in trial I.

The aetiology of the CKD in trials III and IV was glomerulonephritis in nine/eight patients, diabetic nephropathy in three/two, polycystic kidney disease in one/three, interstitial nephritis (in one/zero) and an unknown cause (in one/one). Four patients were the same in both of these trials. The patients had been on dialysis for a mean of 3.8 (range 0.5–16) years in trial III and 46.8 (range 9–117) months in trial IV. During the trials the patients received calcium carbonate (2 in trial III/14 in trial IV), non-calcium phosphate binder (7/12), cinacalcet (4/6) and active vitamin D analogues (paricalcitol 1/6 and alphacalcidol 1/1). The CKD patients in trial IV, but not in III, had been taking 20 μg oral cholecalciferol for a mean of 5.3 (range 1–16) months before the course of NB-UVB.

The protocols were approved by the ethics committee of Tampere University Hospital (code numbers R07149, R09186, R10112 and R11172) and all the subjects gave their informed consent. The authors followed the principles of the Declaration of Helsinki.

7.2 Methods

7.2.1 Narrow-band UVB exposures (I–IV)

In trial I, 18 patients with psoriasis, 18 with atopic dermatitis and 15 healthy hospital employees, all volunteers, received a total of 15 NB-UVB exposures each, given three times a week, to the entire body area with a Waldmann UV 7001 cabin (Waldmann, Villingen-Schwenningen, Germany) equipped with 40 (not 20 as erroneously expressed in paper I) TL-01 tubes (Schulze & Böhm, Brühl, Germany). The initial unweighted NB-UVB dose for all participants was 0.13 J/cm^2, corresponding to one standard erythema dose (SED), which in turn is equivalent to 10 mJ/cm^2 CIE (Commission Internationale de l'Eclairage) erythema-weighted irradiance. Thereafter the dose was gradually increased up to 1.19 J/cm^2 (9.5 SED). The mean theoretical cumulative UVB dose after 15 NB-UVB exposures in the protocol was 8.88 J/cm^2, which corresponds to 71.5 SED, but in practise the patients and healthy subjects received a somewhat lower cumulative UVB dose, from 62 to 66 SED (Table 2).

The 67 healthy subjects in trial II had serum 25(OH)D concentrations below 75.0 nmol/L and were randomly allocated to receive NB-UVB or oral cholecalciferol. The NB-UVB group, comprising 33 subjects, received 12 exposures given three times a week over four weeks to the entire body area with a Waldmann UV 7001 cabin. The spectral irradiance of the cabin was measured with a stray light-corrected single-monochromator spectroradiometer. The initial physical NB-UVB dose was 0.21 J/cm^2 of CIE erythema-weighted irradiance (1.25 SED), and this was increased gradually according to a fixed protocol up to 1.45 J/cm^2 CIE (8.55 SED). If the subject experienced itching or slight erythema the NB-UVB dose was either lowered or the same dose was repeated before any further increase. The mean cumulative amount of NB-UVB given to the subjects was 8.23 (range 6.14–9.34) J/cm^2 CIE, which is equivalent to 48.4 (range 36.1–54.9) SED. The oral cholecalciferol group received 20 μg (800 IU) daily for 4 weeks. This group consisted of 30 subjects because four subjects had to be excluded due to excessively high baseline serum 25(OH)D concentrations (>75 nmol/L).

The 15 dialysis patients and 12 healthy subjects participating in trial III received NB-UVB exposure with a Medisun 700 UVB-311 apparatus equipped with TL01 tubes three times a week. A total of nine NB-UVB exposures were given during three weeks, to the face, arms, chest and abdomen, accounting for approximately 25% of the total body area. The cumulative dose of NB-UVB was 15 SED. The exposure time ranged from 48 to 96 seconds, and the exposure for the patients took place just before their dialysis session.

The 14 dialysis patients and 15 healthy subjects in trial IV received nine NB-UVB exposures given three times a week for three weeks to the entire body area with a Waldmann UV 7001 cabin. Again these were given to the patients just prior to their dialysis. The first NB-UVB dose was 0.19 J/cm^2 CIE (1.11 SED), and this was increased incrementally according to a fixed gradual protocol up to 0.97 J/cm^2 (5.70 SED). The highest NB-UVB dose took approximately 90 seconds. If the subjects experienced mild itching or erythema, the dose was not increased or was reduced on the next occation. The mean cumulative dose of NB-UVB given to the CKD patients was 4.53 (range 2.97–4.75) J/cm^2 CIE, which is equivalent to 26.6 (range 17.5–27.9) SED, while that given to the healthy subjects was 4.37 (range 3.21–4.75) J/cm^2 CIE, which is equivalent to 25.7 (range 18.9–27.9) SED. The cumulative NB-UVB doses did not differ between the dialysis patients and healthy subjects ($p = 0.46$).

7.2.2 Measurement of serum 25(OH)D and 1,25$(OH)_2$D concentrations and dietary intake of vitamin D (I–IV)

Blood samples were taken before, during and after the NB-UVB exposures, as described in papers I–IV. The samples were protected from light, centrifuged and the serum was frozen at -20°C. Serum 25(OH)D concentrations were measured as in duplicate by radioimmunoassay (I–IV), and serum 1,25$(OH)_2$D concentrations were analysed in duplicate by immunoextraction followed by radioimmunoassay (III). The methods included the measurement of the hydroxylated metabolites of both vitamin D_2 and D_3. The dietary intake of vitamin D was assessed with a Food Frequency Questionnaire (II).

7.2.3 Skin biopsies and mRNA expression of enzymes hydroxylating vitamin D, antimicrobial peptides and cytokines (I, III, IV)

Punch biopsies of same representative skin lesions were taken from seven patients with psoriasis and eight patients with atopic dermatitis before treatment and after six NB-UVB exposures, with seven healthy subjects serving as controls. The samples were frozen immediately and stored at -70°C. Total messenger ribonucleic acid (mRNA) was extracted from the biopsies, and the transcript levels of two antimicrobial peptides, cathelicidin and human β-defensin 2 (HBD2), and the mRNA expression levels of various cytokines in the psoriasis and atopic dermatitis lesions were analysed by the real-time quantitative polymerase chain reaction (PCR) technique. The methods are described in detail in paper I.

For trial III, punch biopsies were taken from the abdominal skin of 12 dialysis patients before the first and ninth NB-UVB exposure, with skin biopsies from 12 additional healthy subjects not treated with NB-UVB serving as controls. The biopsies were frozen immediately and stored at -70°C. Total RNA was isolated from them and 1g of RNA was reverse transcribed to complementary deoxyribonucleic acid (cDNA). The mRNA expressions of CYP24A1, CYP27B1 and cathelicidin were evaluated, and fold induction relative to that in healthy volunteers was calculated.

For trial IV, punch biopsies were taken from the skin of the buttocks of 10 dialysis patients and 13 healthy subjects before the first and ninth NB-UVB exposure. The mRNA expression levels of the enzymes CYP27A1 and CYP27B1 and of the antimicrobial peptide cathelicidin were evaluated.

7.2.4 Statistical analyses (I–IV)

The changes in serum 25(OH)D concentration in the three groups and in PASI and SCORAD where applicable were analysed in paper I by means of a Monte-Carlo p-value using the permutation test. Confidence intervals for the changes were obtained by bootstrapping (5000 replicates). Statistical analyses of cathelicidin and HBD2 expression in the psoriasis and atopic dermatitis skin lesions before and after NB-UVB treatment were compared with results for healthy controls using the Mann–Whitney test. Cathelicidin and HBD2 expression levels were compared between the untreated and NB-UVB treated skin lesions with the Wilcoxon matched pairs test, and cytokine expression in the psoriasis skin vs. the atopic dermatitis skin was analysed with the non-parametric Mann–Whitney test.

In paper II, 67 subjects with a serum 25(OH)D concentration below 75.0 nmol/L were randomly allocated to receive NB-UVB or oral cholecalciferol. The sample size needed to discover significant differences in 25(OH)D concentrations, i.e. 30 subjects per group, was calculated with a Power and Sample Size Calculations program. The results were expressed as means ± SD and 95% confidence intervals. Statistically significant differences between the groups were tested using the permutation test. Repeated measures were analysed using generalizing estimating equations models with an unstructured correlation structure. No adjustment was made for multiple testing.

The difference in serum 25(OH)D concentration between the dialysis patients and healthy subjects and the differences between the serum 25(OH)D and $1,25(OH)_2D$ concentrations measured before and after the NB-UVB exposures were analysed in paper III with a Monte-Carlo p-value by means of the permutation test. Confidence intervals were obtained by bias-corrected bootstrapping (5000 replications). The differences in the mRNA expression levels of CYP24A1, CYP27B1 and cathelicidin between the dialysis patients and healthy subjects were analysed with an unpaired t-test and the differences between the levels before and after NB-UVB exposure with a paired t-test.

Statistical comparisons between the groups in paper IV were performed using the t-test, permutation test or Chi-square test. Repeated measures were analysed using generalizing estimating equations models with an unstructured correlation structure using bootstrap-type standard error. The changes within the group of dialysis patients were analysed by applying a t-test and a permutation test to the relevant samples.

8 RESULTS

The 25(OH)D baseline levels and responses to NB-UVB exposures in the four trials are presented in Table 3.

Table 3. Mean serum 25-hydroxyvitamin D (25(OH)D) concentrations at baseline and at the end of the narrow-band UVB (NB-UVB) exposures in the four trials included in the present thesis.

Trial	Subjects	Mean serum 25(OH)D at baseline, nmol/L	Mean serum 25(OH)D at end, nmol/L	Change from baseline, nmol/L (95% CI), p-value	Increase from baseline, %
I*					
• Psoriasis patients	N=18	36.8	96.7	59.9 (53.5; 66.9), <0.001	163
• Atopic dermatitis patients	N=18	32.2	100.4	68.2 (55.4; 80.1), <0.001	212
• Healthy subjects	N=15	60.5	151.2	90.7 (63.8; 123.4), <0.001	150
II*					
Healthy subjects:					
• NB-UVB group	N=33	52.9	93.9	41.0 (34.8; 47.2), <0.001	78
• Cholecalciferol group	N=30	53.5	73.7	20.2 (14.6; 26.0), <0.001	38
III#					
• Dialysis patients	N=15	32.5	46.3	13.8 (8.1; 19.8), <0.001	42
• Healthy subjects	N=12	60.2	69.2	9.0 (1.4; 15.8), 0.032	15
IV*					
• Dialysis patients	N=14	57.6	71.7	14.0 (8.7; 19.5), <0.001	24
• Healthy subjects	N=15	74.3	91.3	17.0 (13.7; 20.2), <0.001	23

NB-UVB source * Waldmann UV 7001 cabin; # Medisun 700 UVB-311

The mean 25(OH)D concentrations in trial I were low at baseline in the patients with atopic dermatitis (32.2 nmol/L) and psoriasis (36.8 nmol/L) but markedly higher (60.5 nmol/L) in the healthy subjects, who were doctors, nurses and other employees

at our university hospital (Table 3). Overall, 89% of the psoriasis patients, 94% of the atopic dermatitis patients and 57% of the healthy subjects had vitamin D insufficiency (25(OH)D <50.0 nmol/L). The inclusion criterion for the healthy subjects in trial II was a serum 25(OH)D concentration below 75 nmol/L, after which they were allocated to two groups. The mean baseline serum 25(OH)D concentration was 52.9 nmol/L in the NB-UVB group and 53.5 nmol/L in the cholecalciferol group (Table 3).

As expected, the mean baseline 25(OH)D concentration for the dialysis patients in trial III was as low as 32.5 nmol/L (Table 3), with five of them (33%) having a concentration below 25.0 nmol/L and 14 (93%) below 50.0 nmol/L. The corresponding figure for the dialysis patients receiving a supplement of 20 μg oral cholecalciferol daily in trial IV was markedly higher, i.e. 57.6 nmol/L (Table 3), but the level was still below 50.0 nmol/L in six patients (43%) and between 50.0 nmol/L and 70.0 nmol/L in another six (43%). The mean baseline serum 25(OH)D concentration in the healthy subjects was 74.3 nmol/L in trial III and 60.2 nmol/L in trial IV (Table 3).

8.1 Effect of narrow-band UVB treatment on serum 25(OH)D concentrations in patients with psoriasis and atopic dermatitis (I)

NB-UVB exposures given three times a week for a total of 15 times markedly increased mean serum 25(OH)D concentrations by 68.2 nmol/L in the patients with atopic dermatitis, 59.9 nmol/L in the patients with psoriasis and 90.7 nmol/L in the healthy subjects (Table 3, Fig. 2). The clinical improvements in psoriasis and atopic dermatitis were statistically significant (PASI from 8.0 to 3.8. p <0.001; SCORAD from 37.1 to 14.1. p <0.001), but they did not correlate with the increases in serum 25(OH)D.

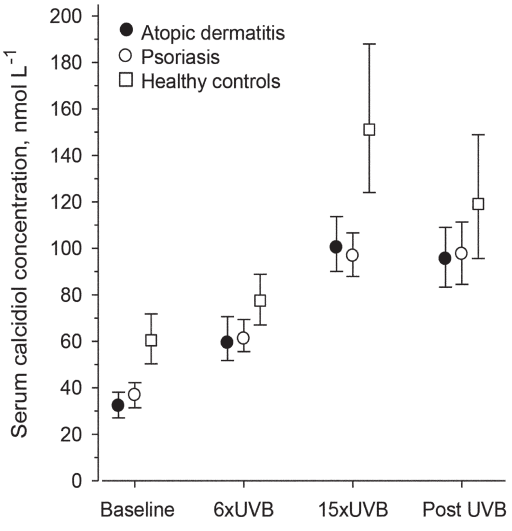

Figure 2. Effect of narrow-band ultraviolet B (NB-UVB) treatment on serum 25-hydroxyvitamin D (25(OH)D, calcidiol) concentration. Fifteen NB-UVB exposures significantly increased the serum 25(OH)D concentration (mean; 95% confidence intervals) in 18 patients with psoriasis, 18 patients with atopic dermatitis and 15 healthy subjects (p <0.001). The serum 25(OH)D level was still elevated in the patients and healthy subjects 1 month after the last NB-UVB exposure. (Original figure in paper I, Fig. 1)

The serum 25(OH)D concentration remained at an elevated level for at least one month in the patients with psoriasis and atopic dermatitis but showed some decrease in the healthy subjects (Fig. 2, Table 4).

Table 4. Mean serum 25-hydroxyvitamin D (25(OH)D) concentrations 1 month and 2 months after the narrow-band UVB (NB-UVB) exposures in the four trials included in the present thesis.

Trial	Subjects	Mean serum 25(OH)D after 1 month/2 months, nmol/L	Change from baseline at 1 month/2 months, nmol/L	Increase from baseline at 1 month/2 months, %
I*				
• Psoriasis patients	N=18	97.6/nd	60.8/nd	165/nd
• Atopic dermatitis patients	N=18	95.5/nd	63.3/nd	197/nd
• Healthy subjects	N=15	119.1/nd	58.6/nd	97/nd
II*				
Healthy subjects:				
• NB-UVB group	N=33	97.2/81.4	44.3/28.5	84/54
• Cholecalciferol group	N=30	84.9/67.7	31.4/14.2	57/27
III#				
• Dialysis patients	N=15	35.7/35.7	3.2/3.2	10/10
• Healthy subjects	N=12	72.9/64.2	12.7/4.2	21/7
IV*				
• Dialysis patients	N=14	69.8/64.5	12.2/6.9	21/12
• Healthy subjects	N=15	88.3/91.8	14.0 /17.5	19/24

NB-UVB source * Waldmann UV 7001 cabin; # Medisun 700 UVB-311
nd = not done

8.2 Comparison of the effects of narrow-band UVB and oral vitamin D supplementation on serum 25(OH)D concentrations in healthy subjects (II)

Twelve NB-UVB exposures increased the mean serum 25(OH)D by 41.0 nmol/L (95% CI 34.8–47.2, p <0.001; Table 3, Fig. 3), while oral cholecalciferol achieved an increase of 20.2 nmol/L (95% CI 14.6–26.0, p <0.001; Table 3, Fig. 3). The difference between the two treatments was therefore a significant one: 20.7 nmol/L (95% CI 12.2–29.2, p <0.001). In fact this was already evident after two weeks of treatment (p = 0.033). Altogether 28 (85%) subjects in the NB-UVB group and 13 (43%) subjects in the oral

cholecalciferol group had an 25(OH)D concentration above 75.0 nmol/L at the end of the active treatment regime.

