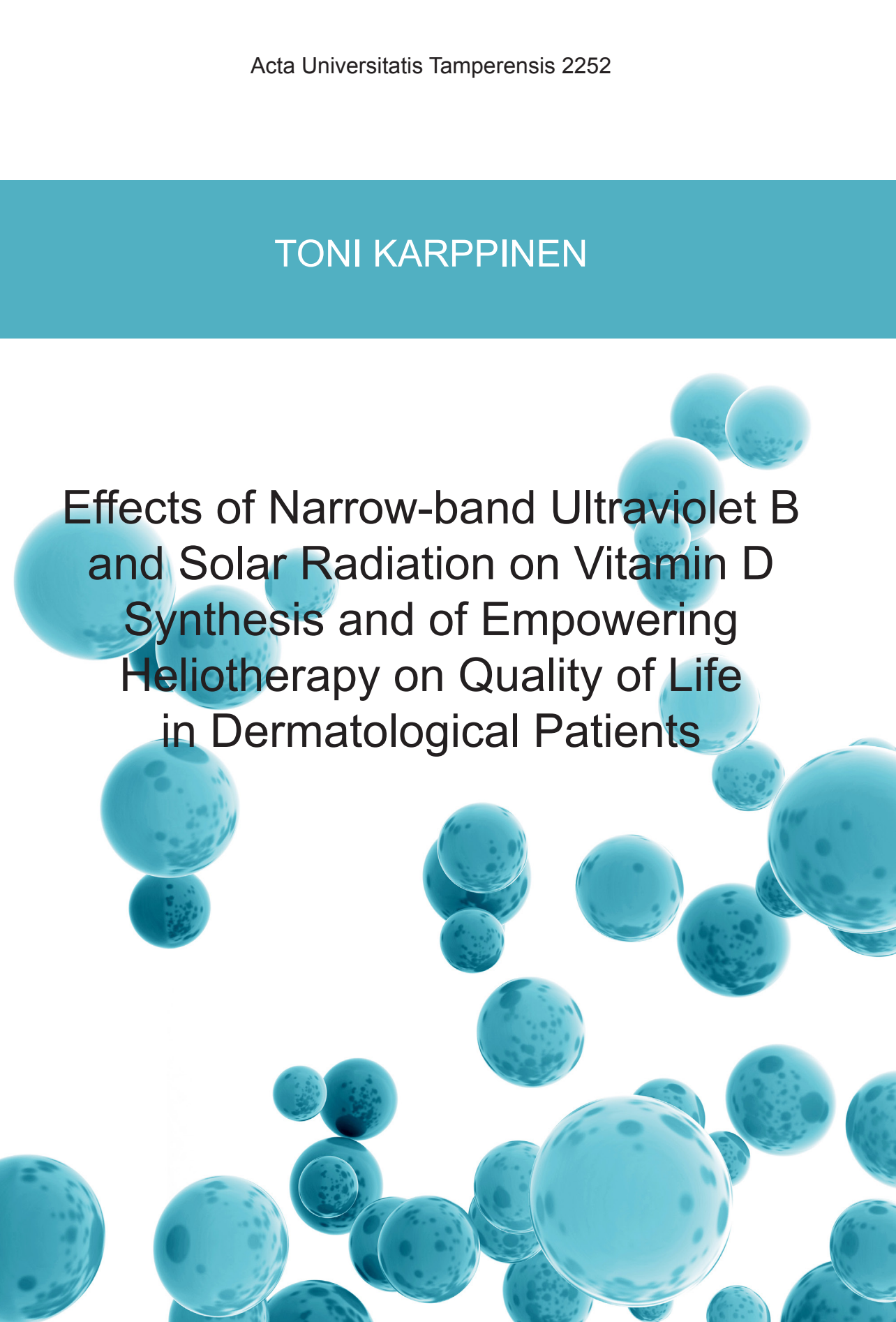


TONI KARPPINEN

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Effects of Narrow-band Ultraviolet B
and Solar Radiation on Vitamin D
Synthesis and of Empowering
Heliotherapy on Quality of Life
in Dermatological Patients



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Effects of Narrow-band Ultraviolet B
and Solar Radiation on Vitamin D
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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 27 January 2017, at 12 o'clock.

UNIVERSITY OF TAMPERE

TONI KARPPINEN

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1 List of original publications

This thesis is based on the following papers, which will be referred to in the text by the Roman numerals I-V. It also includes previously unpublished data.

- I. Ala-Houhala MJ*, **Karppinen T***, Vähävihu K, Kautiainen H, Dombrowski Y, Snellman E, Schaubert J, Reunala T. Narrow-band UVB treatment boosts serum 25-hydroxyvitamin D in psoriasis patients on oral vitamin D supplementation. *Acta Derm Venereol* 2014; 94:146-51.
- II. **Karppinen T**, Ala-Houhala MJ, Ylianttila L, Kautiainen H, Viljakainen H, Reunala T, Snellman E. Narrowband ultraviolet B exposures maintain vitamin D levels during winter: a randomized controlled trial. *Acta Derm Venereol* 2016; 96:490-3.
- III. **Karppinen T**, Ala-Houhala MJ, Ylianttila L, Kautiainen H, Lakkala K, Hannula H-R, Turunen E, Viljakainen H, Reunala T, Snellman E. The effect of vernal solar UV radiation on serum 25-hydroxyvitamin D concentration depends on the baseline level: observations from a high latitude in Finland. *Int J Circumpolar Health*. Accepted for publication.
- IV. **Karppinen T**, Ylianttila L, Kautiainen H, Reunala T, Snellman E. Empowering heliotherapy improves clinical outcome and quality of life of psoriasis and atopic dermatitis patients. *Acta Derm Venereol* 2015; 95:579-82.
- V. **Karppinen T**, Laine J-P, Kautiainen H, Pasternack R, Reunala T, Snellman E. Empowering heliotherapy improves clinical outcome and quality of life in psoriasis and atopic dermatitis patients: an observational study of 186 subjects. *Acta Derm Venereol*. Accepted May 31 (*ePub ahead of print*). doi: 10.2340/00015555-2506.

* both authors provided an equal input to the study

2 Abbreviations

AD	atopic dermatitis
BB-UVB	broad-band ultraviolet B
BMI	body mass index
CIE	Commission Internationale de l'Eclairage
DEQAS	Vitamin D External Quality Assessment Scheme
DLQI	dermatology life quality index
EHT	empowering heliotherapy
ECLIA	electrochemiluminescence immunoassay
HRQoL	health-related quality of life
HT	heliotherapy
HPLC	high performance liquid chromatography
IDS RIA	Immunodiagnosics Systems radioimmunoassay
LC-MS/MS	liquid chromatography tandem mass spectrometry
MCS	mental component summary
NB-UVB	narrow-band ultraviolet B
NIST	National Insitute of Standards and Technology
1,25(OH) ₂ D	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
PASI	psoriasis area and severity index
PCS	physical component summary
PO-SCORAD	patient-oriented scoring of atopic dermatitis
SAPASI	self-administered psoriasis area and severity index
SED	standard erythema dose
S-25(OH)D	serum 25-hydroxyvitamin D
SCORAD	scoring of atopic dermatitis
UV	ultraviolet
UVA	ultraviolet A
UVB	ultraviolet B
UVC	ultraviolet C
UVR	ultraviolet radiation
VAS	visual analogue scale

3 Abstract

Narrow-band ultraviolet B (NB-UVB) phototherapy is used to treat dermatoses such as psoriasis and atopic dermatitis (AD), diseases having a negative impact on the health-related quality of life (HRQoL). NB-UVB can also raise serum 25-hydroxyvitamin D [S-25(OH)D] levels. Since solar ultraviolet radiation (UVR) is not capable of inducing cutaneous vitamin D synthesis in winter, we set out to determine whether NB-UVB exposure can enhance S-25(OH)D levels in subjects receiving cholecalciferol supplementation, and whether NB-UVB can be used to sustain post-summer S-25(OH)D levels during the winter. We also studied whether vernal solar UVR can raise S-25(OH)D levels during outdoor activities in a snow-covered landscape, and what would be the effects of empowering heliotherapy (EHT) on HRQoL and disease severity in psoriasis and AD patients.

The psoriasis patients and healthy subjects described in *paper I* were receiving daily oral cholecalciferol supplements of 20 µg prior to the study and during it. Psoriasis patients received 18 NB-UVB exposures and the healthy subjects 9. After 9 exposures each, their average S-25(OH)D levels had increased by 13 and 17 nmol L⁻¹ ($p < 0.001$), respectively, while after the 18th exposure S-25(OH)D in the psoriasis patients had increased by 49 nmol L⁻¹. One month later the S-25(OH)D level was still 30 nmol L⁻¹ above the baseline in the psoriasis patients and 18 nmol L⁻¹ above in the healthy subjects. Baseline CYP27A1 and CYP27B1 levels were significantly lower in the psoriasis lesions than in the skin of the healthy subjects ($p < 0.001$). Cathelicidin levels were similar in both, whereas human beta defensin 2 levels were significantly higher in the psoriasis lesions ($p < 0.001$). NB-UVB did not alter the CYP27A1, CYP27B1 and cathelicidin levels in the psoriasis patients, but their average human beta defensin 2 level decreased significantly ($p = 0.002$). The NB-UVB exposures significantly reduced CYP27A1, CYP27B1 and cathelicidin levels in the healthy subjects. To conclude, NB-UVB radiation is effective in raising S-25(OH)D levels even in subjects receiving supplementations. The vitamin D hydroxylating enzymes in healthy skin react more actively to NB-UVB than those in psoriasis lesions. Human beta defensin 2 seems to have a role in the pathogenesis of psoriasis. The difference in the

expression of vitamin D-hydroxylating enzymes between psoriasis lesions and healthy skin, and the role of cutaneously synthesized vitamin D in the healing of psoriasis, are subjects which require further investigation since the roles of these effects remain unclear.

The healthy subjects studied in *paper II* were randomized into an intervention group receiving NB-UVB exposures every other week from October to April, or a control group. One standard erythema dose (SED) was administered on the first occasion and 2 SED on all subsequent occasions. Two weeks after the last irradiation the S-25(OH)D in the intervention group had increased by 12 nmol L^{-1} ($p = 0.029$) whereas that in the control group had decreased by 11 nmol L^{-1} ($p = 0.022$). In summary, suberythral NB-UVB exposures maintained and even increased the S-25(OH)D levels in winter. These could be used to maintain S-25(OH)D levels in haemodialysis patients, who typically respond slowly to oral cholecalciferol.

The healthy subjects in *paper III* were exposed to vernal solar UVR in March and April either during their late winter holiday, or at noon on working days. They received a mean cumulative ultraviolet B (UVB) radiation dose of 12 SED on the face and hands, i.e. 7% of the total body surface area, over a mean period of 12 hours spent out of doors. Those whose baseline S-25(OH)D concentrations were below 90 nmol L^{-1} showed significant increases of 6 nmol L^{-1} ($p < 0.001$), while those with a baseline over 90 nmol L^{-1} showed a decrease of 7 nmol L^{-1} ($p < 0.01$). In summary, the 'vitamin D winter' in Finland lasts only until March, encouraging people to engage in vernal outdoor activities.

Papers IV and *V* assess the effects of two-week EHT courses on the HRQoL and disease severity in psoriasis and AD patients. The mean Dermatology Life Quality Index (DLQI) decreased significantly by 5 and 8 units ($p < 0.001$) in these two groups, respectively, after EHT, and remained decreased by 3 and 5 units ($p < 0.001$), respectively, after three months. The Self-Administered Psoriasis Area and Severity Index (SAPASI) decreased by 5.0 units from an initial 7.4 units and was still 2.6 units below the initial level 3 months after EHT ($p < 0.001$). The Patient-Oriented Scoring of Atopic Dermatitis (PO-SCORAD) index decreased by 24.0 units from an initial figure of 36.7, and was still 14.6 units below the initial figure ($p < 0.001$) 3 months after EHT. The RAND-36 physical and mental component summary scores decreased significantly during EHT and remained

decreased 3 months after EHT in both groups. In summary, EHT improves the HRQoL and alleviates disease severity in psoriasis and AD patients.

To conclude, NB-UVB exposures are effective in raising and maintaining S-25(OH)D levels in psoriasis patients and healthy subjects. Vernal solar UVR can elevate S-25(OH)D in subjects with a baseline level below 90 nmol L⁻¹. Two-week EHT courses improve the HRQoL and alleviate disease severity in psoriasis and AD patients for at least 3 months.

Tiivistelmä

Kapeakaistaista ultravioletti B (narrow-band ultraviolet B, NB-UVB) –valohoitoa käytetään useiden ihosairauksien, kuten psoriasisien ja atooppisen dermatiitin hoitamiseen. Näillä sairauksilla on negatiivinen elämänlaatuvaikutus. NB-UVB-valotus nostaa myös seerumin 25-hydroksiviamiini D [S-25(OH)D] –pitoisuutta. Koska auringon ultravioletti(UV)-säteilyn voimakkuus ei riitä käynnistämään ihon D-vitamiinisynteesiä talvella, tutkimme, kykeneekö NB-UVB-valotus nostamaan S-25(OH)D-tasoa kolekalsiferolilisää käyttävillä henkilöillä, ja voidaanko NB-UVB-valotuksilla ylläpitää kesänjälkeistä S-25(OH)D-tasoa talvella. Tutkimme myös, nostaako kevätauringon UV-säteily S-25(OH)D-tasoa lumisilla alueilla ulkoiltaessa. Lisäksi tutkimme aurinkopainotteisen sopeutumisvalmennuskurssin vaikutusta psoriasisista tai atooppista dermatiittia sairastavien potilaiden elämänlaatuun ja iho-oireisiin.

Osatyössä / psoriasispotilaat ja terveet verrokkit käyttivät 20 µg kolekalsiferolilisää ennen tutkimusta ja sen aikana. Psoriasispotilaat saivat 18 ja verrokkit 9 NB-UVB-valotusta. Keskimääräiset S-25(OH)D-tasot nousivat 9. valotuksen jälkeen 13 ja 17 nmol L⁻¹ (p < 0.001), vastaavasti. S-25(OH)D-taso nousi 18. valotuksen jälkeen psoriasispotilailla 49 nmol L⁻¹. Kuukauden kuluttua taso oli psoriasispotilailla edelleen 30 nmol L⁻¹ ja verrokeilla 18 nmol L⁻¹ korkeampi kuin alkutaso. CYP27A1- ja CYP27B1-entsyymien tasot olivat alhaisemmat psoriasisleesioissa kuin verrokkien iholla (p < 0.001). Katelisiidiinitasot olivat yhtä korkeat, mutta human β-defensiini 2:n taso oli korkeampi psoriasisleesioissa (p < 0.001). NB-UVB-valotukset eivät muuttaneet CYP27A1-, CYP27B1- tai katelisiidiinitasoa psoriasispotilailla, mutta human β-defensiini 2:n taso laski merkitsevästi (p = 0.002). Verrokeilla NB-UVB valotukset laskivat CYP27A1-, CYP27B1- ja katelisiidiinitasoa. Yhteenvetona, NB-UVB-valotukset nostavat S-25(OH)D tasoa kolekalsiferolilisää käyttävillä henkilöillä. Terveessä ihossa D-vitamiinihydroksylaatioon osallistuvat entsyymit reagoivat NB-UVB-valotukseen aktiivisemmin kuin psoriasisleesioissa, ja human β-defensiini 2:lla näyttäisi olevan rooli psoriasisien patogeneesissä. D-vitamiinihydroksylaatioon osallistuvien entsyymien erilainen ilmentyminen

terveessä ihossa ja psoriasisleesiossa, ja iholla syntetisoidun D-vitamiinin rooli psoriasisksen paranemisessa, ovat aiheita joiden merkitys jää toistaiseksi epäselväksi ja jotka vaativat jatkotutkimuksia.

Osatyössä II terveitä vapaaehtoisia satunnaistettiin interventioryhmään saamaan NB-UVB-valotuksia joka toinen viikko lokakuusta huhtikuuhun, tai kontrolliryhmään. Ensimmäinen valoannos oli 1 standardieryteemayksikkö (SED) ja jatkoannokset 2 SED. Kaksi viikkoa viimeisen valotuksen jälkeen S-25(OH)D-taso oli noussut 12 nmol L^{-1} ($p = 0.029$) interventioryhmässä ja laskenut 11 nmol L^{-1} ($p = 0.022$) kontrolliryhmässä. Yhteenvetona, punekynnyksen alittavat NB-UVB-valotukset ylläpitävät ja jopa nostavat S-25(OH)D-tasoa talvella. Tällaista valotusprotokollaa voisi käyttää S-25(OH)D-tason ylläpitoon hemodialyysipotilailla, joilla vaste kolekalsiferolilisään on usein heikko.

Osatyössä III terveet vapaaehtoiset ulkoilivat maaliskuussa talvilomallaan tai työpäivän aikana. Kumulatiivinen ultravioletti B (UVB) – säteilyannos oli 12 SED ja se saavutettiin 12 ulkoilutunnin aikana, jolloin kasvot ja kädet eli 7% koko ihon pinta-alasta olivat aurinkoalttiina. Alle 90 nmol L^{-1} S-25(OH)D-tasot nousivat merkitsevästi 6 nmol L^{-1} ($p < 0.001$) ja yli 90 nmol L^{-1} tasot laskivat 7 nmol L^{-1} ($p < 0.01$). Yhteenvetona, Suomen 'D-vitamiinitalvi' kestää vain maaliskuuhun, mikä voi motivoida ihmisiä lisäämään keväistä ulkoilua.

Osatyössä IV ja V arvioitiin 2-viikon aurinkopainotteisten sopeutumisvalmennuskurssien (empowering heliotherapy, EHT) vaikutus psoriasisista tai atooppista dermatiittia sairastavien potilaiden elämänlaatuun ja iho-oireisiin. EHT:n jälkeen elämänlaatumittarin Dermatology Life Quality Index (DLQI) tulos laski merkitsevästi, psoriaatikoilla 5 ja atoopikoilla 8 yksikköä ($p < 0.001$). Kolme kuukautta EHT:n jälkeen DLQI oli 3 ja 5 yksikköä alkutasoa matalampi ($p < 0.001$), vastaavasti. Psoriasispotilailla iho-oireet lievittyivät Self-Administered Psoriasis Area and Severity Index (SAPASI) –mittarilla 5.0 yksikköä arvosta 7.4, ja pysyivät 3 kuukautta EHT:n jälkeen 2.6 yksikköä alemmina ($p < 0.001$). Atoopikoilla iho-oireet lievittyivät Patient-Oriented Scoring of Atopic Dermatitis (PO-SCORAD) –mittarilla 24.0 yksikköä arvosta 36.7, ja pysyivät 3 kuukautta EHT:n jälkeen 14.6 yksikköä alemmina ($p < 0.001$). RAND-36-elämänlaatumittarin fyysisen ja henkisen komponentin

yhteispisteet laskivat merkitsevästi EHT:n aikana, ja pysyivät parantuneina 3 kuukautta EHT:n jälkeen molemmilla potilasryhmillä.

Yhteenvetona, NB-UVB-valotukset nostavat ja ylläpitävät S-25(OH)D-tasoa tehokkaasti psoriasispotilailla ja terveillä henkilöillä. Kevätauringon UV-säteily kykenee nostamaan S-25(OH)D-tasoa henkilöillä, joilla lähtötaso on alle 90 nmol L⁻¹. Kahden viikon EHT parantaa psoriasispotilaiden ja atoopikkojen elämänlaatua ja ihottumaoireita ainakin 3 kuukauden ajan.

4 Introduction

Ultraviolet B (UVB) radiation is responsible for the photoconversion of 7-dehydrocholesterol to previtamin D₃ (Holick et al. 1980, MacLaughlin et al. 1982) and accounts for at least 90% of the total serum 25-hydroxyvitamin D [S-25(OH)D] concentration (Reichrath 2006). However, the intensity of UVB radiation at latitude 52°N and above is not high enough to induce cutaneous photosynthesis of vitamin D between October and March (Webb et al. 1988), although in theory this photosynthesis is possible from early March onwards (Kazantzidis et al. 2009) even at latitude 61°N. Due to the high seasonal variation in UVB radiation and the generally low vitamin D intake, vitamin D insufficiency is common world-wide (Wacker and Holick 2013, Cashman et al. 2016, Lamberg-Allardt et al. 2013), leading in Finland to recent national policies that have succeeded in increasing vitamin D intakes (Helldan et al. 2013). A lack of consensus nevertheless exists as to the optimal S-25(OH)D level (Bouillon et al. 2013), which serves as the best indicator of vitamin D status (Holick 1981). The cut-offs depicting vitamin D deficiency and insufficiency suggested by the Institute of Medicine are $< 30 \text{ nmol L}^{-1}$ and $< 50 \text{ nmol L}^{-1}$ (IOM 2011), but the evidence for the health benefits of vitamin D is conclusive only where bone health and total mortality are concerned (Lamberg-Allardt et al. 2013). Vitamin D insufficiency is more common in patients with psoriasis and atopic dermatitis (AD) than in healthy subjects (Gisondi et al. 2012, Cheng et al. 2014). These two diseases are treated *inter alia* with narrowband ultraviolet B (NB-UVB) phototherapy (Sokolova et al. 2015) and heliotherapy (Snellman et al. 1993,

Autio et al. 2002, Mork and Wahl 2002, Vähävihi et al. 2008), which increase S-25(OH)D concentrations (Vähävihi et al. 2008, Osmanovic et al. 2009a, Vähävihi et al. 2010a). As chronic skin diseases, psoriasis and AD have a negative impact on the health-related quality of life (HRQoL) of affected patients (Maksimovic et al. 2012, Augustin and Radtke 2014), and since self-management plays an essential role in their long-term treatment, patient education is nowadays considered an integral part of successful treatment (De Bes et al. 2011).