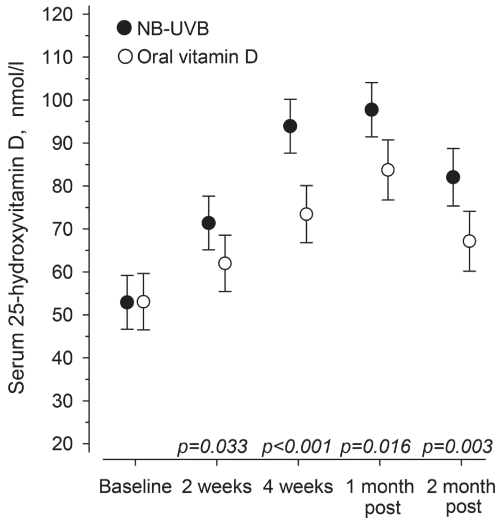

Figure 3. Mean (95% CI) increase in serum 25-hydroxyvitamin D (25(OH)D) from the baseline after 12 narrow-band UVB (NB-UVB) exposures or daily oral cholecalciferol (20 µg) supplementation, both administered over 4 weeks, in healthy subjects. The response to NB-UVB is significantly higher than that to oral cholecalciferol at all time points. The 25(OH)D concentrations had continued to increase by one month after the treatments, and although they had decreased somewhat after 2 months, they were clearly higher than at the baseline. (Original figure in paper II, Fig. 1)

One month after the treatments the 25(OH)D concentrations had increased further in both groups, being 97.2 ± 26.2 nmol/L and 84.9 ± 17.2 nmol/L, respectively ($p < 0.016$; Table 4). Twenty-seven (84%) subjects in the NB-UVB group and 14 (56%) subjects in the oral cholecalciferol group had a 25(OH)D concentration above 75.0 nmol/L. Two months after the treatments the 25(OH)D concentrations had decreased in both groups, to 81.4 ± 21.0 nmol/L and 67.7 ± 17.2 nmol/L ($p < 0.003$, Table 4), respectively, but these concentrations were still clearly higher than at the baseline. The daily dietary intake of vitamin D before the treatment had been 8.14 ± 3.02 µg (mean ± SD) in the NB-UVB group and 8.17 ± 3.35 µg daily in the cholecalciferol group, while two months after the treatments the values were 7.90 ± 2.93 µg and 8.01 ± 3.28 µg, respectively.

8.3 Effect of narrow-band UVB exposures on serum 25(OH)D concentrations in dialysis patients with (IV) or without (III) continuous oral cholecalciferol supplementation

The serum 25(OH)D concentrations of the dialysis patients had increased to 46.3 ± 12.0 nmol/L after eight NB-UVB exposures (Table 3, Fig. 4), a statistically significant change ($p < 0.001$). After intervention, none of these patients had serum

25(OH)D below 25.0 nmol/L, but the concentration was still below 50.0 nmol/L in 11 patients (73%). Serum $1,25(OH)_2D$ increased from 12.4 ± 2.5 to 15.7 ± 4.0 pmol/L (p = 0.002). In the healthy subjects NB-UVB exposure increased the serum 25(OH)D concentration from 60.2 ± 18.0 to 69.2 ± 17.7 nmol/L (p = 0.032; Table 3, Fig. 4).

Mean serum 25(OH)D levels in the dialysis patients at follow-up one and two months after NB-UVB exposure had decreased, but were still 10% higher than initially in paper III (Table 4), while those in the healthy subjects were still significantly increased one month after NB-UVB exposure but had decreased to close to the pre-treatment level after two months (Table 4). Plasma intact parathyroid hormone levels in the dialysis patients did not change during the NB-UVB exposures, and the levels of blood haemoglobin, plasma total calcium, serum ionized calcium and plasma phosphorus all remained unchanged.

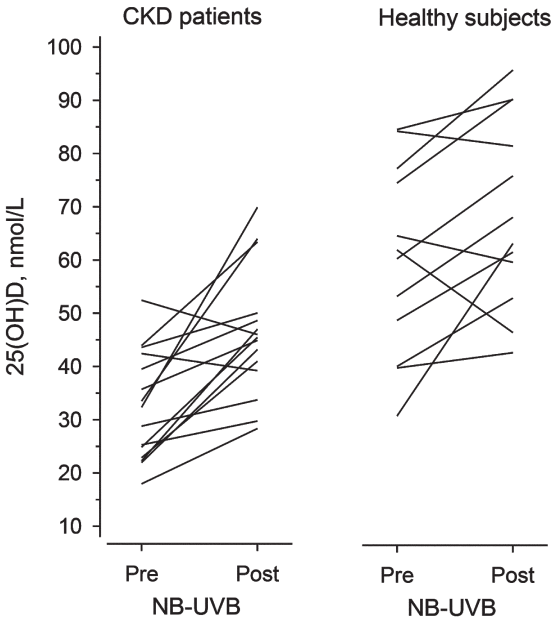

Figure 4. Serum 25-hydroxyvitamin D (25(OH)D) concentrations in 15 chronic kidney disease (CKD) patients on haemodialysis and 12 healthy subjects without oral cholecalciferol supplementation before (pre) and after (post) eight narrow-band ultraviolet B (NB-UVB) exposures. The increase was significant in both the CKD patients (p <0.001) and the healthy subjects (p = 0.032). (Original figure in paper III, Fig. 1)

The NB-UVB course increased serum 25(OH)D by 14.0 nmol/L (95% CI 8.7 to 19.5, p <0.001), or 24.2% (Table 3, Fig. 5). Only one dialysis patient (7%) had a serum 25(OH)D concentration below 50.0 nmol/L after treatment. The increase in 25(OH)D did not differ between the seven patients taking active vitamin D analogues (mean 13.2 nmol/L) and those not doing so (mean 15.0 nmol/L, p = 0.72). In the healthy subjects the NB-UVB course increased serum 25(OH)D by 17.0 nmol/L (CI 13.7 to 20.2, p <0.001), or 22.8% (Table 3, Fig. 5).

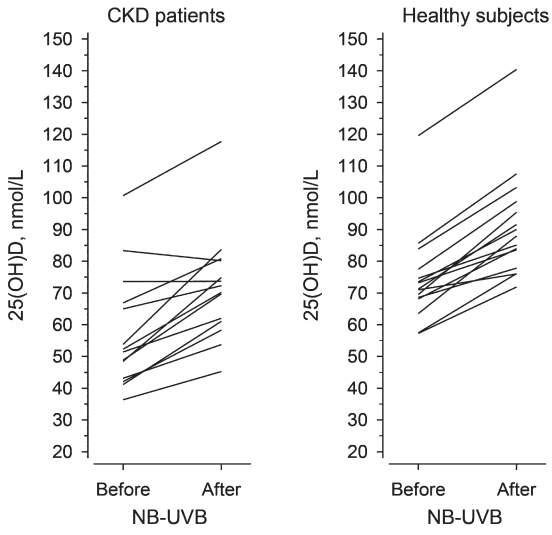

Figure 5. Serum 25-hydroxyvitamin D (25(OH)D) concentrations in 14 chronic kidney disease (CKD) patients on haemodialysis and 15 healthy subjects before and after the narrow-band ultraviolet B (NB-UVB) course. Both groups had been receiving oral cholecalciferol supplementation at a daily dose of 20 µg. The increase was significant ($p < 0.001$) in both groups. (Original figure in paper IV, Fig. 1)

As reported in paper IV, follow-up serum 25(OH)D levels one and two months after the NB-UVB course (Table 4) were still significantly higher than at the baseline in both the dialysis patients (mean 69.8 ± 18.1 and 64.5 ± 22.3 nmol/L; $p < 0.001$ and $p = 0.031$) and the healthy subjects (88.3 ± 19.9 and 91.8 ± 19.7 nmol/L; $p = 0.002$ and $p < 0.001$).

Excluding the serum 25(OH)D levels, NB-UVB treatment had only marginal effects on the laboratory findings in the case of the dialysis patients (IV). Serum ionized calcium decreased, whereas intact parathyroid hormone, phosphorous and haemoglobin did not show any significant changes.

8.4 Effect of narrow-band UVB exposures on cutaneous antimicrobial peptides, cytokines and vitamin D hydroxylating enzymes (I, III, IV)

Cathelicidin and human β-defensin expression (I, III)

Analysis of the mRNA expression levels of cathelicidin before NB-UVB treatment showed significantly elevated levels in psoriasis lesions as compared with the normal skin of healthy subjects (I: Fig. 2a), while those in the atopic dermatitis lesions were also increased, but the difference was not significant (I: Fig. 3a). After six NB-UVB treatments cathelicidin expression had increased markedly in the psoriasis skin lesions and slightly in the atopic dermatitis ones, but the increases did not reach statistical significance. HBD2 mRNA expression levels were also significantly higher in the psoriasis and atopic dermatitis lesions than in the healthy control skin before NB-UVB

treatment (I: Fig. 2b and Fig. 3b), and this treatment significantly reduced the levels in both types of lesions, although the change did not reach statistical significance.

The baseline cathelicidin mRNA expression in the dialysis patients in trial III was somewhat lower than in the healthy subjects, and the nine NB-UVB exposures further reduced it to a significant extent (III: Fig. 4).

Cytokine and chemokine expression in psoriasis and atopic dermatitis (I)

The psoriasis lesions expressed significantly higher amounts of interleukin (IL)-1β, IL-17A and interferon (IFN)-γ before NB-UVB treatment than did the atopic dermatitis lesions (I: Fig. 4), whereas CCL17 (thymus and activation regulated chemokine, TARC) was significantly higher in the atopic dermatitis lesions and IL-10, transforming growth factor (TGF)-β1, tumour necrosis factor (TNF)-α and CCL20 (macrophage inflammatory protein-3a, MIP-3a) were expressed similarly in both diseases. No IL-4 expression was found in the psoriasis and atopic dermatitis lesions. The expression of IL-1β and IL-17A was markedly, but not significantly, reduced in the psoriasis lesions after six NB-UVB treatments (I: Fig. 4), but no changes were observed in the expression of IL-4, IL-10, IFN-γ, TGF-β1, TNF-α, CCL17 or CCL20.

CYP27A1 and CYP27B1 expression in dialysis patients (III, IV)

The normal skin of the dialysis patients in trial IV showed a significantly lower baseline level of CYP27A1 mRNA expression than that of the healthy subjects, whereas their expression of CYP27B1 mRNA was somewhat higher in trial III and significantly higher in trial IV when these patients received oral cholecalciferol supplementation. The nine NB-UVB exposures caused a significant decrease in the CYP27A1 and CYP27B1 mRNA expression levels of the dialysis patients (IV: Fig. 3A and B), and a similar result was found in the healthy subjects, but they did not cause any change in CYP24A1 mRNA expression level in the dialysis patients without cholecalciferol supplementation (III: Fig. 3B), showing that there was no major degradation of 25(OH)D and $1,25(OH)_2D$. CYP24A1 was erroneously interpreted as a synthesizing enzyme in the original paper (III).

9 DISCUSSION

9.1 Narrow-band UVB treatment increases serum 25(OH)D concentrations and affects cutaneous antimicrobial peptides and cytokines in patients with psoriasis and atopic dermatitis (I)

The mean serum 25(OH)D increase during the course of NB-UVB treatment was highest in the patients with atopic dermatitis (212%) and psoriasis (163%; Table 3), probably due to the fact that these patients received more NB-UVB exposures and larger cumulative NB-UVB doses than the others considered in the present thesis (Table 2). It should be noted, however, that these cumulative doses were somewhat lower than those we usually give to patients with psoriasis in our department during a course of 15 NB-UVB exposures, only 84% of the total dose, and that the increase in 25(OH)D after UVB exposure is dependent on the dose given (Bogh et al. 2011a). Moreover, the patients with atopic dermatitis and psoriasis had very low mean serum 25(OH)D concentrations, and the increase is known to be greatest when baseline levels are low (Bogh et al. 2010, Romaní et al. 2012). An analogous situation has been noted with oral vitamin D supplementation, in that subjects with a lower baseline 25(OH)D concentration were found to respond more than those with a higher baseline concentration (Viljakainen et al. 2006).

Several studies have been published in recent years on the effect of NB-UVB treatment on serum 25(OH)D concentrations in psoriasis patients in winter (Osmancevic et al. 2009, Ryan et al. 2010, Lesiak et al. 2011, Romaní et al. 2012), and although the total dose of NB-UVB given has varied greatly, the increases in serum 25(OH)D concentration have been significant in all these reports, as also in the present instance (I). In a more recent examination of how 12 psoriasis patients receiving 20 μg supplementary oral cholecalciferol daily responded to a course of NB-UVB treatment (Ala-Houhala et al. 2013) the mean baseline serum 25(OH)D concentration, 74 nmol/L, was much higher than in trial I, when no supplementation was given, but in spite of this, serum 25(OH)D had increased by 13 nmol/L at the 9th NB-UVB exposure and by 49 nmol/L at the 18th exposure and the PASI scores improved as expected. It is also important to note that serum 25(OH)D concentrations remained far from the

toxicity level in these psoriasis patients who received both vitamin D supplementation and NB-UVB treatment.

Analysis of the baseline mRNA expression levels of the antimicrobial peptides cathelicidin and HBD2 in paper I showed significantly elevated levels in the psoriasis lesions and elevated levels in atopic dermatitis relative to the normal skin of healthy subjects. These results are in agreement with earlier reports concerning psoriasis and atopic dermatitis (Gambichler et al. 2006, Hollox et al. 2008). It is of interest that cathelicidin and HBD2 can act as proinflammatory mediators, or "alarmins", and seem to have a role in the pathogenesis of skin inflammation in psoriasis and atopic dermatitis (Ong et al. 2002, Hollox et al. 2008). After six NB-UVB treatments cathelicidin expression had increased markedly in the psoriasis skin lesions and slightly in the atopic dermatitis lesions. In our more recent NB-UVB treatment trial, however (Ala-Houhala et al. 2013), where the psoriasis patients received supplementary oral cholecalciferol, we did not find any change in cathelidicin expression, while NB-UVB treatment significantly reduced HBD2 expression in healing psoriasis lesions both in the present patient series (I) and in our more recent one (Ala-Houhala et al. 2013) and slight decrease of HBD2 was found in atopic dermatitis lesions, as in previous reports (Gambichler et al. 2006, Ballardini et al. 2009, Peric et al. 2009). Alongside this decrease in HBD2 we saw a significant increase in serum 25(OH)D, the best indicator of the hormonally active form of vitamin D, i.e. 1,25(OH)2D. This exerts potent anti-inflammatory action through the inhibition of NfκB activation and inhibits IL-17A-induced HBD2 in keratinocytes *in vitro* (Peric et al. 2008). As NB-UVB is known to induce local synthesis of 25(OH)D and 1,25(OH)2D in keratinocytes (Lehmann et al. 2007), we propose that the effects of NB-UVB-triggered vitamin D production may outweigh the HBD2-inducing effect of short-duration UVB in our patient series.

We were also able to show that NB-UVB treatment reduced the expression of IL-1β and IL-17A in psoriatic skin lesions. IL-1β is a proinflammatory cytokine and is thought to be a critical mediator of the differentiation of human T cells that produce IL-17 (McKenzie et al. 2006, Wilson et al. 2007). In turn, IL-17 has been shown to enhance vitamin D-induced expression of the antimicrobial peptide cathelicidin (Peric et al. 2008). Although the expression of cathelicidin did not immediately decrease along with IL-17A in our material (I), lowered IL-17 levels may later reduce inflammation and neutrophil activation at lesional skin sites (Nickoloff 2007, Di Cesare et al. 2009). Our results suggest that the interplay between cytokines and vitamin D in the regulation of antimicrobial peptides in keratinocytes is complex and is still only partly understood, so that further research would be warranted.

9.2 Comparison of the effects of narrow-band UVB course and oral vitamin D supplementation on serum 25(OH)D concentrations in healthy subjects (II)

Paper II contains a comparison of the effects of NB-UVB exposures and oral vitamin D supplementation on healthy adult hospital employees in winter. Subjects with 25(OH) D below 75 nmol/L were randomly given either a course of 12 NB-UVB exposures or 20 μg of oral cholecalciferol daily for 4 weeks. The results showed that the NB-UVB exposures increased the mean serum 25(OH)D concentration by 41 nmol/L (Table 2) and oral cholecalciferol by 20 nmol/L, a significant difference. One month after the treatments the 25(OH)D concentrations in both groups were still higher than the baseline values.

Soon after our paper was published, a Swedish group reported similar results (Bogh et al. 2012a). They had given total body NB-UVB exposures three times a week for six weeks and were also able to show that NB-UVB treatment was more effective than a daily oral intake of 40 μg cholecalciferol, as mean serum 25(OH)D increased from 19 to 75 nmol/L in the NB-UVB group and from 23 to 61 nmol/L in the oral cholecalciferol group. They treated 73 participants with vitamin D deficiency (25(OH)D <25 nmol/L), but only 32 completed the treatment. Moreover, the participants were not described in any detail.