The key targets of the present work were to examine the effects of NB-UVB phototherapy and solar ultraviolet radiation (UVR) on the vitamin D status of dermatological patients and healthy subjects, and the effects of UVR during empowering heliotherapy on HRQoL and disease activity in psoriasis and AD patients. Another target was to determine whether vernal solar UVR is capable of raising S-25(OH)D concentrations.

5 Review of the literature

5.1 Ultraviolet radiation

5.1.1 Seasonal and geographical variation in solar UV radiation

Ultraviolet radiation (UVR) represents the wavelength range 400 to 100 nm, within which the biological effects of the radiation vary greatly. Thus, the spectrum was further divided in 1932 into three subregions, defined as ultraviolet A (UVA, 400–315 nm), ultraviolet B (UVB, 315–280 nm) and ultraviolet C (UVC, 280–100 nm). Environmental and dermatological photobiologists tend to use slightly different ranges, however: UVA (400–320 nm), UVB (320–290 nm) and UVC (290–200 nm) (Diffey 2002). The ozone layer absorbs wavelengths shorter than 310 nm, and thus 95% of our terrestrial UVR is UVA, 5% is UVB, and UVC is not detected at all (Reichrath et al. 2006). UVB is differentiated from UVA at 320 nm, because wavelengths shorter than this are generally more photobiologically active (Diffey 2002). UVC radiation causes significant damage to biological organisms, since the absorption spectrum of DNA reaches a maximum at 260 nm, and UVB radiation similarly causes DNA mutations and increases the risk of skin cancer (Reichrath et al. 2006). UVB radiation is also responsible for the photoconversion of 7-dehydrocholesterol to previtamin D₃, with a maximum spectral effectiveness at about 297 nm (Holick

et al. 1980, MacLaughlin et al. 1982). Longer UVA wavelengths penetrate deeper into the dermis and can cause photoageing and solar elastosis (Reichrath et al. 2006).

Factors that affect the ambient UVR level can be either cyclical and predictable or more random and less predictable. The most important cyclical factor is the solar zenith angle, i.e. the angle between the vertical axis and the sun, which depends on the time of day, season of the year and latitude. As the angle increases, the UVR has a longer distance to travel through the atmosphere and therefore has more chance of being absorbed or scattered and spreads over a larger area, which reduces the amount reaching the skin. Small solar zenith angles are associated with summer, noon and low latitudes, whereas large angles are associated with winter, mornings and evenings, and high latitudes (Webb 2006).

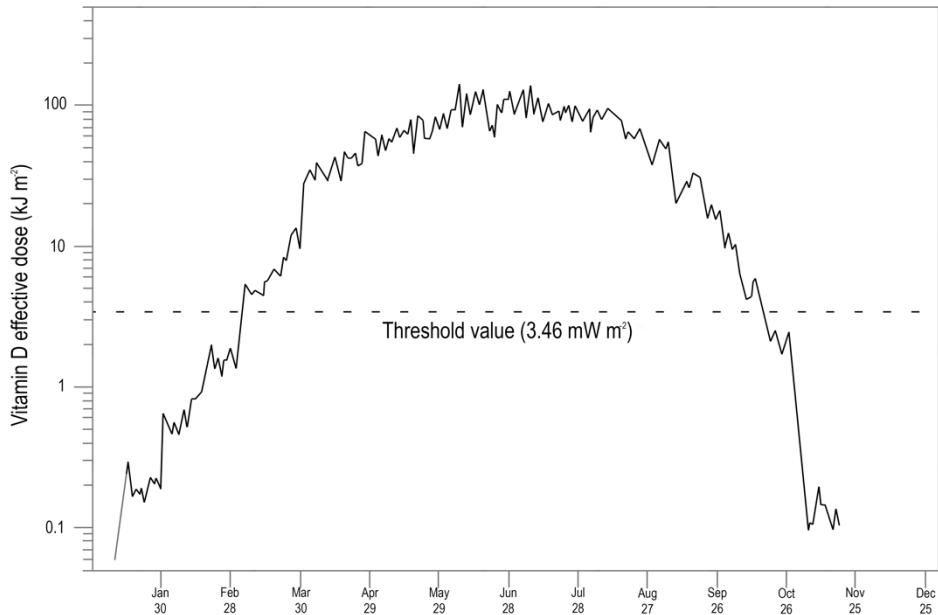
Ozone in the stratosphere is a major absorber of UVB radiation, and this also is cyclical in nature but with a more random seasonal variation. At mid-latitudes the effect of ozone increases in spring, but at high latitudes there is a long-term trend in ozone depletion that is gradually increasing UVR levels. Ozone and altitude influence the shorter UVB wavelengths more than the longer UVA wavelengths and thus alter the spectral shape and intensity of the UVR. More UVR reaches the ground surface at high altitudes and the proportion of UVB increases (Webb 2006).

Cloudiness, and also aerosols in polluted areas, can reduce the irradiance of the surface by causing scattering and absorption (Engelsen et al. 2005, Webb 2006), and a surface such as snow can itself increase the UVR level substantially due to reflections between the ground and the sky, as well as through direct reflections from the ground to vertical surfaces (Jokela et al. 1993). The albedo of snow varies between 0.5 to 0.7 (Meinander et al. 2008 and 2013), the

reflection effect being highest for vertical surfaces such as the face (Jokela et al. 1993).

Due to the high seasonal variation in available UVB radiation, no cutaneous photosynthesis of vitamin D can be detected between October and March at latitude 52°N and above, a phenomenon referred to as the 'vitamin D winter' (Webb et al. 1988). Variations in ozone levels can nevertheless alter the latitude of this 'winter' by up to 10 degrees and extend or shorten its duration by up to 2 months (Engelsen et al. 2005). In theory, cutaneous photosynthesis of vitamin D is possible at latitude 61°N from early March, but this is a mathematically calculated estimate based on experimental UVR spectroradiometer irradiance measurements (Fig. 1) (Kazantzidis et al. 2009). Due to seasonal variation in available UVB radiation, the lowest S-25(OH)D levels are typically measured between February and April in subjects living at high latitudes (Edvardsen et al. 2007, Brustad et al. 2007, Datta et al. 2012, Klingberg et al. 2015).

Figure 1. Mean annual effective doses of vitamin D at Jokioinen (61°N) at 4 pm.



5.1.2 Measurement of UV radiation

An established physical terminology exists for expressing quantities of UVR. A radiation beam has a certain *radiant energy* (J) and *radiant flux* (W), while a radiation source has a certain *radiant intensity* (W sr^{-1}) and *radiance* ($\text{W m}^{-2} \text{sr}^{-1}$). *Irradiance* (W m^{-2}) describes the amount of radiance reaching a given object and the time integral of *irradiance* is *radiant exposure* (J m^{-2}). A prefix *spectral* is used when the quantities are expressed in terms of wavelengths. As the biological effects of UVR vary according to the wavelength, *irradiance* of a radiation source has to be multiplied by an *action spectrum* to create a weighted irradiance (Diffey et al. 1997, CIE 1999, Diffey 2002) for interpreting the consequences. Action spectra are defined by measuring the threshold energy of

each wavelength inducing an end-point of interest. Different action spectra have been developed by the Commission Internationale de l'Eclairage (CIE), such as the erythema reference action spectrum (CIE 1999) and the previtamin D₃ action spectrum (Boullion et al. 2006).

Erythemally weighted doses can be expressed as either CIE erythema weighted doses (mJ cm^{-2} or J m^{-2}), Minimal Erythema Doses or Standard Erythema Doses (SED). A Minimal Erythema Dose is the smallest dose causing well-defined skin erythema 24 hours after irradiation. It is not a standard measure, however, as it only expresses the sensitivity of a single individual to UVR, and the result can be affected by the characteristics of the radiation source, the pigmentation of the skin, previous light exposure, the anatomical site and observational factors. The concept of Minimal Erythema Dose is confined to observational studies (Diffey et al. 1997, Diffey 2002), while SED has been developed for use when referring to natural and artificial UVR sources (Diffey et al. 1997, CIE 1999). One SED is equivalent to 10 mJ cm^{-2} CIE erythema weighted irradiance. The ambient solar UVR exposure on a clear European summer day, for example, is about 30–40 SED. Four SED will produce moderate erythema on unexposed fair skin, but only minimal erythema on previously exposed skin (Diffey et al. 1997). The average Dane receives a dose of 1.5 SED of solar UVR daily in July (Thieden et al. 2004).

The measurement of radiation doses, termed dosimetry, serves as a means of ensuring the consistent administration of UVR, the assessment of UVR exposures gained in the course of different activities, and for the publication of results and their comparison between research groups. The devices used to measure ambient UVR are known as radiometers. A radiometer used as a reference monitor has to be stable, so as to provide precise and reproducible data. If the measured data are to be published and compared between

laboratories or research groups, the radiometer used has to be accurate, i.e. calibrated against an accepted absolute standard (Diffey 2002).

There are different kinds of radiometers in existence. Spectroradiometers can measure spectral power distributions and irradiance, and have to be calibrated over the wavelength that is being measured using standard lamps. Radiometers typically operate in the 200–1600 nm range and are capable of scanning wavelengths at a speed of 0.1–2 nm per second (Diffey 2002).

Narrowband radiometers are used for measuring a narrower spectrum of radiation, for example 280–315 nm, with no response to wavelengths outside that range. The method provides data on the total power received by the sensor in the range concerned, but no information is provided on the power distribution spectrum. For that reason, narrowband radiometers have to be calibrated spectroradiometrically for the type of UVR source being measured. Narrowband radiometers are typically used to measure UVR from the sky, and thus the sensor is often a hemisphere, and is not collimated. The sensor must be cosine-weighted to cope with variation in the angle of the incoming radiation (Diffey 2002).

Broadband radiometers respond to all optical wavelengths. One example of a broadband radiometer is the Robertson-Berger UVR meter, the spectral response of which corresponds to the erythemal action spectrum. This meter was introduced by Berger in 1976 (Robertson 1968, Berger 1976). A Robertson-Berger meter encompassing a hemispheric sensor and cosine weighting can be used to measure ambient UVR in the sky. Both narrowband and broadband radiometers measure UVR continuously and save the data at short intervals, e.g. every ten minutes on the case of a Robertson-Berger meter (Diffey 2002, di Sarra et al. 2002).

To measure the amount of UVR actually received by a subject, personal UVR dosimeters have been developed and have been in use since the 1970s. Nowadays there are chemical, biological and electronic dosimeters, the mostly common chemical dosimeters being those that use polysulphone and polyphenylene oxide (Davis et al. 1976), which have maintained their suitability because they are economical and simple (Amar and Parisi 2013). The spectral sensitivity of a polysulphone dosimeter is close to the erythema action spectrum, and measurements of solar UVB with polysulphone films have been found to correlate closely with spectroradiometric measurements ($R^2 > 0.95$) (Kollias et al. 2003). The maximum UVR measurement times of polysulphone and polyphenylene oxide films are typically 8 hours and 5 days, respectively, which can limit their use, but recently a polyvinyl chloride-based dosimeter has been developed which can measure doses linearly up to 900 SED, corresponding to an UVR exposure of 3 weeks at a subtropical location during summer (Amar and Parisi 2013).

Biological dosimeters are based on UV-induced DNA damage. Validated biological dosimeters include *Bacillus subtilis* spore films, polycrystalline uracil and DNA-virus called bacteriophage T7 (Berces et al. 1999). The spectral response of *B. subtilis* dosimeter equipped with a cut-off filter is similar to the erythema action spectrum in human skin after UVB exposure (Quintern et al. 1992, Quintern et al. 1997). These dosimeters can detect a dose ranging from 0.5 to 360 SED (www.biosense.de, Biosense, Bornheim, Germany). Spore film dosimeters have been found feasible for UVR dose monitoring in practice, and the measured data correlates strongly with UV exposure diary data (Vähävihi et al. 2010b). Vitamin D can also be used as a dosimeter, but this method provides the previtamin D₃ weighted UVR dose rather than the erythema-weighted dose (Galkin and Terenetskaya 1999). These doses are different, because of the

different action spectra for erythema and previtamin D₃ (CIE 1999, Boullion et al. 2006). Contrary to chemical and biological dosimeters, electronic dosimeters such as the SunSaver, can provide time stamped data, which is useful when assessing compliance in observational studies. SunSaver has a silicon carbide photodiode sensor with a built-in diffuser and a cosine response following the CIE erythema action spectrum. The measurement range is 0.1–23.0 SED per hour (CIE 1999, Thieden et al. 2004).

Personal dosimeter exposure levels can vary greatly depending on the outdoor activity. Exposures measured by polysulphone films can range from 9% to 71% of ambient UVR with highest doses acquired during sunbathing, boating and swimming at the ocean (Holman et al. 1983). The top of the head receives typically the highest UVR doses, 50% of the ambient UVR. Despite with considerable individual variation, Thieden et al. (2000) found that, on average, the wrist can receive the same dose as the head, and thus the wrist can be suggested as a dosimeter site for research purposes.

5.2 Vitamin D

5.2.1 Historical background of vitamin D

The first description of the bone deforming disease rickets was drawn up in the 17th century (Rajakumar et al. 2007), and its association with a lack of sunlight was recognized in 1822, but the usefulness of the UVB radiation emitted by a mercury vapour lamp for treating rickets was not observed until 1919. The first scientific definition of rickets as a nutritional deficiency-related disease was published by Edward Mellanby in 1918 (Rajakumar et al. 2007). The vitamin D precursor substrate was searched for in plant sources and finally identified as ergosterol by three research groups simultaneously in 1931 (Wolf 2004), although a certain discrepancy remained, as animal organisms lack ergosterol and can still produce vitamin D from sunlight. Finally, 7-dehydrocholesterol was isolated from hog skin in 1937 by Windaus and Bock and its irradiation product was named cholecalciferol (Wolf 2004). The exact reaction steps from 7-dehydrocholesterol to cholecalciferol were reported by Holick et al. in 1980.

In the 1930s a US government agency provided recommendations for sensible sunlight exposure to prevent childhood rickets (Holick and Chen 2008). Vitamin D-fortified milk was used to prevent rickets in the US and Europe in the 1930s, but several cases of severe hypercalcaemia in Great Britain in the 1950s due to overfortification led to the banning of fortification practices over most of Europe (Holick and Chen 2008). Children in Finland, however, have been continuously receiving vitamin D supplementation since the 1930s. National recommendations for the vitamin D fortification of milk, margarines and spreads were laid down in 2003 (Lehtonen-Veromaa et al. 2008).

5.2.2 Photosynthesis, dietary sources and metabolism

The vitamin D sources available to humans are UVR-induced cutaneous photosynthesis, vitamin D-rich food and supplements. Cutaneous vitamin D synthesis normally contributes more than 90% of the total 5-25(OH)D concentration (Reichrath 2006). Solar UVB radiation (290–315 nm) penetrates the skin and converts 7-dehydrocholesterol to pre-vitamin D₃ (Holick 2007) with a maximum spectral effectiveness at about 297 nm (Holick et al. 1980, MacLaughlin et al. 1982). The formation of pre-vitamin D₃ in fair skin reaches a plateau after 15 min of exposure to equatorial UVR at noon, all the surplus being isomerized to biologically inactive lumisterol and tachysterol (Holick 1981, MacLaughlin et al. 1982). Pre-vitamin D₃ then undergoes heat isomerization, in which 50% of it is converted to vitamin D₃, i.e. cholecalciferol, in 2.5 hours, reaching an equilibrium in 24 hours (Tian et al. 1993).

Only a few foods contain vitamin D, which comprises both vitamin D₂ (ergocalciferol) and D₃ (cholecalciferol). Vitamin D₂ is manufactured by UV irradiating the ergosterol present in yeast, and vitamin D₃ by UV irradiating the 7-dehydrocholesterol found in lanolin (Holick 2007). Both end products are sold as vitamin D supplements in Finland. Even though plants, yeasts and fungi contain ergosterol naturally, they are poor sources of vitamin D unless irradiated (Lehmann and Meurer 2010). Significant dietary vitamin D₃ sources include oily fish (i.e. salmon, sardines, bluefish, mackerel and herring), fish liver, oils from fish, mushrooms and egg yolk (Holick 2007, Lehmann and Meurer 2010).

Vitamin D (D₂ or D₃) synthesized in the skin or acquired from the diet is hydroxylated in the liver to 25(OH)D, the best indicator of vitamin D status (Holick 1981), with a half-life of about 15 days (Jones 2008). The hydroxylating

enzyme is vitamin D-25-hydroxylase (25-hydroxylase, CYP27A1) (Lehmann and Meurer 2010). Even when supplies of vitamin D are high, an effective homeostatic control system functions up to a certain limit to ensure a stable availability of 25(OH)D in order to maintain its serum concentration within a narrow range between 75–220 nmol L⁻¹ (Vieth 1999). The points where this regulation takes place include i) the liver concentration of 25-hydroxylase (Bhattacharyya and DeLuca 1973) and ii) catabolism of 25(OH)D to breakdown products in the liver (Clements et al. 1987) and in other tissues (Tomon et al. 1990). Most laboratories consider serum levels of 50–250 nmol L⁻¹ to be normal (Holick 2007).

25(OH)D is hydroxylated in the kidneys to its hormonally active form, 1 α ,25-dihydroxyvitamin D [1,25(OH)₂D] by the enzyme 25-hydroxyvitamin D-1 α -hydroxylase (1 α -hydroxylase, CYP27B1). This hydroxylation is regulated mainly by parathyroid hormone, but also by calcium, phosphate, calcitonin, fibroblast growth factor 23 and a self-regulation mechanism. 1,25(OH)₂D has biological effects in vitamin D receptor-positive target tissues such as the kidneys, bone, intestine and parathyroid gland (Lehmann and Meurer 2010).

The key role of 1,25(OH)₂D is to optimize the serum calcium level for metabolic functions, signal transduction and neuromuscular activity. It regulates the intestinal calcium uptake and in the case of hypocalcaemia induces osteoclasts to mobilize calcium from the bones (Holick and Chen 2008). The serum levels of 1,25(OH)₂D are in the range 75–200 pmol L⁻¹ and have a half-life of 10–24 hours (Lehmann and Meurer 2010). Several extrarenal tissues such as macrophages, the brain, the colon, the prostate and the breast have enzymatic properties with respect to 1,25(OH)₂D synthesis (Holick and Chen 2008). Keratinocytes express both 25-hydroxylase and 1 α -hydroxylase activity, and cutaneous production of 1,25(OH)₂D is thought to exert autocrine effects on keratinocytes and paracrine

effects on neighbouring cells. This could be one of the therapeutic links between UVB radiation and psoriasis (Lehmann and Meurer 2010).

Both heliotherapy (HT) and artificial UVB treatment raise S-25(OH)D levels. Heliotherapy has been shown to do so by 13–57 nmol L⁻¹, depending on the season, from a baseline level of 43–57 nmol L⁻¹ and artificial UVB by 9–91 nmol L⁻¹ from a baseline of 19–87 nmol L⁻¹ depending on the exposure modality, cumulative dose and irradiated skin area. BB-UVB has proved to be more effective in raising S-25(OH)D than NB-UVB, and NB-UVB more effective than 20 or 40 µg oral cholecalciferol daily (Table I)

Table I. List of studies of the effects of heliotherapy and artificial UVB exposure on S-25(OH)D concentrations.