The main differences between the Swedish report and the present paper II concerned the subjects, their baseline serum 25(OH)D concentrations (<25 nmol/L vs. <75 nmol/L) and the oral cholecalciferol dose (40 μg vs. 20 μg daily). The total cumulative NB-UVB dose was similar in both cases, however, 9 J/cm^2 vs. 8 J/cm^2. It should be noted that the NB-UVB doses used in both trials were smaller than those conventionally used when treating psoriasis patients. The findings reported in these two papers, that 12–18 whole-body NB-UVB exposures given over 4–6 weeks were more efficient in both treating vitamin D deficiency and improving the vitamin D status of healthy subjects in winter than a daily oral vitamin D intake of 20–40 μg, were noted in a British Journal of Dermatology editorial (Diffey 2012), where it was also pointed out that it is controversial as to whether someone exhibiting a "normal" 25(OH)D concentration, i.e. from 50 to 75 nmol/L, should be subjected to a medical intervention by NB-UVB treatment.

9.3 Narrow-band UVB exposures increase serum 25(OH)D concentrations in dialysis patients and affect cutaneous enzymes hydroxylating vitamin D (III, IV)

In advanced kidney disease the kidney is unable to produce $1,25(OH)_2D$ from 25(OH)D due to the loss of renal CYP27B1 activity (Pitts et al. 1988, Nigwekar et al. 2012). It has been shown in UVB-treated skin cultures that keratinocytes are able to hydroxylate 25(OH)D to $1,25(OH)_2D$ (Lehmann et al. 2007). The finding that the CYP27B1 enzyme also exists outside the kidney is of interest with regard to oral vitamin D treatment for CKD patients (Melamed & Thadhani 2012).

In trial III, nine NB-UVB exposures given to dialysis patients increased serum 25(OH)D by 42% (Table 3) and serum $1,25(OH)_2D$ by 27%. The NB-UVB exposures were given only to a body surface area of approximately 25%, whereas in the other trials (I, II, IV) whole-body exposures were given. If the exposed body area had been larger, e.g. the whole body, the serum 25(OH)D response might have been even more substantial. In the healthy subjects the NB-UVB course increased serum 25(OH)D by 15% (III; Table 3).

In trial IV the subjects were given an oral cholecalciferol supplement of 20 μg daily before, during and after the NB-UVB course, so that and the baseline serum 25(OH)D concentrations were clearly higher than in Study III (Table 2). In spite of this, nine NB-UVB whole-body exposures given to the dialysis patients increased their serum 25(OH)D significantly, i.e. by 24% (Table 3), in spite of the fact that they had been taking 20 μg oral cholecalciferol daily for a mean of 5 months beforehand. The NB-UVB course also increased serum 25(OH)D in the healthy subjects, by 23% (Table 3).

It should be noted that though the NB-UVB courses given to the dialysis patients in trials III and IV were quite short, the increase in serum 25(OH)D was significant. NB-UVB exposures have been used to relieve uraemic pruritus (Ko et al. 2011), but to our knowledge, these present trials are the first to show that NB-UVB exposures can also be used to improve the vitamin D balance in CKD patients undergoing dialysis.

Although the mean serum 25(OH)D concentration in the dialysis patients in trial III had decreased one month after the NB-UVB exposures, it was still 10% higher than at the baseline (Table 3). This kind of rapid decrease in serum 25(OH)D was not seen in the healthy subjects in either the present trial or in the psoriasis and atopic dermatitis patients and healthy subjects in our previous ones (I, II). Similarly, 25(OH)D concentrations began to decrease in the dialysis patients in trial IV during the follow-up of two months, in contrast to the situation in the NB-UVB-treated healthy subjects. The more profound decrease in 25(OH)D may be due to the higher body mass index (BMI) of the dialysis patients than in the healthy subjects, i.e. linked to active metabolism of vitamin D precursors in the fat tissue (Lehmann & Meurer 2010, Forsythe et al. 2012).

Although our dialysis patient series were small and the NB-UVB courses given short, the relatively rapid decrease in serum 25(OH)D suggests that dialysis patients may need a longer course of NB-UVB exposures or cyclic NB-UVB exposures to maintain their increased serum 25(OH)D concentrations. It has been shown in healthy subjects that BB-UVB exposures every second week are sufficient to maintain summer 25(OH)D levels during the winter (Bogh et al. 2012b). It would be worth trying continuous NB-UVB exposures also of this kind with dialysis patients in order to maintain their increased 25(OH)D concentrations.

Since as few as nine NB-UVB exposures were shown to cause significant increases in serum 25(OH)D concentrations in dialysis patients, it was of interest to study the effect of NB-UVB exposures on the enzymes hydrolyzing vitamin D in the skin. mRNA expression of the CYP27B1 enzyme was already elevated at the baseline, not significantly in trial III but significantly in trial IV, in which the dialysis patients had received cholecalciferol supplementation. This important observation suggests that the loss of renal CYP27B1 activity in the dialysis patients leads to activation of this enzyme in the skin, and possibly also in other organs, this activity in the skin being further intensified by the NB-UVB exposures in our patients. Interestingly, the NB-UVB exposures themselves caused a significant decrease in the mRNA expression of CYP27B1 and CYP27A1 in both the dialysis patients and the healthy subjects in trial IV, where they all received 20 μg of supplementary oral cholecalciferol. Such a decrease can be expected, because there is a very sensitive natural feedback controlling mechanism caused by the UVB-induced increase in cutaneous vitamin D synthesis (Holick 2007, Schauber et al. 2007, Lehmann & Meurer 2010).

10 CONCLUSIONS AND FUTURE PROSPECTS

The conclusions to be drawn from the present trials concerning the effects of NB-UVB treatment on the vitamin D balance of dermatological and dialysis patients and healthy subjects performed at Tampere University Hospital in 2008–2012 and the future prospects for research in this field may be summarized as follows:

Narrow-band UVB treatment increases serum 25(OH)D concentrations and affects cutaneous antimicrobial peptides and cytokines in patients with psoriasis and atopic dermatitis (I)

NB-UVB treatment is widely used for psoriasis and atopic dermatitis, but its effects on patients' vitamin D balance, i.e. serum 25(OH)D concentrations, had previously been examined only occasionally. In the present series 89% of the patients with psoriasis, 94% of those with atopic dermatitis and 53% of the healthy subjects were found to have baseline vitamin D insufficiency (serum 25(OH)D <50 nmol/L). This finding is in agreement with recent information on the frequent occurrence of vitamin D insufficiency in the general population (Holick 2007, Hyppönen & Power 2007). Fifteen whole-body NB-UVB exposures significantly increased serum 25(OH)D ($p < 0.001$), by 59.9 nmol/L in the psoriasis cases, 68.2 nmol/L in atopic dermatitis and 90.7 nmol/L in the healthy subjects. It was concluded that, in addition to a significant improvement in the status of psoriasis and atopic dermatitis, NB-UVB treatment effectively corrects vitamin D insufficiency. This finding is in agreement with several recent studies of psoriasis in winter (Osmancevic et al. 2009, Ryan et al. 2010, Lesiak et al. 2011, Romaní et al. 2012, Ala-Houhala et al. 2013).

It would be of interest in the future to study systemically how much the serum 25(OH)D concentration would increase, e.g. after the 25–30 NB-UVB exposures which are often needed for complete clearance of moderate or severe psoriasis. The fact that the PASI score denoting the status of psoriasis, did not show any correlation with the increase in serum 25(OH)D as reported here and previously (Romaní et al. 2012), suggests that vitamin D balance is not directly linked to an improvement in psoriasis, but further investigations, especially with regard to the skin lesions themselves, would be needed to confirm or exclude this possibility.

Antimicrobial peptides such as cathelicidin and HBD2 seem to have a role in the pathogenesis of skin inflammation in psoriasis (Schauber & Gallo 2008), and as expected, we found increased expression of these in the untreated psoriasis lesions and

we also able to show that NB-UVB exposure further increased cathelidicin and reduced HBD2 in healing lesions. Similar effects of NB-UVB were not seen in our more recent trial (Ala-Houhala et al. 2013), in which the psoriasis patients received supplementary oral vitamin D. Whether these different effects of NB-UVB could be mediated by the improved vitamin D balance in the psoriasis lesions should be examined in more detail.

Comparison of the effects of narrow-band UVB exposures and oral vitamin D supplementation on serum 25(OH)D concentrations in healthy subjects (II)

Since there had been no direct comparisons on the effects of NB-UVB and oral vitamin D supplementation on serum 25(OH)D concentrations, we assigned healthy adult subjects with serum 25(OH)D below 75 nmol/L to receive randomly either a course of 12 whole-body NB-UVB exposures or 20 μg of oral cholecalciferol daily for 4 weeks. The increase in serum 25(OH)D was significantly greater in the NB-UVB group than in the cholecalciferol group (mean 41.0 nmol/L vs. 20.2 nmol/L). We concluded that a short course of low-dose NB-UVB is an effective way of improving vitamin D balance in winter, and toted that the response was still in evidence after 2 months. Soon after this trial, a Swedish group published similar results concerning subjects who had an initial serum 25(OH)D concentration below 25 nmol/L (Bogh 2012a). It would be of interest in the future to compare NB-UVB exposures with higher oral cholecalciferol doses than 40 μg daily. In addition, it would be of interest to know how high serum 25(OH)D concentrations would rise if NB-UVB courses longer than 4–6 weeks were given. This is important, because it is known that exposure of the skin to UVB triggers a feedback mechanism that controls vitamin D synthesis in such a way that an overdose is not possible (Holick 2007).

Narrow-band UVB exposures increase serum 25(OH)D concentrations and affect cutaneous enzymes hydroxylating vitamin D in dialysis patients (III, IV)

Most chronic kidney patients on dialysis are known to have vitamin D insufficiency because the loss of renal CYP27B1 activity means that the kidney is unable to convert 25(OH)D to 1,25$(OH)_2$D (LaClair et al. 2005, Bhan et al. 2010, Nigwekar et al. 2012). In agreement with this, we found low serum 25(OH)D concentrations in our dialysis patients who did not receive supplementary oral cholecalciferol, 32.5 nmol/L, and in those who received such supplements, 57.6 nmol/L. Eight NB-UVB exposures significantly increased serum 25(OH)D by 43%, in our first dialysis trial (III) and by 24% in the second (IV), and serum 1,25$(OH)_2$D also increased significantly, by 27% (III). To our knowledge, these trials are the first to show that NB-UVB exposures can be used to improve the vitamin D balance of dialysis patients.

Although the serum 25(OH)D concentrations had started to decrease one and two months after the NB-UVB exposures, they were still higher than initially (Table 4). Thus although our dialysis patient series were small and the NB-UVB courses were short, the relatively rapid decrease in serum 25(OH)D suggests that dialysis patients

would need a longer course of NB-UVB treatment or cyclic NB-UVB exposures to maintain their increased serum 25(OH)D concentrations. It would also be worthwhile trying to give dialysis patients regular NB-UVB exposures in order to maintain higher 25(OH)D concentrations.

We also examined the mRNA expression of the enzymes CYP27A1 and CYP27B1 that hydroxylate vitamin D in skin biopsy samples taken from dialysis patients before and after a course of eight NB-UVB exposures. In first dialysis trial (III) the NB-UVB exposures significantly increased the mRNA expression of CYP27B1, whereas in the second (IV), baseline CYP27B1 was already higher. The increased cutaneous CYP27B1 levels in the dialysis patients suggest that loss of the renal activity of this enzyme is at least partially compensated for by the skin, but further trials with more NB-UVB exposures would be needed to confirm this observation.

In conclusion, NB-UVB exposures were shown to be an efficient way of increasing serum 25(OH)D concentrations in dermatological and dialysis patients, and in healthy subjects in winter. A short NB-UVB course was shown to increase serum 25(OH)D in healthy subjects significantly more than does daily supplementation with 20 μg oral cholecalciferol. NB-UVB exposures offer a new possibility for improving the vitamin D balance dialysis patients with vitamin D insufficiency. NB-UVB treatment is currently considered safe, but a longer follow-up would be needed.

11 ACKNOWLEDGEMENTS

This research was carried out at the Department of Dermatology, Tampere University Hospital, and at the School of Medicine, Tampere University. I am most grateful to my supervisors, Professor Emeritus Timo Reunala, M.D., and Professor Erna Snellman, M.D. Professor Reunala has supervised and supported me throughout my scientific career. I was already impressed by his enthusiasm for science when I was a medical student, and it was he who introduced me to scientific thinking and proposed collaboration with nephrologists. Professor Snellman is especially qualified in photodermatology, and her practical advice and experience with regard to phototherapy were essential for the present work. Her encouragement during these years has made me believe in myself. I especially wish to thank both supervisors for being available for consultation at any time.

I owe a great debt of gratitude to my follow-up group and co-workers, Docent Heikki Saha, M.D., and Taina Hasan, M.D. Docent Saha made a significant contribution to the planning and accomplishment of the trials involving dialysis patients, and Dr. Hasan had a great impact on the UVB treatments and doses. I am also happy to have had an additional supervisor, co-worker and friend in Katja Vähävihu, M.D., who has advised me in many difficult situations.

I am particularly indebted to Docent Annikki Vaalasti, M.D., Head of the Department of Dermatology at Tampere University Hospital, for her positive support. She has enabled me to carry on with my scientific work during specialization as a dermatologist. I cannot emphasise enough the influence that she has had on this project.

I am deeply grateful to my reviewers, Professor Emeritus Aarne Oikarinen, M.D., and Docent Pauli Karhapää, M.D., for their constructive criticism, convincing comments and relevant revisions, which refined my dissertation in its final form.

Special thanks are due to all my skilful co-workers on this thesis: to Docent Jürgen Schauber, M.D., Yvonne Dombrowski, Ph.D. and Mark Peric, Ph.D., who carried out the trials concerning the enzymes hydroxylating vitamin D and antimicrobial peptides at the Department of Dermatology and Allergy, Ludwig Maximilian University, Munich, especially Docent Schauber, who gave me much friendly counselling in these challenging subjects; to Professor Harri Alenius, Ph.D., and Piia Karisola, Ph.D., who carried out the cytokine tests at the Unit of Systems Toxicology, Finnish Institute of Occupational Health; to Lasse Ylianttila, M.Sc. (Tech.), working at the Radiation and Nuclear Safety Authority's Non-Ionizing Radiation Laboratory, with his amazing skills

in UVB dose measurement; to Heli Viljakainen, Ph.D., who investigated the amount of vitamin D obtained from food and gave advice on nutritional matters; and to Hannu Kautiainen, Ph.D., for his expertise in statistical matters and illustrations.

I am grateful to the Department of Dermatology and the Dialysis Unit at our hospital, especially head nurses Anne Rintala and Johanna Mörö, who arranged facilities for examining the dermatological and dialysis patients and the healthy subjects, and for performing the NB-UVB exposures. I also warmly thank the research nurses Pirjo Honko, Tuija Valjakka and Heidi Hällström for taking care of the subjects during NB-UVB treatments. I similarly thank the laboratory staff, especially Marianne Kuuslahti and Arja Ahola, for carrying out the vitamin D analyses. Indeed, I wish to express my gratitude for all the people working in our cosy dermatology department, and the other staff who have been working with me and have made it so pleasant to do things together.

I sincerely thank Malcolm Hicks, M.A., for his thorough and swift revision of the English language of the thesis.

It is good that we now have a new member in our group, Toni Karppinen, who is excited about research into vitamin D. I warmly thank Anita, Hanna-Mari and Nora for their close friendship; they have known me for decades, we have had good times together and they have supported me at weak moments. I also thank my wonderful parents Marja and Ilpo for their lifelong caring and unconditional love, and my sweet sister Mari for her thoughtful conversations and pleasant company.

I feel privileged to have been a student in the National Graduate School of Clinical Investigation (CLIGS), which provided me with a scientific education and the necessary financial support. I also gratefully acknowledge the finance received from the Tampere University Hospital Competitive Research Funding and from the Finnish Dermatological Society.

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ORIGINAL PUBLICATIONS

BJD
British Journal of Dermatology

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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Accepted for publication
9 March 2010

Key words
antimicrobial peptides, atopic dermatitis, calcidiol, psoriasis, ultraviolet B radiation, vitamin D

Conflicts of interest
None declared.

DOI 10.1111/j.1365-2133.2010.09767.x

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)].

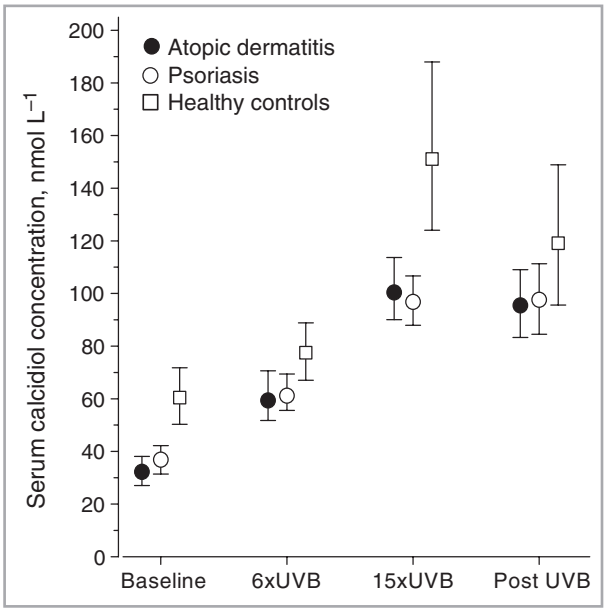

Fig 1. Effect of narrowband ultraviolet B (NB-UVB) treatment on serum calcidiol concentration. Fifteen NB-UVB exposures significantly increased serum calcidiol concentration (mean; 95% confidence intervals) in 18 patients with psoriasis, 18 patients with atopic dermatitis and 15 healthy subjects ($P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

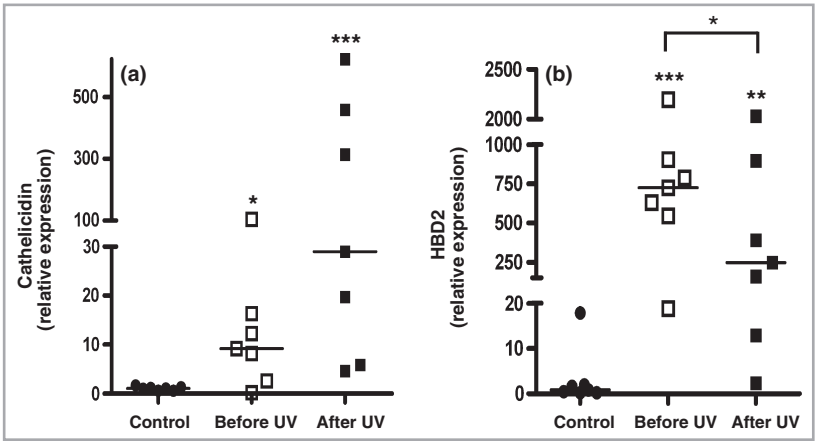

Fig 2. Expression of cathelicidin and human β-defensin 2 (HBD2) in psoriasis. Biopsies of a marker psoriatic plaque (n = 7) were taken before and after six narrowband ultraviolet (UV) B treatments. Total mRNA was extracted and transcript levels of cathelicidin (a) and HBD2 (b) analysed by real-time quantitative 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.

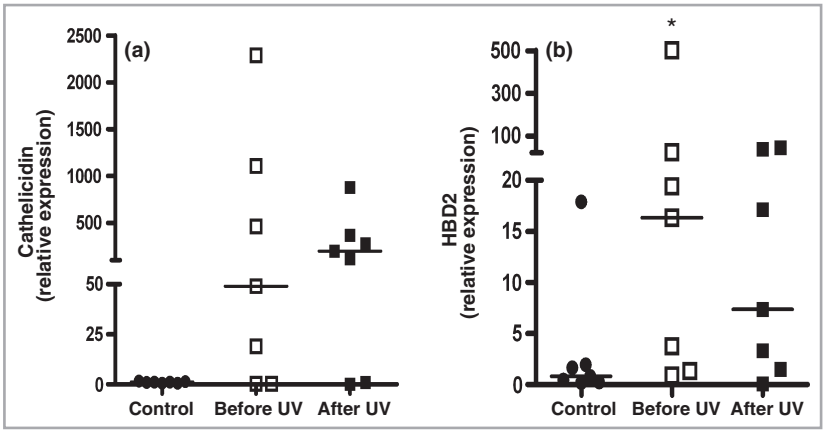

Fig 3. Expression of cathelicidin and human β-defensin 2 (HBD2) in atopic dermatitis. Biopsies of atopic dermatitis skin lesions (n = 7) were taken before and after six narrowband ultraviolet (UV) B treatments. Total mRNA was extracted and transcript levels of cathelicidin (a) and HBD2 (b) analysed by 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

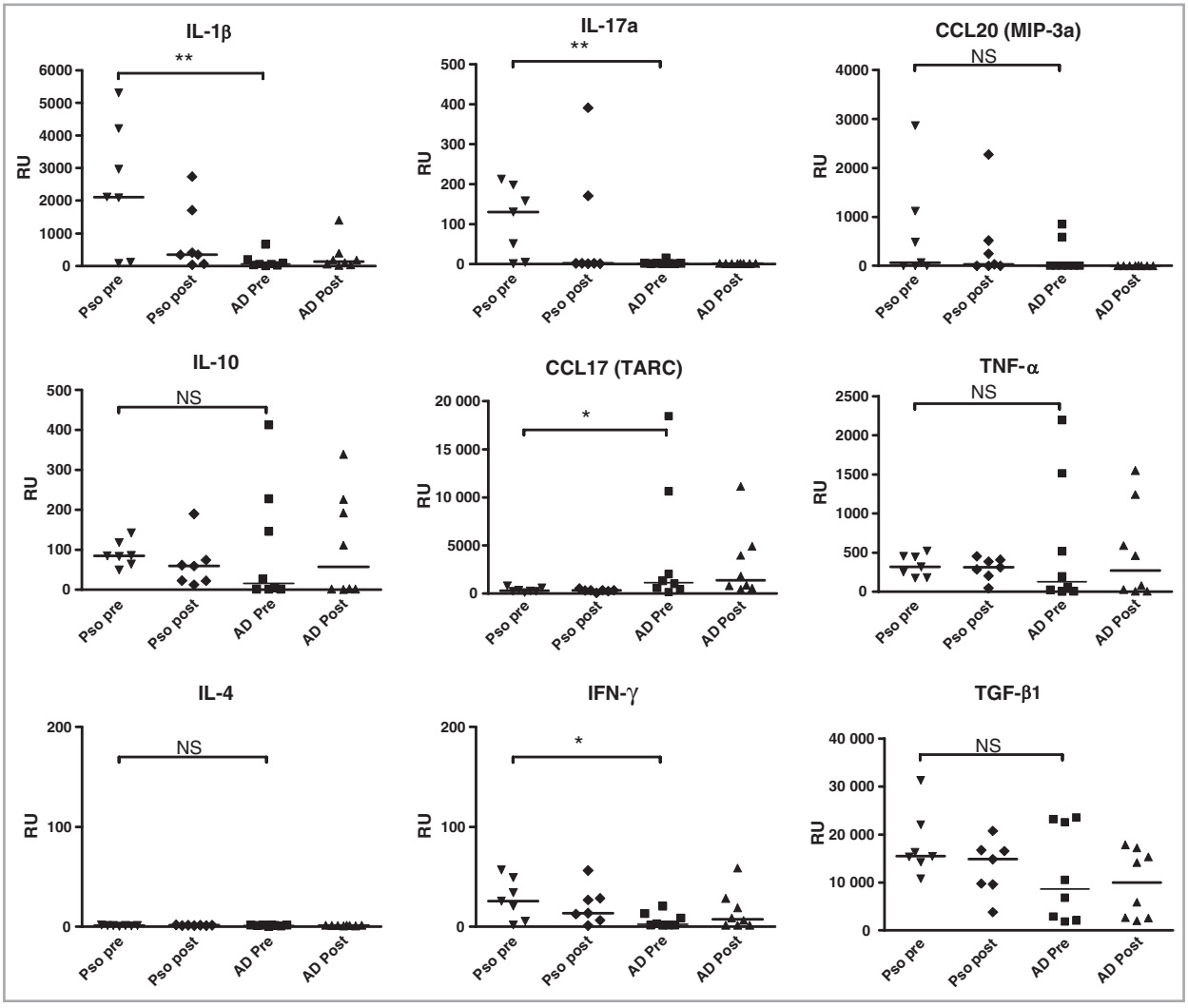

Fig 4. Expression of cytokines and chemokines in the skin lesions of psoriasis (Pso) and atopic dermatitis (AD) before and after narrowband ultraviolet B (NB-UVB) treatment. Real-time quantitative polymerase chain reaction was used to analyse the mRNA expression levels of different cytokines and chemokines 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.

Acknowledgments

This study was supported by the National Graduate School of Clinical Investigation, by the Medical Research Funds of Tampere University Hospital, by the Academy of Finland and by the Deutsche Forschungsgemeinschaft. We thank nurses Pirjo Honko and Tuija Valjakka for taking care of the patients during NB-UVB treatments.

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Comparison of narrowband ultraviolet B exposure and oral vitamin D substitution on serum 25-hydroxyvitamin D concentration

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Accepted for publication
3 April 2012

Funding sources
This study was supported by National Graduate School of Clinical Investigation (M.A.-H.), Finnish Dermatological Society (M.A.-H.) and Competitive Research Funding of the Tampere University Hospital (Grants 9J103 and 9K104).

Conflicts of interest
None declared.

DOI 10.1111/j.1365-2133.2012.10990.x

Summary

Background A short course of narrowband ultraviolet B (NB-UVB) exposures increases the serum 25-hydroxyvitamin D [25(OH)D] concentration in patients with psoriasis and healthy subjects.

Objectives To compare the effects of NB-UVB and oral vitamin D substitution in healthy subjects in winter.

Methods Healthy adult hospital employees and medical students were screened for serum 25(OH)D concentration. Those with 25(OH)D below 75 nmol L^{-1} were randomly given either 12 NB-UVB exposures or 20 μg of oral cholecalciferol daily for 4 weeks. The NB-UVB exposures were given with a Waldmann UV 7001 cabin and the mean cumulative dose was 48·4 standard erythema doses. Serum 25(OH)D was measured before and after the treatments by radioimmunoassay.

Results The baseline serum 25(OH)D concentrations were 52·9 ± 10·4 (mean ± SD) in the 33 NB-UVB-treated and 53·5 ± 12·7 nmol L^{-1} in the 30 oral cholecalciferol-treated subjects. The mean increase in serum 25(OH)D was 41·0 nmol L^{-1} [95% confidence interval (CI) 34·8–47·2; $P < 0·001$] in the NB-UVB group and 20·2 nmol L^{-1} (95% CI 14·6–26·0; $P < 0·001$) in the cholecalciferol group. The difference between the two treatments was significant at 2 weeks ($P = 0·033$) and at 4 weeks ($P < 0·001$). One month after the treatments the 25(OH)D concentrations had increased further.

Conclusions The present study shows that 12 NB-UVB exposures given during 4 weeks increase serum 25(OH)D concentration significantly more than 20 μg of oral cholecalciferol daily. A short NB-UVB course is an effective way to improve vitamin D balance in winter and the response is still evident 2 months after the course.

Vitamin D insufficiency is common worldwide and this condition affects people especially during winter when vitamin D synthesis induced by sun is zero.[1–3] The desirable concentration of serum 25-hydroxyvitamin D [25(OH)D], which is the best indicator of vitamin D status, is still under debate but a concentration of below 75 nmol L^{-1} is considered to be insufficient for bone fracture prevention.[4,5] In addition to osteoporosis, low serum 25(OH)D concentration has recently been associated with the risk of colorectal and prostate cancers,[6] cardiovascular disease[7] and with all-cause mortality in the general population.[8] In addition, vitamin D is known to affect skin inflammation and cutaneous innate or adaptive immune responses.[9,10] Thus, vitamin D seems to have numerous functions in addition to maintaining bone health.

Although milk products and margarine are fortified with vitamin D in many countries, dietary intake seems still insufficient, e.g. in Britain and the Nordic countries.[2,11,12] To prevent vitamin D deficiency or insufficiency, especially in winter, national recommendations in Finland advise children and elderly persons to use regularly oral vitamin D substitution.[13] A recent large study of healthy adults in Scotland[14] found that use of vitamin D supplements improved serum 25(OH)D concentration. However, 79% of the study participants still had May-standardized 25(OH)D concentrations below 50 nmol L^{-1}. Viljakainen *et al.*[15] showed that in Finland as much as 17·5–20 μg of oral vitamin D supplement use daily seems to be required to maintain stable bone turnover in healthy men in winter.

Narrowband UVB (NB-UVB) phototherapy is a widely used dermatological treatment and it is known to suppress a broad range of important molecular pathways in psoriatic skin.[16] NB-UVB light emitting at 311 nm is also capable of activating vitamin D synthesis in cultured keratinocytes.[17] We showed previously that a short NB-UVB course given in winter improves vitamin D balance in healthy women, and also in patients with psoriasis and atopic dermatitis.[18,19] Recently, Bogh *et al.*[20] reported that a small suberythemal UVB dose every second week is sufficient to maintain summer vitamin D levels in healthy subjects. In the present study we were interested to know whether a short NB-UVB course given in winter to healthy subjects could correct vitamin D balance as well as daily vitamin D substitution given orally.

Materials and methods

Subjects, narrowband ultraviolet B exposure and oral cholecalciferol

Healthy adult hospital employees and medical students were screened for 25(OH)D concentration below 75 nmol L^{-1}. Inclusion criteria were age 18–65 years, no vitamin D supplement use during the preceding 2 months and no sun holidays or solarium visits during the preceding 3 months. Ninety-nine subjects participated in the screening. Sixty-seven subjects had serum 25(OH)D concentration below 75 nmol L^{-1} and were randomly allocated to NB-UVB or oral cholecalciferol study groups. The sample sizes, i.e. 30 subjects per group, needed to discover significant differences in 25(OH)D concentrations were calculated with a Power and Sample Size Calculations program. Treatments were given in winter months from December 2010 to March 2011. The ethics committee of Tampere University Hospital approved the study protocol and all subjects gave informed consent to participate. The authors followed the principles of the Declaration of Helsinki.

The first group of 33 subjects (four men, 29 women, mean age 43·8 years, Table 1) were allocated to receive 12 NB-UVB exposures given three times a week on the whole body area with a Waldmann UV 7001 cabin equipped with 40 TL01 tubes (Schulze & Böhm, Brühl, Germany). The spectral irradiance of the cabin was measured with a stray light corrected single-monochromator spectroradiometer as previously described.[18] The initial physical NB-UVB dose was 0·21 J cm^{-2} (1·25 SED). One standard erythema dose (SED) is equivalent to 10 mJ cm^{-2} CIE (Commission Internationale de l'Eclairage) erythema-weighted irradiance.[21] The dose was increased according to a fixed protocol gradually up to 1·45 J cm^{-2} (8·55 SED). However, if the subjects experienced itching or slight erythema the NB-UVB dose was either lowered or the same NB-UVB dose was repeated before increasing the dose. Due to this, only two subjects received the intended maximum dose. The mean cumulative amount of NB-UVB given to the 33 subjects was 8·23 J cm^{-2} (range 6·14–9·34 J cm^{-2}), which is equivalent to 48·4 SED (range 36·1–54·9 SED). The second group was allocated to receive oral cholecalciferol 20 μg (Devisol 20 μg®; Orion Pharma, Espoo, Finland) daily for 4 weeks (Table 1). Cholecalciferol 20 μg is equivalent to 800 international units (IU). This group consisted of 30 subjects (five men, 25 women, mean age 40·2 years) because four subjects had to be excluded due to too high (over 75 nmol L^{-1}) baseline serum 25(OH)D concentrations.

Measurement of serum 25(OH)D concentrations

Blood samples for serum 25(OH)D measurements were taken from both study groups before the study, after 2 weeks (before the 7th NB-UVB exposure) and at the end of the 4-week treatment (before the 12th NB-UVB exposure). Follow-up samples were taken 1 and 2 months after the treatments. The serum samples were protected from light, centrifuged and then stored at −70 °C. Serum 25(OH)D concentrations were analysed in duplicate by using radioimmunoassay (Immunodiagnostic Systems, Boldon, U.K.) as in our previous study.[19]

Table 1 Demographic data of the healthy subjects in the narrowband ultraviolet B (NB-UVB) exposure and oral cholecalciferol study groups

	NB-UVB group (n = 33)	Oral cholecalciferol group (n = 30)
Male/female	4/29	5/25
Mean age (range), years	43·8 (23–59)	40·2 (25–62)
Body mass index (range), kg m^{-2}	26·2 (17·9–41·2)	25·8 (19·1–33·1)[a]
Fitzpatrick skin type II/III/IV	5/23/5	5/18/5[b]
Daily dietary vitamin D intake at baseline	8·14 ± 3·02 μg	8·17 ± 3·35 μg[a]
Serum 25-hydroxyvitamin D at baseline	52·9 ± 10·4 nmol L^{-1}	53·5 ± 12·7 nmol L^{-1}

[a]Not determined in three subjects. [b]Not determined in two subjects.