Subjects	Mean baseline S-25(OH)D (nmol L ⁻¹)	Alterations of mean S-25(OH)D, (nmol L ⁻¹)	Intervention type and mean number of exposures	UVB dose, mean (SED)	Author
Atopic dermatitis	43 42	13 24	Heliotherapy January Heliotherapy March	60 109	Vähävihi 2008
Psoriasis	57	47	Heliotherapy March	166	Osmancevic 2009a
Psoriasis	92	57	23 BB-UVB exposures	N.D.	Osmancevic 2007
Psoriasis	95 87	79 51	27 BB-UVB exposures 28 NB-UVB exposures	N.D. N.D.	Osmancevic 2009b
Psoriasis Atopic dermatitis Healthy subjects	37 32 61	60 68 91	15 NB-UVB exposures	72	Vähävihi 2010a
Healthy subjects	53 54	41 20	12 NB-UVB exposures Oral cholecalciferol 20 µg	48	Ala-Houhala 2012a
Healthy subjects	19 23	56 37	18 NB-UVB exposures Oral cholecalciferol 40 µg	53	Bogh 2012a
Healthy subjects	51 54	20 25	10 UVB exposures Oral cholecalciferol 50 µg	23.8	Lagunova 2013
Dialysis patients Healthy subjects	33 60	14 9	9 NB-UVB exposures on 25% of body surface area	15	Ala-Houhala 2012b
Dialysis patients Healthy subjects	58 74	14 17	Oral cholecalciferol 20 µg + 9 NB-UVB exposures	26	Ala-Houhala 2013

Vähävihiu et al. (2010c) studied the ability of different skin areas to photosynthesize vitamin D. They gave women NB-UVB exposures for seven consecutive days in winter, totalling 13 SED, and showed that total body irradiations raised S-25(OH)D by 11.4 nmol L⁻¹ and irradiating the face and arms, i.e. 25% of total skin area, was almost as effective, with an increase of 11.0 nmol L⁻¹. Osmanovic et al. (2015) gave suberythemal BB-UVB exposures for 3 consecutive days on the face and hands (5% of the total skin area), whole body and upper body, and showed that although the exposure of larger skin areas resulted in greater increases in S-25(OH)D levels, exposure of the face and hands was already capable of increasing these levels by 6.1 nmol L⁻¹. Bogh et al. (2011) exposed 6%, 12% or 24% of the total skin area to either a 0.75, 1.5 or 3.0 SED dose of BB-UVB irradiation on nine occasions over a period of 16 weeks and found that the increase in 25(OH)D levels was mainly dose-dependent, except that at smaller BB-UVB doses the area of the irradiated surface was also important. No significant relationship with body surface area was found for the higher BB-UVB doses of 1.5 and 3.0 SED because a state of saturation was reached. They also showed that 1 SED exposures to BB-UVB every second week are capable of maintaining S-25(OH)D levels during the winter.

The Institute of Medicine recommends a dietary intake of vitamin D - including supplements of 15 µg daily for people aged 1-70 years and 20 µg daily for those older than 70 years (IOM 2011). The Nordic Nutrition Recommendations (NORDEN 2012), and consequently the Finnish Nutrition Recommendations (National Nutrition Council 2014), are more conservative, suggesting 10 µg for people aged under 75 years, 20 µg for those older than 75, and 10 µg for pregnant and breastfeeding women. The recommendations are based on a review by Lamberg-Allardt et al. (2013), where conclusive and convincing evidence for the benefits of vitamin D was found only in terms of bone health

and total mortality. A concentration of $\geq 50 \text{ nmol L}^{-1}$ was considered optimal, and to reach that in 97.5% of individuals, a vitamin D intake of $15 \mu\text{g/d}$ is warranted. The recommended intake of $10 \mu\text{g/d}$ ensures adequacy in 50% of individuals with limited sun exposure during the summer, but vitamin D is typically stored up during the summertime (Lamberg-Allardt et al. 2013). The amount of outdoor activity and the level of 7-dehydrocholesterol in the skin usually decline with age, which is the reason for the higher intake recommendation for people older than 75 years (Lamberg-Allardt et al. 2013).

National surveys suggest that actual dietary intakes of vitamin D in Europe are typically below $5 \mu\text{g/d}$. They vary somewhat, however, according to national fortification practices, age, gender and the use of vitamin D supplements, the last being most important (Kiely and Black 2012). Due to the gap between vitamin D intake and the recommendations, many countries allow the fortification of vitamin D in foods. In the USA this is optional, but milk, cheese, juice, yogurt, cereals, noodles and rice can be fortified, for example. In Canada liquid milk fortification is mandatory (Calvo and Whiting 2013). In Finland, margarines have been fortified since 1941 and fat-free milk since the 1980s (Valtion ravitsemusneuvottelukunnan historiikki). In 2002 the fortification policy was reviewed by an expert meeting due to the high prevalence of vitamin D deficiency in peripubertal girls in winter (Lehtonen-Veromaa et al. 2002), and the committee decided to fortify milk, sour milk and yogurt with $0.5 \mu\text{g}/100 \text{ g}$ of vitamin D and increase the fortification of margarine and spreads from $7.5 \mu\text{g}$ to $10 \mu\text{g}/100 \text{ g}$ (Tylavsky et al. 2006). The Ministry of Social Affairs and Health agreed to the new recommendations, which came into force in February 2003, but even the new fortification policy failed to raise the vitamin D intake of adolescent Finnish females to $7.5 \mu\text{g/d}$, which was the recommended dietary allowance at that time, and consequently 65.5% of the subjects still had S-

25(OH)D < 50 nmol L⁻¹ (Lehtonen-Veromaa et al. 2008). Wintertime vitamin D levels in young Finnish men did improve significantly, but still remained insufficient (Laaksi et al. 2006, Välimäki et al. 2007). The most recent national survey, FINDIET 2012, has finally shown, that the food fortification policy has succeeded in increasing the vitamin D intakes of Finnish men above the recommendations, to 11 µg/d, whereas women received 9 µg/d. Men taking supplements received 29 µg/d and women 25 µg/d, clearly exceeding the recommendations (Helldan et al. 2013). A recent review underlines food fortification as a useful way of improving the vitamin D status of a population and highlights the need to re-evaluate food fortification practices globally as well (Cashman 2015).

Although vitamin D supplementation has resulted in a 20-fold increase in S-25(OH)D levels above 125 nmol L⁻¹ in 10 years, vitamin D toxicity and hypercalcaemia are rare events (Dudenkov et al. 2015). This is not surprising, as the concentrations of both 25(OH)D and 1,25(OH)₂D are tightly regulated (Vieth 1999, Biancuzzo et al. 2013). A practical demonstration of the feedback system is the dose-response curve between vitamin D supplementation and S-25(OH)D concentration, which follows an exponential curve in healthy non-insufficient subjects, with a plateau effect at higher doses. On average, the S-25(OH)D concentration increases by 0.48 nmol L⁻¹ per 1 µg in the supplementation range 0–25 µg per day and by 0.04 nmol L⁻¹ per 1 µg in the range 375–500 µg per day (Ekwaru et al. 2014). Daily vitamin D doses of more than 1250 µg raise S-25(OH)D levels to over 375 nmol L⁻¹ (Holick 2007), but up to 500 µg can be taken daily without signs of toxicity or elevation of S-25(OH)D levels over 250 nmol L⁻¹ (Ekwaru et al. 2014), which is considered the upper limit of a normal concentration by the Institute of Medicine, the Endocrine Society and many laboratories (Holick 2015). Even though relatively high doses of vitamin D can be

taken without toxicity, the Institute of Medicine has suggested a daily upper intake level of 100 µg for subjects aged nine years or more, a dose which is not likely to cause any adverse effects in that age group (IOM 2011).

5.2.3 Vitamin D insufficiency and health

Vitamin D insufficiency

Vitamin D insufficiency is common world-wide (Wacker and Holick 2013), in Europe (Cashman et al. 2015) and in all the Nordic Countries (Lamberg-Allardt et al. 2013). A large pooled-data analysis showed that an average of 13.0% of all Europeans are deficient in vitamin D, i.e. they have S-25(OH)D < 30 nmol L⁻¹ and 40.4% have insufficient vitamin D with S-25(OH)D < 50 nmol L⁻¹ (Cashman et al. 2015). Correspondingly, Lamberg-Allardt et al. (2001) showed that S-25(OH)D levels in February and March were < 25 nmol L⁻¹ in over 25% of a cohort of 328 healthy Finnish volunteers, while Välimäki et al. (2004) reported that 38.9% of healthy men recruited into the Finnish Army had S-25(OH)D < 20 nmol L⁻¹ in January 2001. As a result of the new vitamin D fortification policy introduced in 2003, wintertime S-25(OH)D levels in young military conscripts increased, and the prevalence of values < 40 nmol L⁻¹ decreased from 78% to 35% (Laaksi et al. 2006). On the other hand, Välimäki et al. (2007) reported that there were still 73.8% of young men who had January S-25(OH)D levels < 37.5 nmol L⁻¹ even under the new fortification policy, and Lehtonen-Veromaa et al. (2008) reported that 65.5% of adolescent females had S-25(OH)D < 50 nmol L⁻¹. Similarly, Vähävihu et al. (2010c) showed that 77% of women had S-25(OH)D < 50 nmol L⁻¹ in mid-winter.

A recent Danish study showed that about 90% of adolescent girls had S-25(OH)D levels $< 50 \text{ nmol L}^{-1}$ during the winter and 20% during the summer, the respective findings for older women being about 50% and 20%. The authors stated that a summertime S-25(OH)D level of about 100 nmol L^{-1} is required to avoid vitamin D insufficiency in winter (Andersen et al. 2013). In this context, an Icelandic study showed that adults not taking vitamin D supplements, including cod liver oil, had S-25(OH)D levels $< 50 \text{ nmol L}^{-1}$ throughout the year and reached a mean high of 45 nmol L^{-1} during the summer (Steingrimsdottir et al. 2005). Adequate S-25(OH)D levels were reported at a high latitude (63°N) in a recent Swedish study, where 79.2% of the subjects were sufficient in vitamin D. This was thought to result from successful vitamin D supplementation and high consumption of fatty fish (Ramnemark et al. 2015). Another Swedish study showed that 50% of healthy subjects had S-25(OH)D $< 50 \text{ nmol L}^{-1}$ for 50% of the year (Klingberg et al. 2015). The lowest S-25(OH)D levels in subjects living at high latitudes are consistently measured between February and April (Edvardsen et al. 2007, Brustad et al. 2007, Datta et al. 2012, Klingberg et al. 2015).