Dietary intake of vitamin D

Dietary intake of vitamin D was assessed at baseline and 2 months after the active treatments with a Food Frequency Questionnaire (FFQ). The FFQ contains 14 quantified food items known as sources of vitamin D. The frequency of these food items consumed during a previous month was investigated. Vitamin D content of the foods is based on the Fineli® (Finnish Food Composition Database, version 2010), which is maintained by the National Institute for Health and Welfare. In addition, the FFQ contains specific questions about the type of dairy products, fish and fat in the diet or cooking to quantify the intake of vitamin D.

Statistics

The results were expressed as means ± SD and 95% confidence intervals (CIs). Statistically significant differences between the groups were tested by permutation test. Repeated measures were analysed using generalizing estimating equations models with the unstructured correlation structure. Generalized estimating equations were developed as an extension of the general linear model (e.g. ordinary least squares regression analysis) to analyse longitudinal and other correlated data. Generalized estimated equations models take into account the correlation between repeated measurements in the same subject; models do not require complete data and can be fit even when individuals do not have observations at all time points.[22] No adjustment was made for multiple testing.

Results

The baseline serum 25(OH)D concentration value was 52·9 ± 10·4 (mean ± SD) in the NB-UVB exposure group and 53·5 ± 12·7 nmol L^{-1} in the oral cholecalciferol group ($P = 0·96$, Table 1). Twelve NB-UVB exposures given during 4 weeks increased the mean serum 25(OH)D concentration by 41·0 nmol L^{-1} (95% CI 34·8–47·2; $P < 0·001$, Fig. 1). In the oral cholecalciferol group the increase of serum 25(OH)D concentration was 20·2 nmol L^{-1} (95% CI 14·6–26·0; $P < 0·001$). The difference between the two treatments was 20·7 nmol L^{-1} (95% CI 12·2–29·2; $P < 0·001$). This was already evident at 2 weeks ($P = 0·033$, Fig. 1). At the end of active treatments 28 (85%) subjects in the NB-UVB group and 13 (43%) subjects in the oral cholecalciferol group had 25(OH)D concentrations over 75 nmol L^{-1}.

In the NB-UVB group follow-up samples were available from 32 subjects at 1 month and from 28 subjects at 2 months after the treatment. In the oral cholecalciferol group the samples were available from 25 and 25 subjects, respectively. One month after the treatments the 25(OH)D concentrations had increased further in the both groups and were 97·2 ± 26·2 nmol L^{-1} and 84·9 ± 17·2 nmol L^{-1} ($P < 0·016$, Fig. 1). Twenty-seven (84%) subjects in the NB-UVB group and 14 (56%) subjects in the oral cholecalciferol group had 25(OH)D concentrations over 75 nmol L^{-1}. Two months after the treatments the 25(OH)D concentrations had decreased in the both groups and were 81·4 ± 21·0 nmol L^{-1} and 67·7 ± 17·2 nmol L^{-1} ($P < 0·003$), respectively. These concentrations were still clearly higher than at the baseline (Fig. 1).

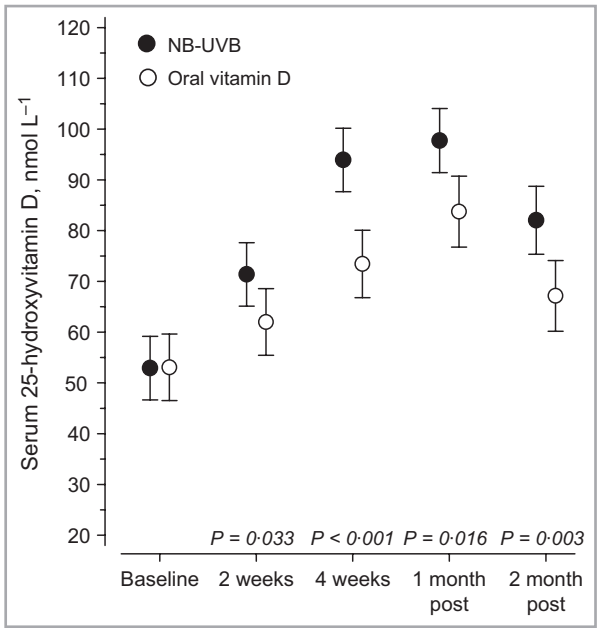

Fig 1. Mean (95% confidence interval) increase of serum 25-hydroxyvitamin D [25(OH)D] from baseline by 12 narrowband ultraviolet B(NB-UVB) exposures and daily oral cholecalciferol (20 μg) substitution given for 4 weeks. The response to NB-UVB is significantly higher than that of oral cholecalciferol at all time points. One month after the treatments 25(OH)D concentrations had increased further and after 2 months they had decreased but were clearly higher than at the baseline.

The daily dietary intake of vitamin D before the treatment was 8·14 ± 3·02 μg (mean ± SD) in the NB-UVB treated group and 8·17 ± 3·35 μg daily in the cholecalciferol-treated group (Table 1). Two months after the treatments the values were 7·90 ± 2·93 μg and 8·01 ± 3·28 μg, respectively.

Discussion

Vitamin D insufficiency affects people especially during winter when vitamin D synthesis induced by the sun is zero.[1–3,15] Previous studies in healthy subjects have shown that a short NB-UVB course improves vitamin D balance in women in winter,[18] and that a small broadband UVB dose every second week is sufficient to maintain summer vitamin D levels.[20] In the present study we have shown that a short NB-UVB course improves vitamin D balance even better than orally given vitamin D substitution in healthy subjects in winter. Twelve NB-UVB exposures given during 4 weeks increased serum 25(OH)D by 77·5% (41·0 nmol L^{-1}) whereas the increase was 37·8% (20·2 nmol L^{-1}, $P < 0·001$) in the subjects who received oral cholecalciferol 20 μg daily. The NB-UVB exposures were given with a Waldmann UV 7001 cabin on the whole body and the total UVB dose was 48·4 SED. In our previous study[19] we used

the same UV 7001 cabin to give 15 NB-UVB exposures (71·5 SED) on the whole body and found that 25(OH)D concentration increased as much as 90·7 nmol L^{-1} in healthy subjects. These results show that a short NB-UVB course is a very efficient way to improve vitamin D balance in winter. Moreover, the response to broadband UVB is highest in the subjects with lowest 25(OH)D concentrations[20,23] and a similar effect seems to be valid also for NB-UVB.

In previous Finnish studies[18,24] and in the present study daily dietary vitamin D intake was 7–8 μg. This amount meets the current Finnish recommendation for vitamin D intake in adults. However, it seems not to be enough to maintain a sufficient vitamin D balance in winter. To obtain the recommended 25(OH)D concentrations of over 75 nmol L^{-1},[3–5] daily intake of 17–20 μg of vitamin D supplements is required.[14,25] Previously, Vieth *et al.*[26] gave 25 μg of oral vitamin D_3, i.e. cholecalciferol, to healthy adults in winter in Canada and found that their serum 25(OH)D increased by 28·0 nmol L^{-1}. Burgaz *et al.*[11] observed that vitamin D supplement intake increased serum 25(OH)D concentration by 11·0 nmol L^{-1} in Swedish women in winter. In the present study oral cholecalciferol 20 μg daily increased serum 25(OH)D concentration by 20·2 nmol L^{-1} although the substitution was given for only 4 weeks. Even higher increases could have been obtained if the substitution had lasted longer. In agreement with this, Vieth *et al.*[26] showed that a continuous cholecalciferol supplementation increased serum 25(OH)D concentration slowly up to 3 months but not thereafter. Similarly, continuous NB-UVB exposures might also increase 25(OH)D concentrations but to what extent should be examined in a further study. Overall, it seems clear that in comparison, a 4-week period of 12 NB-UVB exposures is significantly more efficient in improving vitamin D balance in winter than 4 weeks of daily oral cholecalciferol 20-μg supplementation. The explanation for this might be the efficient enzyme system for vitamin D synthesis present in the skin, which is naturally induced by UVB radiation from the sun.

One month after the NB-UVB course and oral cholecalciferol supplementation the 25(OH)D concentrations had increased further in both treatment groups. Previously we have found similar increases after short NB-UVB courses[18,19] but are not aware of this kind of marked increase after a short cholecalciferol substitution. It seems evident that vitamin D synthesis continues actively for 1 month after stopping the NB-UVB course and oral cholecalciferol supplementation. It is possible that the liver is the major site for this post-treatment synthesis indicated by increased serum 25(OH)D concentration. However, other tissues might also participate. It is of interest that in cultured keratinocytes UVB is capable of activating vitamin D synthesis by converting vitamin D_3 by successive hydroxylation to active calcitriol, i.e.$1,25(OH)_2D$,[17,27] and the same seems to be true *in vivo*. We recently showed[28] that NB-UVB exposures increase CYP27B1 expression in the skin and this enzyme is known to be important in the final step of vitamin D synthesis.

Two months after the treatments 25(OH)D concentrations were decreasing but still clearly higher than at baseline in both study groups. This shows that in winter the maintenance of vitamin D concentration at recommended levels, i.e. over 75 nmol L^{-1}, needs continuous NB-UVB exposure or oral supplementation. As shown recently by Bogh *et al.*[20] with broadband UVB, a maintenance treatment of low-dose NB-UVB exposures given every second week during winter months seems to be enough for this purpose.

The NB-UVB doses used in the present study were smaller than those used when treating psoriasis and the mean cumulative amount was 48·4 SED. This is comparable to a dose received during a sunny day in the summer.[21] The NB-UVB exposures in the cabin were of short duration, below 2 min. No harmful erythema reactions were encountered and no dropouts occurred during the study. NB-UVB is a widely used dermatological treatment. When treating psoriasis higher doses and longer courses are usually needed for clearing than used in the present study. It is important that the dermatological NB-UVB treatment has not shown any risk for skin cancer in a large British study.[29] Due to this we consider the present low-dose NB-UVB course to be an effective and safe way to improve vitamin D balance in healthy subjects in winter.

The limitations of the present study were short duration, i.e. 4 weeks, and only one NB-UVB schedule and cholecalciferol dose used in the study. NB-UVB exposures were given three times a week as in our previous study when NB-UVB was used for the treatment of patients with psoriasis and atopic dermatitis.[19] A daily exposure schedule seems too frequent[18] but NB-UVB given twice weekly could be optimal to increase serum 25(OH)D concentration. In the present study the oral cholecalciferol dose was 20 μg daily, which has proven sufficient to increase serum 25(OH)D concentration to the recommended levels in winter.[5,15,26] The maximum increase after supplementation of oral cholecalciferol 20–25 μg daily lasts as long as 1·5–3 months. Therefore, a higher cholecalciferol dose, e.g. 100 μg daily, could also have been compared with the NB-UVB exposures because this high dose has not shown any adverse effects.[15,30] However, the risk for vitamin D toxicity is worthy of consideration because the absorption of this fat-soluble agent from the gut is high. In contrast, after UVB exposures to the skin a feedback mechanism controls vitamin D synthesis in a way that overdosing is not possible.[10]

In conclusion, the present study showed that a short NB-UVB course increases serum 25(OH)D concentration significantly more than a daily intake of 20-μg oral cholecalciferol. The NB-UVB response was still evident 2 months after exposure.

What's already known about this topic?

- Vitamin D insufficiency affects people especially during winter when vitamin D synthesis induced by the sun is zero.
- Narrowband ultraviolet B (NB-UVB) exposures have been shown to increase serum 25-hydroxyvitamin D [25(OH)D] in dermatological patients and healthy subjects in winter.
- No comparisons of the effects of NB-UVB exposures and oral vitamin D substitution are available.

What does this study add?

- The study shows that a short course of NB-UVB exposure increases serum 25(OH)D concentration significantly more than oral cholecalciferol 20 μg given daily.
- The effect of NB-UVB on the vitamin D balance was still evident 2 months after the treatment course.

Acknowledgment

We thank nurses Pirjo Honko and Tuija Valjakka for giving NB-UVB exposures in the study.

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Narrow-band ultraviolet B exposures increase serum vitamin D levels in haemodialysis patients

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Type: Original article

Running title: UVB and vitamin D in dialysis patients

Subject of manuscript: Chronic kidney disease

Abstract

Background: Chronic kidney disease (CKD) patients are especially prone to vitamin D insufficiency. Narrow-band UVB (NB-UVB) treatment increases serum 25-hydroxyvitamin D [25(OH)D] in dermatologic patients and we studied whether it improves vitamin D balance also in CKD patients on haemodialysis.

Methods: Fifteen dialysis patients (mean age 48.3 years) and twelve healthy subjects (mean age 43.6 years) received nine NB-UVB exposures on the upper body. Serum 25(OH)D and $1,25(OH)_2D$ were measured before and after the exposures. From skin biopsy specimen mRNA expression levels of CYP24A1 and CYP27B1, two enzymes needed for hydroxylation of vitamin D into its active metabolites, and of antimicrobial peptide cathelicidin, were examined.

Results: Before NB-UVB mean serum 25(OH)D was 32.5 ± 10.2 nmol/l in the dialysis patients and 60.2 ± 18.0 nmol/l in the healthy subjects ($p < 0.001$). After eight NB-UVB exposures serum 25(OH)D increased by 13.8 nmol/l (43%; $p < 0.001$) and serum $1,25(OH)_2D$ by 3.3 pmol/l (27%; $p = 0.002$) in the dialysis patients. After NB-UVB exposures CYP27B1 mRNA was increased ($p = 0.04$) whereas cathelicidin mRNA was decreased ($p < 0.0001$) compared to non-treated healthy subjects. One and two months after NB-UVB exposures serum 25(OH)D was still 10% higher than initially in the dialysis patients.

Conclusions: The present study shows that a short course of NB-UVB exposures increases significantly serum 25(OH)D and $1,25(OH)_2D$ in dialysis patients. The effect is, however, short-lasting suggesting that the patients need cyclic NB-UVB exposures to maintain their improved vitamin D concentration.

Key words: chronic kidney disease, haemodialysis, ultraviolet B radiation, vitamin D

Abbreviations:

CKD	chronic kidney disease
NB-UVB	narrow-band ultraviolet B radiation
PTH	parathyroid hormone
$1,25(OH)_2D$	1,25-dihydroxyvitamin D (calcitriol)
25(OH)D	25-hydroxyvitamin D (calcidiol)

Introduction

Vitamin D deficiency is common in the general population worldwide (1). This condition frequently affects people especially during winter when vitamin D synthesis induced by the sun is zero and dietary sources alone are not able to maintain adequate vitamin D balance (2,3). The desirable concentration of serum 25-hydroxyvitamin D [25(OH)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 bone health (4,5). In addition to osteoporosis, vitamin D deficiency has been recently associated with the risk of colorectal and other cancers (6), cardiovascular disease (7), multiple sclerosis (8) and for all-cause mortality in general population (9).

Vitamin D deficiency is very common in chronic kidney disease (CKD) patients both in pre-dialysis and dialysis populations (10-13). In advanced renal failure, the kidney is due to loss of renal 1alpha-hydroxylase activity, unable to convert 25(OH)D to $1,25(OH)_2D$, which is the active form of vitamin D (14). It has been recommended that serum 25(OH)D concentration should be >75 nmol/l in CKD patients (15). However, at present, there is an ongoing debate about the definition of sufficient vitamin D level (16). Quinibi et al. (17) found that the recommended level, >75nmol/l, is difficult to achieve by a 6-month oral ergocalciferol supplementation. Nevertheless, based on observational and randomized controlled studies, there is an agreement that vitamin D supplementation improves serum 25(OH)D concentration in CKD patients but the response seems to be rather slow (18).

Artificial ultraviolet B (UVB) exposures on the skin could be another possibility to improve vitamin D balance in CKD patients because solar UVB radiation is a potent inducer of photosynthesis of vitamin D. The synthesis starts from 7-dehydrocholesterol in the skin and proceeds rapidly into vitamin D_3 (19). The next steps of synthesis are programmed in the liver and kidney. Interestingly, also the keratinocytes in the skin have the enzymes (CYP24A1 and CYP27B1)

to hydroxylate vitamin D_3 to 25(OH)D and further to $1,25(OH)_2D$ which is the active form of vitamin D (20,21).

We recently showed that narrow-band ultraviolet B (NB-UVB) exposures, a widely used treatment for psoriasis (22), increased significantly serum 25(OH)D concentration in this disorder and it also is an alternative to improve vitamin D balance in healthy women in winter (23,24). In the present study we examined in CKD patients on haemodialysis the effect of NB-UVB exposures on serum 25(OH)D and $1,25(OH)_2D$ concentrations. We also measured the mRNA expression levels of CYP24A1 and CYP27B1 enzymes and cathelicidin, an antimicrobial peptide known to be regulated by vitamin D (25).

Subjects and methods

Patients and narrow-band UVB exposures

There were 32 CKD stage 5 patients in our self-care dialysis unit. Fourteen of them used vitamin D supplementation (cholecalciferol or ergocalciferol) or were older than 65 years old, so they did not met the inclusion criteria and were excluded. Three of the remaining patients refused from the study and thus 15 CKD stage 5 patients (ten men, five women, mean age 48.3 years) on haemodialysis started and also completed the study.

CKD was due to glomerulonephritis in nine, diabetic nephropathy in three, interstitial nephritis in one, polycystic kidney disease in one, and unknown in one patient. The patients had been on dialysis a mean of 3.8 years (range 0.5 - 16 years). During the study, three dialysis patients used synthetic vitamin D analoques (alphacalcidol in two, paricalcitol in one), four calcimimetic drugs, two calcium carbonate and seven non-calcium containing phosphate binder.

Twelve healthy nurses and other hospital employees (two men, ten women, mean age 43.6 years) volunteered as NB-UVB treated controls in the study. None of the patients or controls received vitamin D supplementation (cholecalciferol or ergocalciferol) at the time of the study. The CKD patients and healthy subjects were at the beginning of the study informed not to change their personal medication or dietary habits but their nutritional vitamin D intake was not evaluated.

The ethics committee of the Tampere University Hospital approved the study protocol and all subjects gave an informed consent to participate. Inclusion criteria were age of 18 – 65 years and no sun holidays or solarium visits during the two preceding months. The authors adhered during the study to the Declaration of Helsinki.

The study was performed between December 2009 and March 2010. The dialysis patients and controls received three times a week NB-UVB exposures with Medisun 700 UVB-311 apparatus equipped with TL01 tubes (Schulze & Böhm, Brühl, Germany). A total of nine NB-UVB exposures were given during three weeks on the face, arms, chest and abdomen, which is about 25% of the

total body area. The cumulative amount of NB-UVB was 15 standard erythema doses (26). The time for NB-UVB exposures ranged from 48 to 96 seconds and the exposures were given just before the dialysis.

Measurement of serum 25-hydroxy- and 1,25-dihydroxyvitamin D concentrations

Blood samples for serum 25(OH)D and $1,25(OH)_2D$ measurements were taken before the first and the ninth NB-UVB exposure. Follow-up samples were taken one and two months after the NB-UVB course. The serum samples were protected from light, centrifuged and then stored at -70°C. Serum 25(OH)D concentration was analyzed in duplicates by using radioimmunoassay (Immunodiagnostic Systems, Boldon, UK) as in our previous study (23). Serum $1,25(OH)_2D$ concentration was analyzed in duplicates by using immunoextraction followed by radioimmunoassay (27). The methods included measurements of hydroxylated metabolites of both vitamin D2 and D3.

Skin biopsies and quantitative real-time PCR

Punch biopsies were taken from the abdominal skin of the dialysis patients (n = 12) before the first and ninth NB-UVB exposure. Skin biopsies from additional healthy subjects (n = 12) not treated with NB-UVB served as controls. The biopsies were immediately frozen and stored at -70°C before examined. Total RNA from biopsies was isolated using TRIsure Reagent (Bioline, Luckenwalde, Germany) and 1 µg of RNA was reverse transcribed with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) to cDNA. The mRNA expressions of CYP24A1, CYP27B1 and cathelicidin were evaluated using a LightCycler® 2.0 system and the corresponding human Universal Probe Library Set (Roche), and fold induction relative to the healthy volunteers was calculated as previously described (23).

Statistics

The difference between serum 25(OH)D concentration in the dialysis patients and healthy subjects, as well as the differences of the serum 25(OH)D and $1,25(OH)_2D$ concentrations measured before and after NB-UVB exposures were analyzed by a permutation test with Monte-Carlo p-value. Confidence intervals were obtained by bias corrected bootstrap confidence bootstrapping (5000 replications).

The differences in the mRNA expression levels of CYP24A1, CYP27B1 and cathelicidin between the dialysis patients and healthy subjects were analyzed with unpaired t-test, and the differences before and after NB-UVB exposures with paired t-test.

Results

Narrow-band UVB treatment and serum 25(OH)D and 1,25(OH)$_2$D concentrations

Before NB-UVB exposures serum 25(OH)D was 32.5 ± 10.2 nmol/l (mean ± SD) in fifteen dialysis patients and 60.2 ± 18.0 nmol/l in twelve healthy subjects (Table 1, Fig. 1). Serum 25(OH)D was below 25.0 nmol/l in five (33%) and below 50.0 nmol/l in fourteen (93%) dialysis patients. After eight NB-UVB exposures serum 25(OH)D concentration was 46.3 ± 12.0 nmol/l in the dialysis patients. The increase was statistically significant ($p < 0.001$; Table 1, Fig. 1). At this point, none of the dialysis patients had serum 25(OH)D below 25.0 nmol/l but in eleven (73%) patients the concentration was still below 50.0 nmol/l. Serum 1,25(OH)$_2$D increased from 12.4 ± 2.5 pmol/l to 15.7 ± 4.0 pmol/l ($p = 0.002$; Table 1). In the healthy subjects NB-UVB exposures increased serum 25(OH)D concentration from 60.2 ± 18.0 nmol/l to 69.2 ± 17.7 nmol/l ($p = 0.032$; Fig. 1).

At follow-up, one and two months after NB-UVB exposures, mean serum 25(OH)D concentration had decreased in the dialysis patients but, still, it was 10% higher than initially (Fig. 2). In the healthy subjects, one month after NB-UVB exposures serum 25(OH)D concentration was still significantly increased but after two months it had decreased almost to the pre-treatment level (Fig. 2).

The plasma intact parathyroid hormone (PTH) levels did not change during NB-UVB exposures in the dialysis patients. Also the levels of blood haemoglobin, plasma total calcium, serum ionized calcium or plasma phosphorus remained unchanged (Table 1).

CYP24A1, CYP27B1 and cathelicidin mRNA expression in skin biopsy specimen

At baseline the expression of CYP27B1 mRNA was somewhat higher ($p = 0.055$) in the dialysis patients compared to the healthy subjects, and after NB-UVB exposures the increase was significant ($p = 0.04$; Fig. 3A). No change was found in the expression of CYP24A1 mRNA (Fig. 3B). At baseline the cathelicidin mRNA expression was somewhat lower ($p = 0.069$) in the dialysis patients

compared to the healthy subjects, and after NB-UVB exposures the decrease was significant ($p < 0.0001$; Fig. 4).

Discussion

Before NB-UVB treatment fourteen (93%) of the present CKD patients on haemodialysis had vitamin D insufficiency [serum 25(OH)D <50.0 nmol/l] or deficiency [serum 25(OH)D <25.0 nmol/l]. This high prevalence is in agreement with previous studies on vitamin D balance in pre-dialysis and dialysis populations of CKD patients (10-13). The response of vitamin D insufficiency or deficiency to vitamin D supplementation in CKD patients is known to be variable (18). A study on dialysis patients with high-dose oral cholecalciferol for nine months showed that a 25(OH)D concentration of >75 nmol/l, as recommended by K/DOQI, was achieved in only 57% of the patients (28). A recent study by Qunibi et al. (17) showed that a six month treatment with oral ergocalciferol with doses recommended in K/DOQI guidelines increased serum 25(OH)D from 38.8 nmol/l to 58.5 nmol/l ($p < 0.001$) in 88 vitamin D deficient patients with CKD stage 1 to 5.

In the present study we gave eight NB-UVB exposures in connection with dialysis sessions to haemodialysis patients and found that serum 25(OH)D increased about the same magnitude, i.e. from 32.5 nmol/l to 46.3 nmol/l ($p < 0.001$). This increase took place in three weeks. A recent systematic review and meta-analysis by Kandula et al. (18) included 17 observational studies on vitamin D supplementation in a total of 1115 patients with CKD stage 3 to 5 and reported a mean increase of 60.3 nmol/l in their 25(OH)D concentration. However, in most of the referred studies vitamin D supplement doses were very high, such as ergocalciferol 50,000 IU weekly, and the duration of the treatment was in most studies from 3 to 12 months. In the studies reviewed by Kandula et al. (18) the increase of 25(OH)D was associated with a significant decline in serum PTH level. Although NB-UVB exposures increased significantly the serum 25(OH)D in the present study, we found no change in the mean PTH level. This could be due to the short duration of the UVB intervention.

In the present study the NB-UVB exposures were given only on a body surface area of 25%, i.e. on the face, arms, chest and abdomen. In spite of this, we could observe a significant increase in

serum 25(OH)D in the dialysis patients and healthy subjects. If the exposed body area would have been larger, e.g. the whole body like in our previous study in patients with psoriasis and atopic dermatitis (23), the response in serum 25(OH)D might have been even more prominent. It is known that in the healthy subjects, the lower the baseline 25(OH)D concentration, the higher the increase after UVB exposures (29). Most likely this is also valid for the CKD patients, as the increase in 25(OH)D in our dialysis patients was greater than in the healthy control subjects in whom the pre-treatment levels of 25(OH)D were markedly higher.

One month after the NB-UVB exposures mean serum 25(OH)D concentration had decreased in the CKD patients but was still 10% higher than initially. This kind of rapid decrease of serum 25(OH)D was not seen in the healthy subjects in the present study or in the psoriasis and atopic dermatitis patients in our previous study (23). Though the present dialysis patient series was small, the observed rapid decrease of serum 25(OH)D suggests that these patients need a longer NB-UVB course or alternatively cyclic NB-UVB exposures to maintain better their vitamin D balance. This possibility should be investigated in a further study and a comparison between NB-UVB exposures and oral vitamin D supplementation on serum 25(OH)D levels is also warranted in the CKD patients on haemodialysis.

In advanced renal failure, the kidney is unable to convert 25(OH)D to the active form of vitamin D [1,25$(OH)_2$D] due to the loss of renal CYP27B1 activity (14). Lehman et al. (20) showed in UVB treated organ cultures of skin that also keratinocytes are capable to hydroxylate 25(OH)D to 1,25$(OH)_2$D. It is of interest that the present dialysis patients showed after NB-UVB exposures significantly increased mRNA expression of CYP27B1, but not of CYP24A1, in their skin compared to healthy subjects. The mRNA expression of CYP27B1 enzyme was increased, though not significantly, already at the baseline. This observation suggests that the loss of renal CYP27B1 activity in the dialysis patients could lead to the activation of this enzyme in the skin which then is further intensified by NB-UVB exposures. In agreement with the increased activity of cutaneous

CYP27B1 in the dialysis patients, a slight but significant increase of serum $1,25(OH)_2D$ could also be found after NB-UVB exposures. We examined mRNA expression of the antimicrobial peptide cathelicidin, as well. Its expression is known to be regulated by $1,25(OH)_2D$ (25). Surprisingly, we found a significantly decreased mRNA expression of cathelicidin after NB-UVB exposures compared to the healthy controls. This finding is in contrast to our previous study in patients with psoriasis and atopic dermatitis (23) and suggests that the dialysis patients could have some deficiency in the regulation of cutaneous antimicrobial peptide cathelicidin.

Several recent papers have focused attention on vitamin D supplementation in CKD patients (30-34). Available evidence shows that this supplementation improves biochemical endpoints but whether such improvements translate into clinically significant outcomes such as reduced cardiovascular mortality has yet to be determined (35-37). To our knowledge the present study is the first one to show that NB-UVB exposures can also be used to improve vitamin D balance in the dialysis patients. The NB-UVB doses needed to increase serum 25(OH)D were small and this widely used dermatologic treatment has not shown any risk for skin cancer (38). The NB-UVB exposures are easy to give in dialysis units in connection with dialysis sessions and one exposure takes only a couple of minutes. Further NB-UVB studies are warranted to find out whether exposures to the whole body or a longer exposure period such as used in the treatment of dermatological diseases in our previous study (23) would give more marked and permanent responses. It would also be of interest to compare the effects of vitamin D supplementation and NB-UVB exposures in parallel study groups, and also to combine these treatments in order to find out whether such combination would be the most effective way to improve vitamin D insufficiency/ deficiency in CKD patients.

In conclusion, the present study showed that a short course of NB-UVB exposures increased serum 25(OH)D and $1,25(OH)_2D$ concentrations significantly in CKD patients on haemodialysis. The NB-UVB exposures were easy to perform in connection with dialysis sessions. The effect of

NB-UVB was, however, short lasting suggesting that the patients need a longer course or alternatively cyclic NB-UVB exposures to maintain their improved vitamin D concentration.

Acknowledgements

This study was supported by Competitive Research Funding of the Tampere University Hospital (Grants 9J103 and 9K114), by the Deutsche Forschungsgemeinschaft (Emmy Noether Program SCHA 979/3-1) and by the Fritz Thyssen Stiftung (J.S).

We thank research nurse Heidi Hällström for giving NB-UVB exposures to the dialysis patients and healthy subjects.

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Tables

Table 1. Serum 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] concentrations and levels of intact parathyroid hormone (PTH), haemoglobin, calcium and phosphorus before and after eight narrow-band ultraviolet B (NB-UVB) exposures in 15 chronic kidney disease patients on haemodialysis.

		Before NB-UVB Mean ± SD	**After NB-UVB** Mean ± SD	P-value
25(OH)D	nmol/l	32.5 ± 10.2	46.3 ± 12.0	< 0.001
1,25$(OH)_2$D	pmol/l	12.4 ± 2.5 *	15.7 ± 4.0 *	0.002
Intact PTH	pmol/l	32.1 ± 24.9 **	32.7 ± 18.5 **	ns
Haemoglobin	g/l	113.8 ± 19.3	114.7 ± 14.0	ns
Total calcium	mmol/l	2.32 ± 0.19	2.29 ± 0.21	ns
Ionized calcium	mmol/l	1.21 ± 0.07	1.19 ± 0.10	ns
Phosphorus	mmol/l	1.78 ± 0.48	1.75 ± 0.60	ns

*in 13 patients

**in 14 patients

ns = non-significant

Legends to figures

Figure 1. Serum 25-hydroksyvitamin D [25(OH)D] concentrations before (pre) and after (post) eight narrow-band ultraviolet B (NB-UVB) exposures in 15 chronic kidney disease (CKD) patients on haemodialysis and 12 healthy subjects. The increase is significant in the CKD patients ($p < 0.001$) and in the healthy subjects ($p = 0.032$).

Figure 2. Serum 25(OH)D concentration increased by 42% in 15 chronic kidney disease patients on haemodialysis (black dots) and by 14% in 12 healthy control subjects (open dots) after eight NB-UVB exposures (0 month). One and two months after the NB-UVB exposures 25(OH)D concentration had decreased in the dialysis patients but was still 10% over the baseline value. Mean values; 95% confidence intervals are shown by bars.

Figure 3A. CYP27B1 mRNA expression in the skin of healthy subjects (n = 12) and chronic kidney disease patients on haemodialysis (n = 12) before (pre) and after (post) narrow-band UVB (NB-UVB) exposures. Difference between the healthy subjects and the dialysis patients was non-significant ($p = 0.055$) before NB-UVB exposures but significant ($p = 0.04$) after NB-UVB exposures.

Figure 3B. CYP24A1 mRNA expression in the skin of healthy subjects (n = 12) and chronic kidney disease patients on haemodialysis (n = 12) before (pre) and after (post) narrow-band UVB (NB-UVB) exposures. Differences between the healthy subjects and the dialysis patients were non-significant before and after NB-UVB exposures.

Figure 4. Cathelicidin mRNA expression in the skin of healthy subjects (n = 12) and chronic kidney disease patients on haemodialysis (n = 12) before (pre) and after (post) narrow-band UVB

(NB-UVB) exposures. Difference between the healthy subjects and the dialysis patients before NB-UVB non-significant ($p = 0.069$) but significant ($p < 0.0001$) after NB-UVB exposures.

Figures

Figure 1.