Health effects of vitamin D

Since 90% of our vitamin D is produced through cutaneous photosynthesis (Reichrath 2006), subjects who fail to expose their skin to sun on account of illness or frailty run a risk of vitamin D deficiency, the classical manifestation of which is osteomalacia, the failure to mineralize newly-formed bone (Elder and Bishop 2014). Osteomalacia in children is known as rickets, the aetiological role of vitamin D deficiency in the development of which had already been established in the early twentieth century (Rajakumar et al. 2007). Strong clinical evidence exists for the use of vitamin D to treat and prevent osteomalacia in

both adults and children (Elder and Bishop 2014). The agreed cut-off point, at which the likelihood of rickets increases in the UK is S-25(OH)D < 25 nmol L⁻¹ (Elder and Bishop 2014).

The increased availability of assays for S-25(OH)D has resulted in its measurement in connection with several other health conditions, and consequently low levels have been reported to co-exist with certain of these, as reviewed by Reid (2016). The most obvious explanation is that subjects who are unwell for a physical or psychological reason spend less time in outdoor physical activities, which can provide an association with vitamin D insufficiency without any true causality (Reid 2016).

As a fat-soluble hormone, vitamin D is volumetrically diluted in the adipose tissue and thus S-25(OH)D levels are reduced in obese subjects (Drincic et al. 2012). A recent systematic review and metaregression analysis showed that a weight loss of 10 kg will result in an S-25(OH)D increase of 6.4 nmol L⁻¹, whereas a 10 kg loss of fat mass will result in an increase of 10.5 nmol L⁻¹. The authors also stated that the increase in S-25(OH)D levels would have been higher if volumetric dilution had been the only mechanism behind the low S-25(OH)D levels. Thus, a sequestration effect and the degradation of 25(OH)D to inactive metabolites could not be excluded (Pannu et al. 2016). There is strong evidence that vitamin D insufficiency is a consequence of obesity rather than a cause, as vitamin D supplementation does not reduce adiposity (Pathak et al. 2014). A recent Mendelian randomization study has shown that low S-25(OH)D levels are not genetically associated with an increased risk of type 2 diabetes, suggesting no causal relationship (Ye et al. 2015).

Because of volumetric dilution, low S-25(OH)D levels can be seen in conditions associated with obesity such as type 2 diabetes, hypertension, chronic kidney disease and cardiovascular disease (Nakashima et al. 2016). To date vitamin D

supplementation has not been shown to improve glycaemic control or lower insulin resistance in patients with type 2 diabetes, while evaluations of vitamin D supplementation in cases of chronic kidney disease have pointed to conflicting results or no effects at all, and further studies are warranted to assess the improvement in terms of hard end points (Nakashima et al. 2016). A recent systematic review by Papandreou and Hamid (2015) concludes that vitamin D status in the 'sufficient' range entails no harm in the case of diabetes patients, and that vitamin D consumption within the normal range may be considered beneficial. As regards cardiovascular disease, the effect of vitamin D on stroke risk, blood pressure, arterial stiffness and carotid intima and media thickness provided equivocal results, as reviewed by Papandreou and Hamid (2015).

A trial sequential meta-analysis recently performed to investigate the effect of vitamin D supplementation, with or without calcium, on the risk of cerebrovascular diseases, cancer, total fracture, hip fracture and mortality, in which a risk reduction threshold of 5% was used for mortality and 15% for the other end points, indicated on the basis of its pooled analyses that vitamin D supplementation could not provide a risk reduction greater than the threshold in terms of any of the end points. Vitamin D supplementation with calcium was shown to reduce the incidence of hip fracture by 16%, but the benefits were restricted to elderly institutionalized patients with low baseline S-25(OH)D concentrations and calcium intakes and signs of secondary hyperparathyroidism. The total risk of a fracture and the risk of a hip fracture were not reduced in community-dwellers (Bolland et al. 2014a). Snellman et al. (2014) followed 61 433 middle-aged and elderly Swedish women for 19 years and found no correlation between vitamin D intake, the first fracture event, a hip fracture or osteoporosis. The authors stated that theoretically too few women might have had a dietary vitamin D intake low enough to influence their

fracture risk. The patients were mostly vitamin D sufficient, as the mean 25(OH)D level was 52.9 nmol L⁻¹ even in the lowest vitamin D intake quintile (< 2.5 µg/d) (Snellman et al. 2014).

In agreement with Bolland et al. (2014a), vitamin D supplementation has not been shown to reduce the risk of developing breast cancer (Sperati et al. 2013), nor has vitamin D receptor polymorphism been linked to an increased risk (Lu et al. 2016). Neither has vitamin D status been seen to play a causal role in disease progression or mortality in prostate cancer patients (Trummer et al. 2016). On the other hand, vitamin D receptor gene polymorphism has been linked to an increased melanoma risk (Zeljic et al. 2014) and vitamin D deficiency at the diagnosis of melanoma has been shown to be associated with higher Breslow thickness (Wyatt et al. 2015).

The Nordic Nutrient Recommendations for vitamin D intake in the elderly are based on its convincing protective effect on bone health and total mortality, and its potentially protective effect on the risk of falling (NORDEN 2012). While the benefits of vitamin D supplementation in preventing osteomalacia are clear, the protective effects on total mortality and the risk of falling require verification in a large body of pooled data. Several meta-analyses of randomized controlled trials have been performed to demonstrate the reduction in total mortality. In a Cochrane meta-analysis of 56 trials in which the majority of the subjects were older women and the mean duration of treatment was 4.4 years, vitamin D₃ supplementation reduced the total mortality by 6%. It was calculated that 150 individuals have to be treated for over 5 years to prevent one death (Bjelakovic et al. 2014). The analysis by Rejnmark et al. (2012) found that vitamin D with or without calcium reduced the total mortality by 7%, but vitamin D alone did not show any protective effect. A traditional meta-analysis by Bolland et al. (2014a) demonstrated a 4% decrease in the total mortality risk, but the trial sequential

meta-analysis suggested that there remained some uncertainty regarding the significance of the finding.

Concerning the risk of falls, the most comprehensive meta-analysis, including 20 randomised controlled trials and 29535 subjects, has been that performed by Bolland et al. (2014b). Here their traditional meta-analysis found no effect of vitamin D supplementation on the risk of falls, with or without calcium, and the trial sequential analysis provided reliable evidence that vitamin D supplementation does not alter the relative risk of falls by as much as 15%. The analysis was repeated using a risk reduction threshold of 10%, with no change in the results. Most trials included in the analyses had baseline S-25(OH)D levels below 50 nmol L^{-1} , and most had achieved levels greater than 50 nmol L^{-1} following supplementation. The authors concluded that further clinical trials to determine the effect of vitamin D supplements on falls are difficult to justify, but adults at risk of osteomalacia should in any case be treated with vitamin D supplements. Uusi-Rasi et al. (2015) recently determined the effectiveness of exercise training and vitamin D in home-dwelling women aged 70–80 years. Neither vitamin D supplementation nor exercise affected the rate of falling, but the hazard ratio for an injurious fall decreased in the exercise group.

Vitamin D supplementation has recently been shown to raise vitamin D receptor gene expression in human muscle tissue biopsies (Pojednic et al. 2016). In a review by Tomlinson et al. (2015) vitamin D supplementation ranging from $100 \mu\text{g}$ per day to $1500 \mu\text{g}$ per week and lasting for periods of 4 weeks to 6 months was shown to improve muscle strength in healthy adults aged 18 to 40 years.

The role of vitamin D in neurological conditions has recently been reviewed by Mpandzou et al. (2016). Vitamin D has an immunomodulatory role due to its anti-inflammatory and anti-autoimmune actions. In the nervous system, it is

involved in the regulation of calcium-mediated neuronal excitotoxicity, in the reduction of oxidative stress and in the induction of synaptic structural proteins, neurotrophic factors and deficient neurotransmitters, and its deficiency has been shown to be associated with multiple sclerosis, amyotrophic lateral sclerosis, Parkinson's disease and Alzheimer's disease (Mpandzou et al. 2016). Randomized controlled trials would now be required to show a true causal link. Such a trial was performed on multiple sclerosis patients by Åivo et al. (2012), who reported a reduction in the number of T1 enhancing lesions and slower T2 enhancing lesion growth in the vitamin D supplementation group. Clinical improvement scales also favoured this group. The sample size was too small, however, to allow assessment of the impact of vitamin D on clinical measures of disease activity, progression, and function (Åivo et al. 2012). As for the other neurological disorders mentioned previously, no causal link has yet been shown (Mpandzou et al. 2016).

In a review by Li et al. (2014) covering six randomized controlled trials, no effect of vitamin D supplementation on depression scores was found. A meta-analysis by Spedding (2014) found significant improvements in depression scores following vitamin D supplementation, and supplements of ≥ 20 μg daily were considered beneficial in studies that showed a change in vitamin D levels and compared this effect with that of anti-depressant medication. In the meta-analysis of Gowda et al (2015), which included nine trials with a total of 4923 participants, vitamin D supplementation was not shown to affect depression scores, but most of the subjects had sufficient vitamin D, accompanied by mild depression. It was concluded that further trials using depressed vitamin D-deficient individuals were warranted.

The effect of vitamin D intake during early life on the risk of developing type 1 diabetes was assessed in a meta-analysis by Dong et al. (2013) covering two

cohort studies and six case-control studies, and the effects of maternal vitamin D intake were also assessed through two cohort studies and one case-control study. The pooled odds ratio for type 1 diabetes was 0.71 in infants receiving supplementation as compared with those not doing so. The cohort subgroup analysis nevertheless failed to confirm the results. No association between maternal vitamin D intake and a risk of type 1 diabetes in the offspring was found. Thus, no causal link could be confirmed between vitamin D intake and type 1 diabetes.

Some studies have suggested that vitamin D supplementation may reduce the risk of respiratory tract infections in young healthy adults and adolescents, but inconsistency remains between the results with regard to other infectious diseases (Kearns et al. 2015).

5.2.4 25(OH)D measurement techniques and target groups

Circulating 25(OH)D is the best indicator of vitamin D status (Holick 1981). Two metabolites are present, 25(OH)D₃, derived mainly from photosynthesis in the skin, and 25(OH)D₂, derived from plants in the diet. Both can also be present due to vitamin D₂ or D₃ supplementation (Lehmann and Meurer 2010). In the early 1970s a method based on competitive protein binding was developed for measuring 25(OH)D and later HPLC became available. Radioimmunoassay (RIA) was developed in 1985, but has mostly been replaced by chemiluminescent or enzyme immunoassays to avoid handling radioactive labels. Immunoassay kits can be manual, such as the Immunodiagnosics Systems RIA (Immunodiagnosics Systems Ltd., Boldon, UK), or automated, such as the electrochemiluminescent

immunoassay (ECLIA) Elecsys Vitamin D Total (Roche Diagnostics, Mannheim, Germany).

Two methods using direct non-immunological detection are available, high performance liquid chromatography (HPLC) and liquid chromatography/tandem mass spectrometry (LC-MS/MS), which is the most advanced method and is able to differentiate concentrations of 25(OH)D₂ and 25(OH)D₃. In HPLC a chromatographic separation is followed by detection methods based on a UVR or electrochemical detector. The procedure is called LC-MS/MS when mass detectors are being used, and the only comparable method is HPLC, whereupon the former is used as a reference method (Roth et al. 2008, Wallace et al. 2010, DEQAS 2014).

The development of evidence-based guidelines has largely been confounded by the widespread variation to be found in 25(OH)D measurements (Sempos et al. 2012). To increase the standardization of measurement methods, the Vitamin D Standardization Program was established in 2010 by the National Institute of Standards and Technology (NIST) (Gaithersburg, MD, USA) with the aim of establishing a reference procedure for 25(OH)D measurements and assisting investigators in calibrating past measurement data against the reference material provided by NIST. Isotope dilution LC-MS/MS was defined as the reference (Simpson et al. 2015).

An easier and less costly way for laboratories to increase their standardization of 25(OH)D measurements is the Vitamin D External Quality Assessment Scheme (DEQAS), which has been monitoring 25(OH)D assay performance since 1989 (Carter et al. 2010, DEQAS 2014). The reference values are provided by NIST, using the reference procedure. Five serum samples are distributed quarterly to the participant laboratories, which then submit their results to DEQAS for comparison against the NIST reference values. Based on the pooled results from

laboratories using the same analytical method, an All-Laboratory Trimmed Mean with standard deviation (SD) is calculated. The primary aim of DEQAS is to monitor the performance of individual laboratories, but the data can also be used to assess the performance of the assessment methods. DEQAS has indeed succeeded in reducing the variability in 25(OH)D measurements (Carter et al. 2010, Simpson et al. 2015).

Even though several novel 25(OH)D analysis methods exist, some laboratories still use traditional techniques such as the IDS RIA, which can be used to quantify the total 25(OH)D concentration in serum or plasma. 25(OH)D is extracted from a sample and a calibrator using two reagents, centrifuged and incubated with ¹²⁵I-labelled 25(OH)D (tracer) and sheep 25(OH)D antibody. Antisheep IgG cellulose is used to separate the antibody-bound tracer from the free tracer. The bound radioactivity is then inversely proportional to the 25(OH)D concentration (Wallace et al. 2010). The manufacturer of the kit suggests recoveries of 100% for 25(OH)D₃ and 75% for 25(OH)D₂, while recovery rates of 92–95% for 25(OH)D₃ and 21–29% for 25(OH)D₂ were reported by Hollis (2000). The manufacturer reports within and between-batch precisions of < 6.1% and < 8.2% for 25(OH)D₃ and 25(OH)D₂ (Wallace et al. 2010), while Hollis (2000) reports < 10% for both. Roth et al. (2008) reported a bias of –15% compared with LC-MS/MS, while Carter et al. (2004) reported a bias of –5% compared with the DEQAS All-Laboratory Trimmed Mean.

A new automated ECLIA method, Elecsys Vitamin D Total (Roche Diagnostics, Mannheim, Germany), was introduced in 2011. This uses recombinant vitamin D binding protein to measure both 25(OH)D₂ and 25(OH)D₃. The 25(OH)D in the sample is dissociated from its binding protein, the sample is incubated with ruthenium-labelled vitamin D-binding protein, which binds the 25(OH)D. Biotinylated 25(OH)D and streptavidin-coated microparticles are added to form

a complex with the free sites of the ruthenium-labelled vitamin D-binding protein, and the 25(OH)D concentration is measured using electrochemiluminescence (Emmen et al. 2012, Wielders et al. 2015). The method has shown a within-run variation $\leq 7\%$, a within-laboratory variation $\leq 9.5\%$, good correlation with HPLC ($r = 0.91$) and good correlation with LC-MS/MS ($r = 0.93$) (Emmen et al. 2012, Wielders et al. 2015, Kocak et al. 2015).

Since the therapeutic range of vitamin D supplementation is wide, allowing safe dosing up to 100 $\mu\text{g}/\text{d}$ (IOM 2011), there is generally no definite need for S-25(OH)D testing in the case of healthy subjects who use adequate supplementation. Measurements are justified in patients who have been diagnosed with a disease that is a consequence of vitamin D deficiency, such as rickets, osteomalacia or osteoporosis (Souberbielle et al. 2012), and patients with a risk of developing vitamin D insufficiency because of a chronic disease, the medication used to treat a chronic disease, or certain surgical procedures should also be monitored for S-25(OH)D. These include patients with coeliac disease, Crohn's disease, a gastric bypass, chronic kidney disease, hepatic failure or primary hyperparathyroidism, and patients receiving oral glucocorticoids. S-25(OH)D should be measured in patients with symptoms of severe vitamin D deficiency or toxicity, such as diffuse pain, extraskeletal calcifications, nephrocalcinosis or recurrent renal stones. In general, S-25(OH)D should always accompany serum parathyroid hormone measurement, because of their close interaction (Souberbielle et al. 2012). S-25(OH)D should be measured at baseline and after 3-4 months of vitamin D supplementation. If S-25(OH)D is measured in healthy subjects, the best time is between February and April, when the seasonal S-25(OH)D level is lowest. Supplementation should aim at a S-25(OH)D level over 50 nmol L^{-1} in that time period, ensuring vitamin D sufficiency around the year.

5.3 Psoriasis and atopic dermatitis

5.3.1 Psoriasis

Psoriasis is a common immune-mediated chronic disease manifested in the skin and joints (Boehncke and Schön 2015). Its prevalence in adults varies between 0.91 and 8.5% in the western countries (Parisi et al. 2013). The clinical hallmark features of *psoriasis vulgaris*, accounting for 90% of all cases, include well-delineated, typically palm-sized papulosquamous plaques that are red or salmon pink in colour and usually scaly. An acute form known as *psoriasis guttata* presents itself as small fingertip-sized papules usually following a β -haemolytic streptococcal or viral infection. Erythrodermic psoriasis, i.e. psoriasis affecting more than 70% of the total body surface area, is a rare and potentially life-threatening form (Griffiths and Barker 2007), while pustular psoriasis, which can be either generalized or localized to the palmar and plantar areas (Hallopeau psoriasis), is also a rare and a more severe form and is considered a distinct entity from the other forms (Boehncke 2015). Inverse psoriasis is a site-specific variant occurring in flexural and intertriginous areas (Boehncke 2015). Data in the literature suggest that 5.8–30 % of psoriasis patients also have psoriatic arthritis (Henes et al. 2014). Psoriasis has been shown to be associated with an increased risk of metabolic syndrome, the risk being closely associated with the Psoriasis Area and Severity Index (PASI) score (Salihbegovic et al. 2015).

The pathomechanisms of psoriasis are multifactorial, and genetic susceptibility plays an important role. Psoriatic plaques originate from dysregulated interaction between the innate and adaptive immune systems, and cytokines such as nuclear factor- κ B, interferon- γ , and interleukin-23 are the key drivers of inflammation (Boehncke 2015). Chronic systemic inflammation predisposes

psoriasis patients to several co-morbidities, such as psoriatic arthritis, cardiovascular disease and depression (Oliveira et al. 2015).

The physical and psychological burdens of psoriasis are considerable and are comparable to those of cancer, myocardial infarction or congestive heart failure (Rapp et al. 1999). A negative impact on the HRQoL has been reported in 6497 Nordic patients with psoriasis (Zachariae et al. 2002), and the HRQoL impairment has been shown to correlate with the extent of the psoriasis (Uttjek et al. 2004, Gelfand et al. 2004, Grozdev et al. 2012). Skin pain, discomfort and pruritus are significantly related to HRQoL, often mediated by sleep disturbances (Ljosaa et al. 2010, Zhu et al. 2014). It has also been observed that psoriasis particularly affects the emotional and social functioning aspects of HRQoL (Wahl et al. 2000, Weiss et al. 2002).

Visible lesions can cause feelings of stigmatization and troubles in establishing social relationships (Hrehorow et al. 2012), and the psoriasis-related burden clearly impairs relationships with family members and partners, who report life quality impairment due to extra housework, psychological issues, social disruption and their personal relationships with the patient (Eghlileb et al. 2007). HRQoL in relation to sexual health is diminished practical terms in patients with genital lesions (Meeuwis et al. 2011).

Psoriasis impairs social functioning and causes distress at work, leading to reduced work efficiency in the form of presenteeism and absenteeism (Gaikwad et al. 2006, Mattila et al. 2013, Armstrong et al. 2012, Mustonen et al. 2015). Half of all patients have limited career expectations (Ayala et al. 2014) and disease severity has a negative impact on income (Hawro et al. 2015). In addition, a poorer HRQoL is associated with increased use of healthcare resources independently of disease severity (Sato et al. 2011). The cumulative effect of all the consequences of psoriasis may result in failure to achieve a 'full

life potential' (Kimball et al. 2010). Personal resources, adequate coping strategies and disease acceptance correlate with a better HRQoL, and thus patient education and psychomedical interventions should be developed (Wahl et al. 1999, Miniszewska et al. 2013).

Psoriasis tends to worsen in the winter and improve in the summer, as observed in a large sample survey based on the numbers of visits to clinics for various dermatological conditions between 1990 and 1998 (Hancox et al. 2004). Later Balato et al. (2013) found in a questionnaire that 70% of patients reported an improvement in psoriasis during the summer and 13% a worsening, whereas 60% reported worsening during the winter and 1.6% an improvement. Recently Pascoe and Kimball (2015) used the Physician's Global Assessment (PGA) to show the same trend by means of an objective assessment.

The treatment for psoriasis consists of topical therapies, phototherapy, established systemic drugs and biological drugs. The most effective and feasible topical therapies include class III–IV corticosteroids, vitamin D₃ derivatives and also calcineurin inhibitors for facial areas, flexure areas and paediatric use. Established systemic drugs include cyclosporine, methotrexate, fumarate and retinoids. Injectable biological drugs can be used for severe psoriasis if established drugs fail or are contraindicated. Phototherapy with various UVB or PUVA regimens, or heliotherapy, can be used together with topical therapies and retinoids to treat moderate-to-severe psoriasis (Nast et al. 2012).