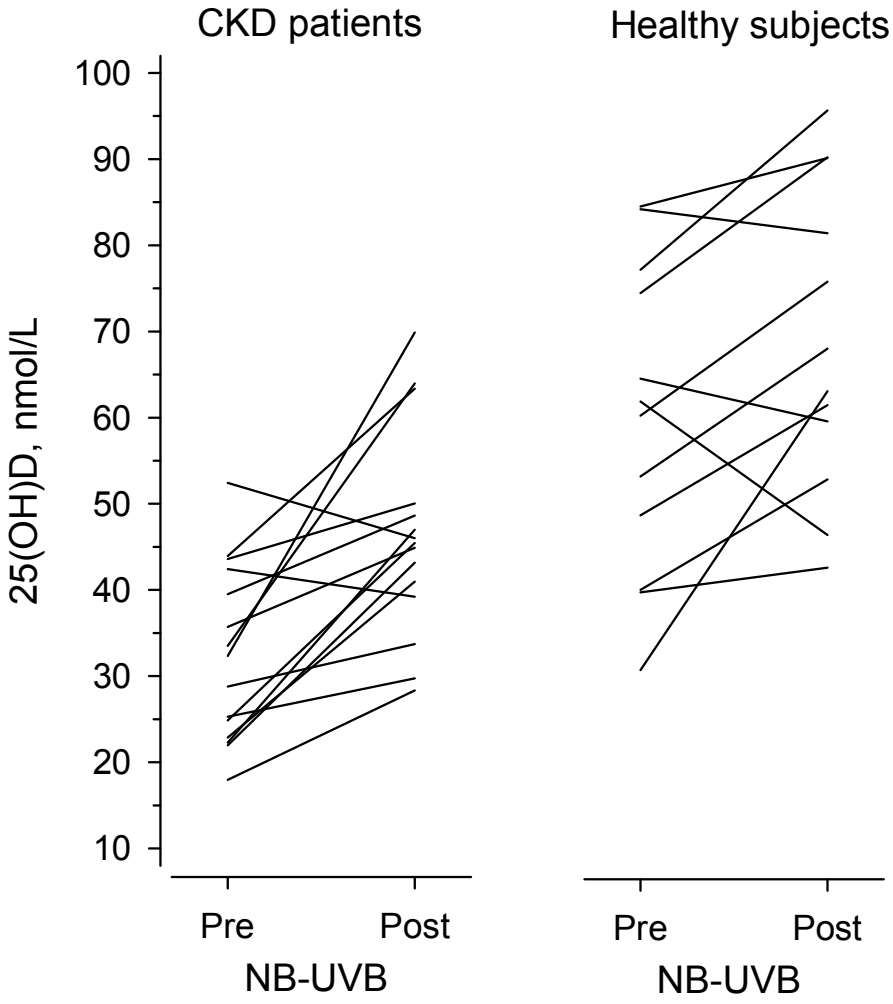


Figure 2.

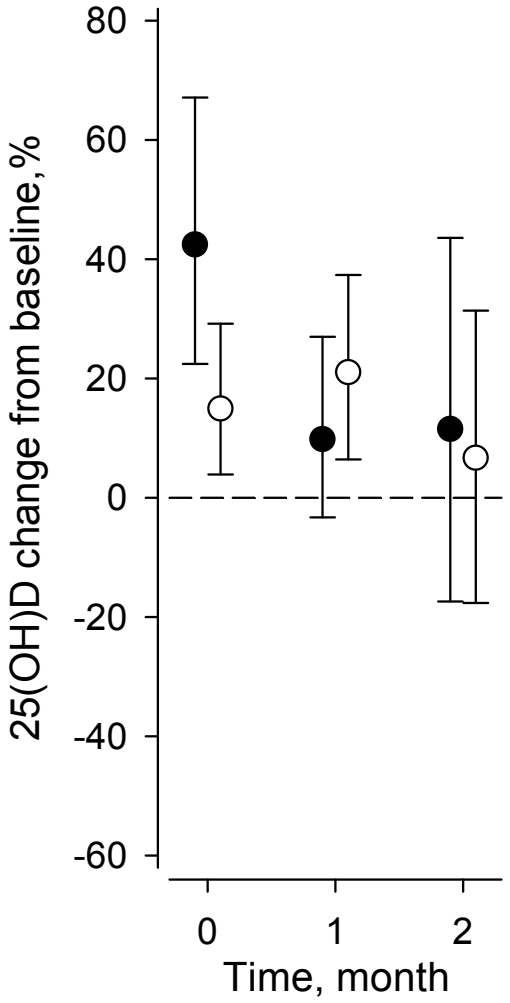

80
60
40
20
0
-20
-40
-60
25(OH)D change from baseline,%
0
1
2
Time, month

Figure 3A.

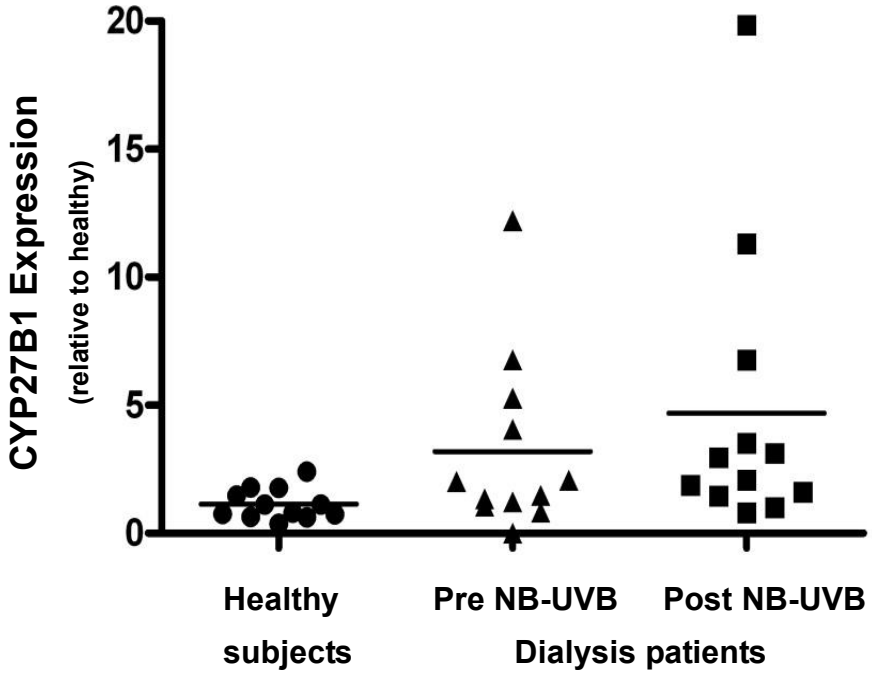

CYP27B1 Expression
(relative to healthy)
20
15
10
5
0
Healthy subjects
Pre NB-UVB
Post NB-UVB
Dialysis patients

Figure 3B.

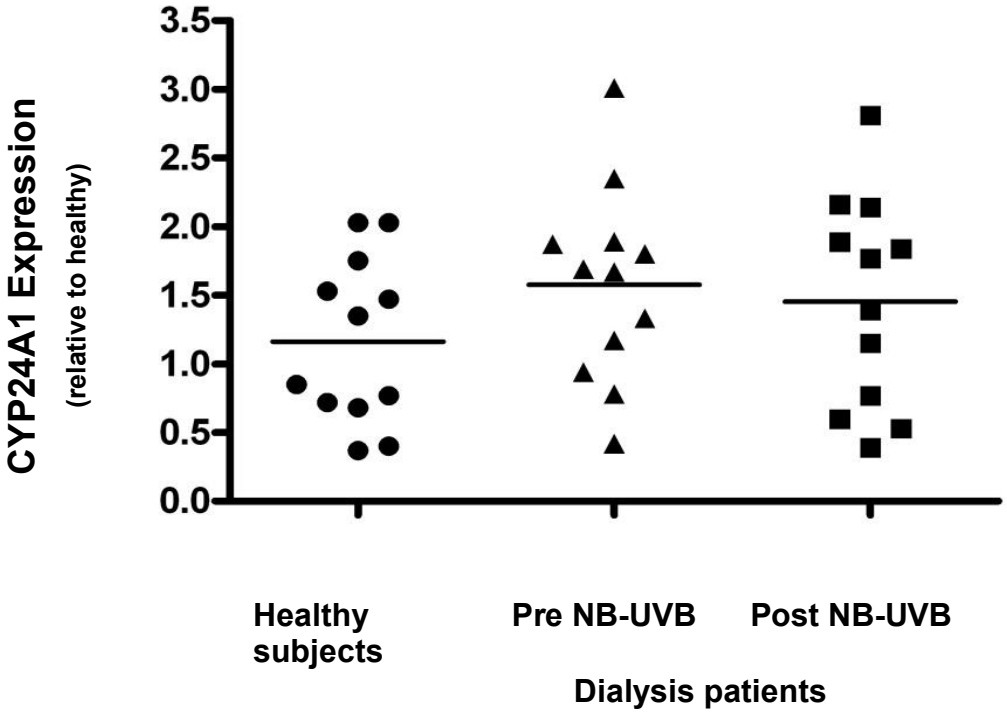
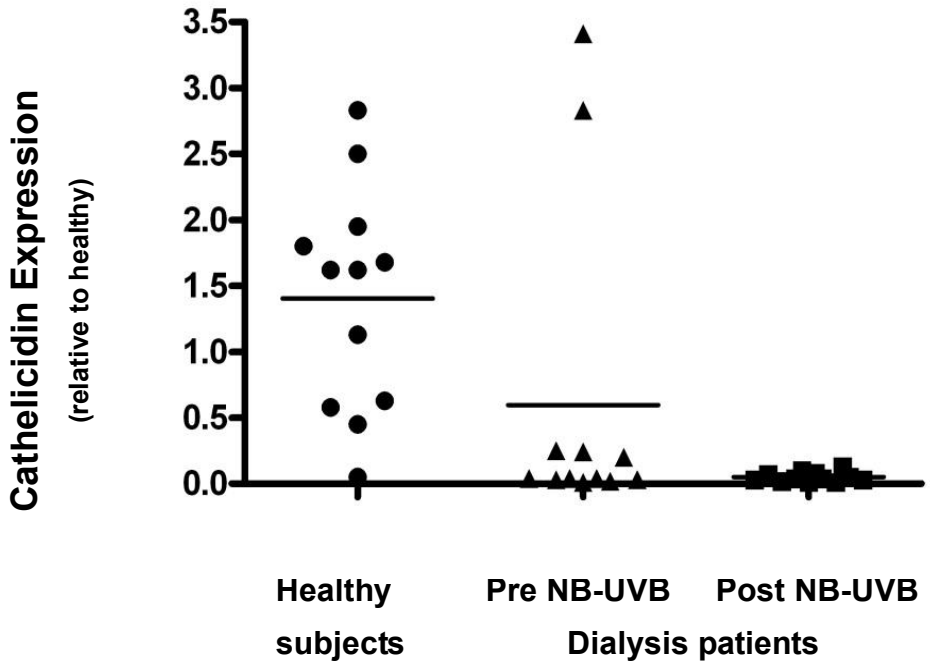

CYP24A1 Expression
(relative to healthy)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Healthy subjects
Pre NB-UVB
Post NB-UVB
Dialysis patients

Figure 4.


Cathelicidin Expression
(relative to healthy)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Healthy subjects
Pre NB-UVB
Post NB-UVB
Dialysis patients

Original Paper

nephron Clinical Practice

Nephron Clin Pract 2013;124:17–22
DOI: 10.1159/000355010

Received: March 18, 2013
Accepted: August 10, 2013
Published online: September 12, 2013

A Narrow-Band Ultraviolet B Course Improves Vitamin D Balance and Alters Cutaneous CYP27A1 and CYP27B1 mRNA Expression Levels in Haemodialysis Patients Supplemented with Oral Vitamin D

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Key Words

Cholecalciferol · Chronic kidney disease · CYP27A1 · CYP27B1 · Haemodialysis · Ultraviolet B radiation · Vitamin D

Abstract

Background/Aims: Chronic kidney disease (CKD) patients on dialysis are prone to vitamin D insufficiency despite oral vitamin D supplementation. Here, we studied whether narrow-band ultraviolet B (NB-UVB) exposures improve vitamin D balance. ***Methods:*** 14 haemodialysis patients and 15 healthy subjects receiving oral cholecalciferol 20 μg daily got nine NB-UVB exposures on the entire body. Serum 25-hydroxyvitamin D (25(OH)D) was measured by radioimmunoassay. Cutaneous mRNA expression levels of CYP27A1 and CYP27B1, two enzymes required for hydroxylation of vitamin D into its active metabolite, were also measured. ***Results:*** The baseline serum 25(OH)D concentration was 57.6 ± 18.2 nmol/l in the CKD patients and 74.3 ± 14.8 nmol/l in the healthy subjects. The NB-UVB course increased serum 25(OH)D by 14.0 nmol/l (95% CI 8.7–19.5) and 17.0 nmol/l (CI 13.7–20.2), respectively. At baseline the CKD patients showed significantly increased CYP27B1 levels compared to the healthy subjects. ***Conclusions:*** A short NB-UVB course is an efficient way to improve vitamin D balance in CKD patients on dialysis who are receiving oral vitamin D supplementation. The increased cutaneous CYP27B1 levels in the CKD patients suggest that the loss of renal activity of this enzyme is at least partially compensated for by the skin.

Introduction

Vitamin D deficiency is a common complication in pre-dialysis and dialysis patients with chronic kidney disease (CKD) [1–4]. In advanced kidney disease, the

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Table 1. Demography, use of oral cholecalciferol before the NB-UVB course and 25(OH)D levels at baseline in 14 CKD patients on haemodialysis and 15 healthy subjects

	CKD patients (n = 14)	Healthy subjects (n = 15)	p value
Male/female	6/8	1/14	0.035
Age, years (mean ± SD)	53.6±12	46.1±11	0.11
Body mass index, kg/m^2 (mean ± SD)	32.1±8.4	23.6±3.8	0.001
Fitzpatrick skin type II/III/IV	5/7/2	3/10/2	0.67
Use of cholecalciferol 20 μg daily before NB-UVB course, months, median (range)	2.3 (1–16)	2.0 (1–24)	0.40
Serum 25(OH)D, nmol/l (mean ± SD)	57.6±18.2	74.3±14.8	0.01

kidney is unable to produce 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) from 25-hydroxyvitamin D (25(OH)D) due to loss of renal 1α-hydroxylase (CYP27B1) activity [5, 6]. An individual's vitamin D status is best evaluated by measuring the level of serum 25(OH)D [6, 7]. CKD patients are recommended to have a serum 25(OH)D concentration >75.0 nmol/l (>30.0 ng/ml) [8]. However, this level is difficult to achieve using oral ergocalciferol supplementation for 6 months [9]. A recent meta-analysis on the use of oral vitamin D compounds in dialysis and non-dialysis CKD patients confirmed significant improvement in serum 25(OH)D concentration, but no effect on bone or cardiovascular outcomes were found [10].

Artificial ultraviolet B (UVB) skin exposures are another possible method of improving vitamin D balance because solar UVB radiation is a potent inducer of vitamin D photosynthesis. The synthesis starts from 7-dehydrocholesterol in the skin and is rapidly processed into vitamin D. The next steps of synthesis are programmed in the liver and kidney, but also occur in skin keratinocytes. These cells have CYP27A1 (25-hydroxylase) and CYP27B1 (1α-hydroxylase) enzymes to hydroxylate vitamin D to 25(OH)D and further to $1,25(OH)_2D$, which is the active form of vitamin D [6, 7]. A narrow-band UVB (NB-UVB) course, which is widely used as treatment for psoriasis, increases serum 25(OH)D levels in vitamin-D-insufficient subjects more efficiently than oral cholecalciferol [11, 12].

We found previously in CKD patients on haemodialysis who had no oral vitamin D supplementation that their serum 25(OH)D levels responded rapidly to a 3-week NB-UVB course [13]. The 25(OH)D concentration increased by 43% and after the NB-UVB course none of the patients were vitamin D deficient. In the present study, we examined whether a similar short NB-UVB course improves vitamin D balance in dialysis patients receiving standard oral vitamin D supplementation, i.e. cholecalciferol 20 μg daily prior to and during the study. In addition, we measured cutaneous messenger RNA (mRNA) expression levels of CYP27A1 and CYP27B1.

Materials and Methods

CKD Patients on Dialysis and Healthy Subjects

There were 32 patients in our self-care haemodialysis unit. The inclusion criteria for the study were: age of 18–70 years; no sun tanning within the 2 preceding months; Fitzpatrick skin type II–IV, which indicates that skin does not burn easily in the sun, and daily use of oral cholecalciferol 20 μg (800 IU) for at least 1 month. 14 CKD stage 5 patients on haemodialysis (6 male, 8 female, mean age 53.6 years; table 1) meeting these criteria were enrolled in the study. The patients had been on dialysis for a mean of 47 (range 9–117) months. During the study, all 14 patients received calcium carbonate, 12 non-calcium-containing phosphate binder, 6 cinacalcet and 7 active vitamin D analogues. The CKD patients had used oral cholecalciferol 20 μg daily for a mean of 5.3 (range 1–16) months.

15 hospital employees (1 male, 14 female, mean age 46.1 years; table 1) volunteered as controls in the study. These subjects had used oral cholecalciferol 20 μg daily for a mean of 3.4 (range 1–24) months. Both groups continued to use cholecalciferol 20 μg daily during and after the NB-UVB course.

The ethics committee of Tampere University Hospital approved the study protocol and all subjects gave an informed consent to participate. The authors followed the Declaration of Helsinki.

NB-UVB Exposures

The study was performed between December 2011 and March 2012. The subjects received nine NB-UVB exposures given three times a week on the entire body with a Waldmann UV 7001 cabin (Schulze & Böhm, Brühl, Germany). The NB-UVB exposures were given to the patients prior to dialysis. The first NB-UVB dose was 0.19 J/cm^2 (1.11 SED), and this dose was increased according to a fixed protocol gradually up to 0.97 J/cm^2 (5.70 SED). One SED is equivalent to 10 mJ/cm^2 CIE (Commission Internationale de l'Eclairage) erythema-weighted irradiance. The mean cumulative dose of NB-UVB given to the CKD patients was 4.53 (range 2.97–

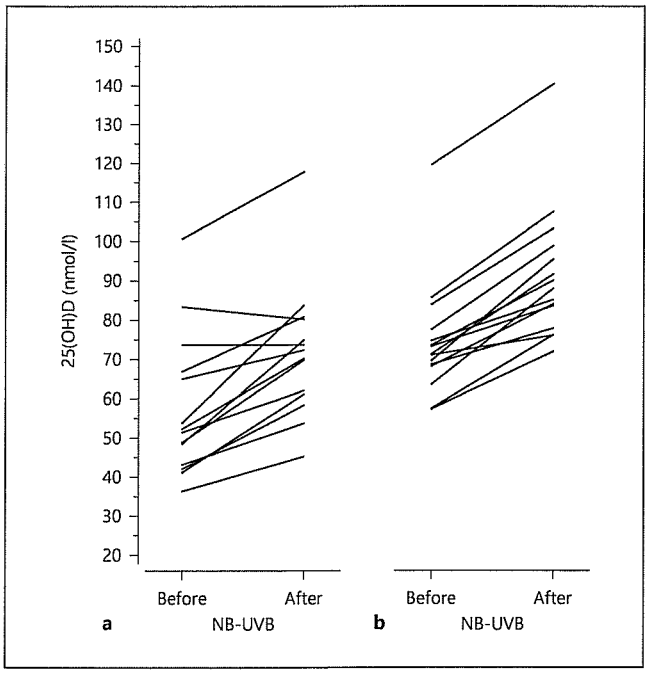

Fig. 1. Serum 25(OH)D concentrations before and after the NB-UVB course in 14 CKD patients on (**a**) haemodialysis and (**b**) 15 healthy subjects. The both groups had been receiving oral vitamin D substitution at a daily dose of 20 μg. The increase is significant ($p < 0.001$) in the both groups.

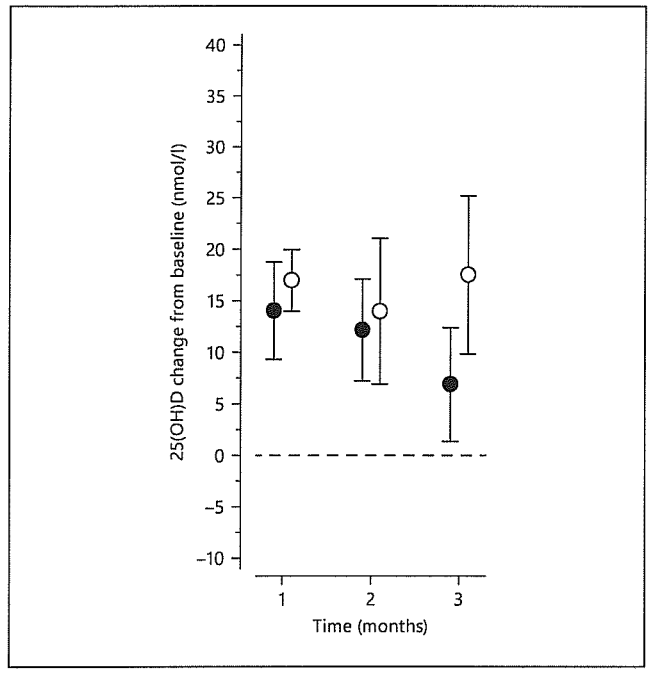

Fig. 2. A NB-UVB course given for 1 month increased serum 25(OH)D concentration by 24.2% in 14 CKD patients on haemodialysis (black dots) and by 22.8% in 15 healthy control subjects (open dots) receiving continuous oral vitamin D substitution. After the NB-UVB exposures, at 2 and 3 months from baseline, 25(OH)D concentrations were still significantly higher than at baseline in the both groups. Mean values; 95% CIs are shown by bars.

4.75) J/cm^2 which is equivalent to 26.6 SED. In the healthy subjects, the mean cumulative dose of NB-UVB was 4.37 J/cm^2 (range 3.21–4.75) which is equivalent to 25.7 SED.

Measurement of Serum 25(OH)D Concentrations

Blood samples for serum 25(OH)D measurements were taken before the first and the ninth NB-UVB exposures, and follow-up samples 1 and 3 months after the NB-UVB course. The samples were protected from light, centrifuged and then stored at –70°C. The 25(OH)D concentration was analysed in duplicates using a radioimmunoassay (Immunodiagnostic Systems, Boldon, UK) as previously described [13].

Skin Biopsies and Quantitative Real-Time PCR

Punch biopsies were taken from the buttocks of 10 CKD patients and 13 healthy subjects before the first and the ninth NB-UVB exposures. The biopsies were frozen and stored at –70°C. Total RNA was isolated using TRIsure Reagent (Bioline, Luckenwalde, Germany), and 1 μg of RNA was reverse transcribed with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, Calif., USA) to cDNA. The mRNA expression levels of CYP27A1 and CYP27B1 were evaluated using a LightCycler® 2.0 system and the corresponding human Universal Probe Library Set (Roche) as previously described [13].

Table 2. 25(OH)D concentrations and levels of intact parathyroid hormone (PTH), haemoglobin, ionized calcium and phosphorus before and after a NB-UVB course in 14 CKD patients on haemodialysis

	Before NB-UVB mean ± SD	After NB-UVB mean ± SD	p value
25(OH)D, nmol/l	57.6±18.2	71.7±17.2	<0.001
Intact PTH, pmol/l	31.8±29.0	26.7±25.6	0.11
Haemoglobin, g/l	114.2±11.3	112.3±9.2	0.39
Ionized calcium, mmol/l	1.18±0.08	1.16±0.07	0.044
Phosphorus, mmol/l	1.88±0.44	1.91±0.32	0.86

Statistics

The statistical comparison between the groups was performed by t test, permutation test or χ^2 test. Repeated measures were analysed using generalizing estimating equations models with the unstructured correlation structure using bootstrap type standard error. The changes within CKD patients were analysed by applying a t test and a permutation test to related samples.

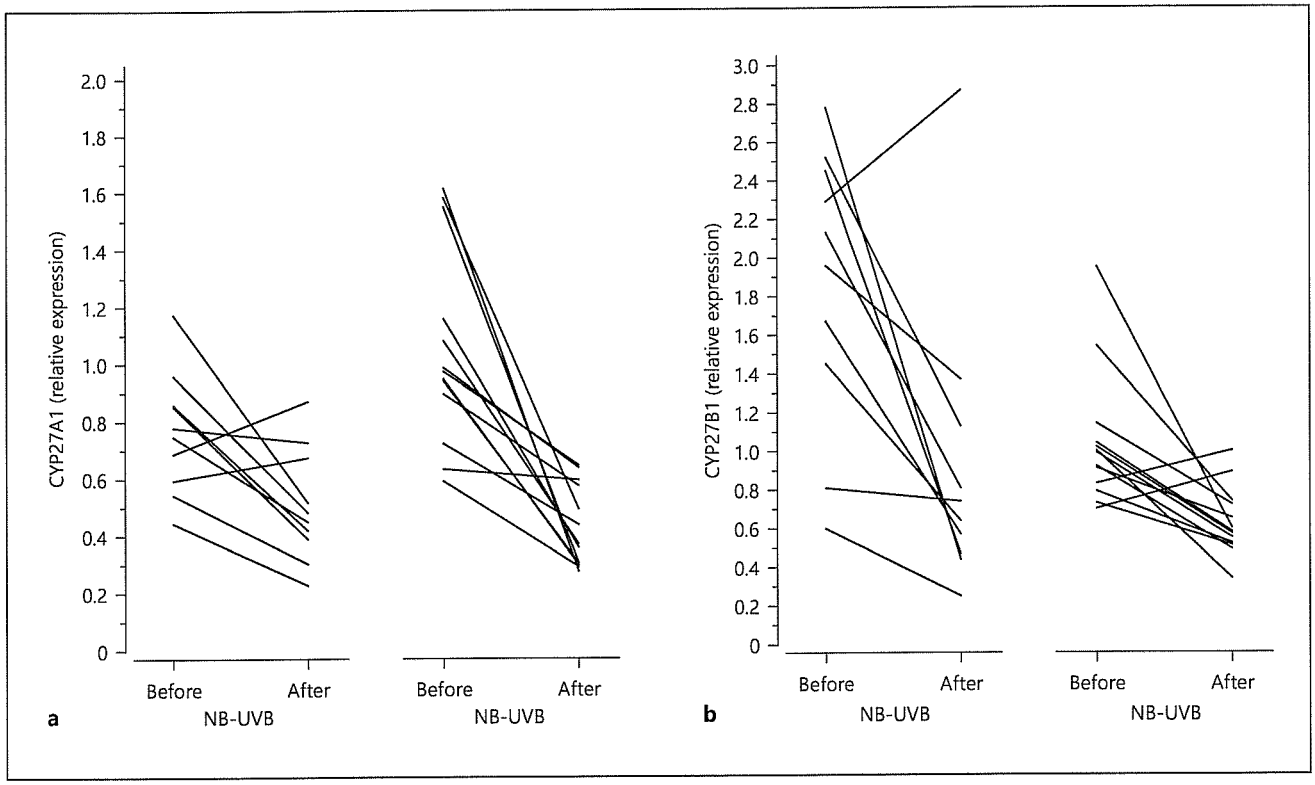

Fig. 3. a CYP27A1 mRNA expression levels in skin biopsies in CKD patients on haemodialysis (n = 10) (left) and healthy subjects (n = 13) (right) before and after the NB-UVB course. Before the NB-UVB course the CYP27A1 levels were significantly (p = 0.028) lower in the CKD patients compared to healthy subjects. The NB-UVB course caused a significant decrease in the CYP27A1 levels in the CKD patients (p = 0.018) and healthy subjects (p < 0.001). **b** CYP27B1 mRNA expression levels in skin biopsies in CKD patients on haemodialysis (n = 10) (left) and healthy subjects (n = 13) (right) before and after a NB-UVB course. Before the NB-UVB course the CYP27B1 levels were significantly (p = 0.003) higher in the CKD patients compared to healthy subjects. The NB-UVB course caused a significant decrease in the CYP27B1 levels in the CKD patients (p = 0.01) and healthy subjects (p = 0.002).

Results

Serum 25(OH)D Concentrations before and after the NB-UVB Course

The baseline serum 25(OH)D concentration was 57.6 ± 18.2 nmol/l (mean ± SD) in the 14 dialysis patients (table 1). The serum 25(OH)D was <50.0 nmol/l in 6 (43%) and <75.0 nmol/l in another 6 (43%) patients (fig. 1). The NB-UVB course increased serum 25(OH)D by 14.0 nmol/l (95% CI 8.7–19.5, p < 0.001), or 24.2% (fig. 1). Only 1 (7%) patient had serum 25(OH)D <50.0 nmol/l. NB-UVB treatment had only marginal effects on the other laboratory findings (table 2).

The baseline serum 25(OH)D was 74.3 ± 14.8 nmol/l in the 15 healthy subjects (table 1). NB-UVB course increased serum 25(OH)D by 17.0 nmol/l (CI 13.7–20.2, p < 0.001), or 22.8% (fig. 1).

Figure 2 shows that 1 and 2 months after the NB-UVB course, serum 25(OH)D levels were still significantly higher than at baseline in the CKD patients (mean 69.8 ± 18.1 and 64.5 ± 22.3 nmol/l; p < 0.001 and p = 0.031), and in the healthy subjects (88.3 ± 19.9 and 91.8 ± 19.7 nmol/l; p = 0.002 and p < 0.001).

CYP27A1 and CYP27B1 mRNA Expression in the Skin

At baseline, the CKD patients showed decreased CYP27A1 mRNA expression (p = 0.028), whereas CYP27B1 mRNA expression was significantly (p = 0.003) increased compared to the healthy subjects (fig. 3a, b). The NB-UVB course caused a significant decrease in the CYP27A1 (p = 0.018) and CYP27B1 (p = 0.010) mRNA expression levels in the CKD patients, and also in the healthy subjects (p < 0.001, p = 0.002, respectively; fig. 3a, b).

Discussion

In the present study, NB-UVB exposures were given to 14 CKD patients on haemodialysis who were receiving oral cholecalciferol 20 μg daily. The supplementation of 20–25 μg of vitamin D daily has been recommended for older people to prevent bone fractures [14], and these amounts are also in common use in Finnish CKD patients on haemodialysis. Despite this supplementation, the mean serum 25(OH)D concentration was at baseline rather low, i.e. 57.6 nmol/l. In 6 (43%) dialysis patients, the serum 25(OH)D was <50.0 nmol/l (20.0 ng/ml), which can be considered as vitamin D insufficient [15]. A NB-UVB course given within a 3-week period significantly increased the serum 25(OH)D. The mean concentration was 71.6 nmol/l, and only 1 patient was still vitamin D insufficient. The increase of 25(OH)D was 24.2%, but this percentage is almost two times lower than in our previous study when the dialysis patients were not on oral vitamin D supplementation [13]. To our knowledge, these studies are the first to show that NB-UVB exposures can be used to improve vitamin D balance in CKD patients on dialysis. The NB-UVB exposures were easy to give in connection with dialysis sessions, and one exposure took only a few minutes. The NB-UVB treatment cabins are usually available in dermatologic outpatient clinics in the same hospitals as dialysis units. In our university hospital the cost of one NB-UVB exposure is approximately 10% of one haemodialysis session showing that the cost is not any potential barrier to this treatment. Moreover, the NB-UVB doses were small. So far, this widely used dermatologic treatment has not shown any increased risk for skin cancer [15, 16].

In the present study, we followed up serum 25(OH)D concentrations for 2 months after the NB-UVB course. In contrast to NB-UVB-treated healthy subjects, 25(OH)D concentrations began to decrease in the CKD patients after 2 months. The more profound decrease of 25(OH)D may be caused by the higher BMI of the CKD patients compared to healthy subjects and linked to the active metabolism of vitamin D precursors in the fat tissue [7]. Though our present and previous CKD patient series were small, the observed relatively rapid decrease of serum 25(OH)D suggests that the CKD patients would need a longer NB-UVB course or cyclic NB-UVB exposures to maintain their vitamin D balance. The further limitation of the present study was that the dialysis patients were supplemented by only one dose of cholecalciferol, and a dose >20 μg daily would also be of interest.

In advanced kidney disease, the kidney is unable to produce $1,25(OH)_2D$ from 25(OH)D due to loss of renal CYP27B1 activity [5, 6]. It has been shown in UVB-treated organ cultures of skin that keratinocytes are able to hydroxylate 25(OH)D to $1,25(OH)_2D$ [17]. The finding that the CYP27B1 enzyme is also outside the kidney is of interest with regard to oral vitamin D treatment in the CKD patients [18]. In the present study, we found that prior to NB-UVB exposures the CKD patients had significantly increased cutaneous mRNA expression of the CYP27B1 enzyme compared to healthy subjects. The increased cutaneous CYP27B1 levels in the CKD patients supplemented with oral cholecalciferol suggest that the loss of renal activity of this enzyme is at least partially compensated for by the skin. This hypothesis was also supported by the previous study demonstrating that dialysis patients on oral vitamin D supplementation had enough extrarenal CYP27B1 activity to influence the serum $1,25(OH)_2D$ levels [19]. Interestingly, the NB-UVB course caused a significant decrease in the mRNA expression of CYP27B1 and CYP27A1 in both the CKD patients and healthy subjects. This type of decrease can be expected because there is a very sensitive natural feedback controlling mechanism caused by the UVB-induced increase in cutaneous vitamin D synthesis [6, 7, 20].

In conclusion, the present study shows that a short NB-UVB course is a rapid and effective way to improve vitamin D balance also in those dialysis patients who have already had oral vitamin D supplementation. The increased CYP27B1 levels in the patients suggest that the loss of renal activity of this enzyme is at least partly compensated for by the skin.

Acknowledgements

This study was financially supported by the National Graduate School of Clinical Investigation (M.A.-H.) and by Competitive Research Funding of the Tampere University Hospital (Grant 9M089). We thank nurses Pirjo Honko and Tuija Valjakka for giving the NB-UVB exposures in the study.

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