5.3.2 Atopic dermatitis

Atopic dermatitis (AD) is the most common inflammatory skin disease, with a world-wide prevalence in children of 0.3–20.5%, the highest rates being recorded in Northern Europe (Beasley et al. 1998). Its clinical features vary with age. Infants show pruritic papules and vesicles on the cheeks, forehead and scalp, while in childhood there are lichenified papules and plaques involving the hands, feet, wrists, ankles and popliteal regions. In adulthood, it is especially flexural folds, the face and neck and the upper arms and back that are involved. The lesions are characterized by dry, scaling erythematous papules and plaques and by chronic lichenified plaques (Akdis et al. 2006). Exacerbations of varying duration and severity are typical, but spontaneous cure is also possible at any time. Nevertheless, at least 30% of children with AD suffer from eczema in adulthood, at least occasionally (Werfel et al. 2016).

AD is a chronic disease with numerous predisposing factors and a complex pathophysiology. Microbial factors associated with it include a large number of siblings, infections, the use of antibiotics, vaccinations and exposure to animals. Non-microbial factors include genetics, tobacco smoke and stress (Torres-Borrego et al. 2008). The most profound patient-related pathogenic factor is skin barrier dysfunction, and thus the strongest single-gene defect related to AD is a mutation in the filaggrin gene, which encodes an important epidermal barrier protein, profilaggrin (Irvine et al. 2011). The immunological mechanisms behind AD include infiltration of T-cells, dendritic cells, macrophages, mast cells and eosinophils and also various cytokines and chemokines (Peng and Novak 2015). A chronic pruritic skin disease which by nature is highly visible will inevitably be a stressful condition and one that causes anxiety and impairs the patient's

HRQoL (Linnet and Jemec 1999, Lundberg et al. 1999). The impact of AD is reflected particularly in social functioning and psychological aspects of the HRQoL (Kiebert et al. 2002, Coghi et al. 2007). Atopic dermatitis in children affects the HRQoL of the patient and of the whole family (Gånemo et al. 2007), while in couples AD has a negative influence on their sex life (Misery et al. 2007). The decrease in HRQoL correlates with the severity of the AD symptoms (Kim et al. 2012, Sanchez-Perez et al. 2013). In addition to detracting from the HRQoL, AD also imposes a financial burden on the patient and the health care system (Fivenson et al. 2002).

Dry skin and mild AD symptoms can be treated with topical emollients and glucocorticosteroids, and topical calcineurin inhibitors can also be used in cases of intolerance and for application to the facial and genital areas. Topical calcineurin inhibitors can also be resorted to if the symptoms tend to relapse or if topical corticosteroids fail to elicit a sufficient response. Moderate symptoms can be treated with more potent glucocorticosteroids and severe symptoms with systemic immunomodulatory therapy, e.g. cyclosporine A. Also, a new biological drug, dupilumab, has proved effective in clinical studies. Phototherapy such as UVB, PUVA and heliotherapy can be used together with topical glucocorticosteroids (Werfel et al. 2016).

5.3.3 Assessment of disease activity and quality of life

Assessment of disease activity

Specific tools have been developed for assessing disease activity in psoriasis and AD patients. In the systematic review by Puzenat et al. (2010) six psoriasis severity assessment tools were considered to meet the methodological validation and quality criteria, but none of them was considered ideal. The Psoriasis Area and Severity Index (PASI) was deemed the most extensively studied and validated, however, and was recommended as the first choice for assessing the severity of psoriasis (Puzenat et al. 2010). PASI was developed by Fredriksson and Pettersson in 1978 to serve as a subtler instrument for the assessment of psoriasis than global evaluation (Fredriksson and Pettersson 1978), but as its measurement is time-consuming and requires trained personnel, its suitability for large epidemiological studies is limited. For that reason, Fleischer and Feldman developed a structured PASI-like instrument to enable patients to self-evaluate the severity of their psoriasis. This new instrument was named the Self-Administered Psoriasis Area and Severity Index (SAPASI) and its scores were found to correlate well with PASI scores (Fleischer et al. 1994, Feldman et al. 1996).

There are currently sixteen instruments available for measuring the clinical signs of AD, of which only the SCORing Atopic Dermatitis (SCORAD) and the Eczema Area and Severity Index (EASI) have shown adequate validity, responsiveness, reliability and interpretability (Schmitt et al. 2013). The European Task Force on Atopic Dermatitis (ETFAD) developed the SCORAD index in 1992 to assess the severity of AD and to provide better management of

patients. The instrument has then been validated in several clinical studies (European Task Force on Atopic Dermatitis 1993, Kunz et al. 1997, Ricci et al. 2009). SCORAD is a doctor-based evaluation method, however, and, in view of the varying nature of the disease, clinical assessment is often insufficient to evaluate its course. A self-assessment scale was developed by the ETFAD to improve disease monitoring and communication between the patient and doctor. This patient-oriented SCORAD (PO-SCORAD), which uses the same assessment criteria as SCORAD and comes with illustrations and instructions, has been validated for use in a large European study (Stalder et al. 2011).

The visual analogue scale (VAS) is a quick unidimensional tool for measuring subjective phenomena and can be used to assess global disease severity in psoriasis and AD patients (Holm et al. 2006, Flytström et al. 2012). It has been shown to have the highest correlation with most of the HRQoL assessments used in dermatology (Holm et al. 2006) and has also been validated for measuring pruritus in pruritic dermatoses (Reich et al. 2012).

Assessment of health-related quality of life

There are generic, dermatology-specific and disease-specific instruments available for measuring HRQoL. The generic instruments can be used to measure HRQoL in all kinds of diseases, whereas the dermatology-specific instruments assess those aspects of HRQoL that are particularly related to skin diseases. Disease-specific instruments can give an insight into particular skin diseases and detect more specific aspects of HRQoL (Prinsen et al. 2013).

The most common dermatology-specific measure is the Dermatology Life Quality Index (DLQI) (Finlay and Khan 1994, Basra et al. 2008), which includes six subscales: symptoms and feelings, daily activities, leisure, work and school,

personal relationships and treatment satisfaction. The total score, ranging from 0 to 30, is based on ten questions, a higher score indicating greater HRQoL impairment. The scores are categorized into bands (Basra et al. 2015) demonstrating the HRQoL impact: 0–1, ‘no effect at all’; 2–5, ‘small effect’; 6–10, ‘moderate effect’; 11–20, ‘very large effect’; 21–30, ‘extremely large effect’. An improvement of 4 points is considered to represent a minimal clinically important difference (MCID) (Basra et al. 2015).

The generic measure RAND-36 (Hays et al. 1993, Hays and Morales 2001) is a widely used 36-item generic HRQoL measure encompassing eight subscales: physical function, physical role, bodily pain, general health perceptions, social function, emotional role, vitality and mental health. These are presented as units on a 0–100 scale, a higher score indicating a better HRQoL. The physical and mental domains can be combined and converted to norm-based T-scores, i.e. summary scores with a mean of 50 and an SD of 10. The physical component summary (PCS) score is calculated from physical function, physical role, bodily pain and general health, whereas the mental component summary (MCS) score is derived from the vitality, social function, emotional role and mental health subscales.

5.3.4 Ultraviolet radiation phototherapy

Artificial phototherapy

Phototherapy for psoriasis began in 1925, when Goeckerman introduced a treatment involving a combination of sunlight and tar (Goeckerman 1925). In 1948 Nexman reported on the beneficial effect of treating atopic dermatitis (AD) with UVR emitted by carbon arc lamps (Patrizi et al. 2015) and later Ingram combined dithranol with UVB and tar bathing as a treatment for psoriasis (Ingram 1953). Fischer & Alsins (1974) and Parrish & Jaenicke (1981) showed that wavelengths of around 311 nm are in theory the most effective for treating psoriasis and cause fewest cases of erythema. A fluorescent bulb (TL-01), emitting a major peak at 311 (± 2 nm) and a minor peak at 305 nm, was developed and the new modality, termed narrowband ultraviolet B (NB-UVB), was shown to be more effective than BB-UVB for treating psoriasis (van Weelden et al. 1988, Green et al. 1988, Karvonen et al. 1989, Picot et al. 1992). Today NB-UVB is considered an efficacious and cost-effective first line of treatment for psoriasis (Racz and Prens 2015) and has remained a cornerstone of psoriasis treatment even in the era of biological drugs (Richard and Hönigsmann 2014). It is typically given three times a week (Dawe et al. 1998) and usually 50–75% of patients achieve a 75% reduction of PASI score in four to six weeks (Nast et al. 2012). In the case of atopic dermatitis NB-UVB represents a valid second-line treatment together with topical therapies. A SCORAD improvement of over 50% can be expected when NB-UVB is administered three times a week for up to 12 weeks (Patrizi et al. 2015). A beneficial effect of NB-UVB on the HRQoL has been

demonstrated in both psoriasis and AD patients (Lim and Brown 2006, Al Robaee and Alzolibani 2011, Darné et al. 2014).

The antipsoriatic effect of NB-UVB results from its suppression of inflammatory IFN- γ and IL-17 signalling pathways (Johnson-Huang et al. 2010, Rácz et al. 2011), and theoretically from the antiproliferative effect of 1,25(OH) $_2$ D on epidermal keratinocytes (Lehmann et al. 2007). It has been debated whether NB-UVB phototherapy alleviates psoriasis via systemic as well as local pathways (Dawe et al. 2002, Gibbs 2003). Evidence of the systemic effects was presented by Milliken et al. (2012), who showed that NB-UVB can reduce systemic immune responses by inducing regulatory T cells.

The effect of UVB in alleviating symptoms of AD is based on its immunosuppressive effects, including apoptosis of infiltrating T-cells and inhibition of the antigen-presenting function of Langerhans cells (Koulu et al. 1985, Patrizi et al. 2015). Other mechanisms of action include thickening of the stratum corneum, antibacterial effects, the reduction of superantigens and alteration of mRNA levels of antimicrobial peptides (Patrizi et al. 2015).

While the increased risk of skin cancer attached to PUVA is well-known, the long-term effects of NB-UVB remain to be seen (Archier et al. 2012). No evidence of any increased skin cancer risk was found in a German study with 195 psoriasis patients treated with BB-UVB or NB-UVB (Weischer et al. 2004), and early follow-up data showed no such evidence in either 484 Irish or 1908 Scottish patients treated with NB-UVB (Man et al. 2005, Black and Gavin 2006). Similarly, no association between NB-UVB and skin cancer was found in a British study involving 3867 patients (Hearn et al. 2008), although only 352 patients received over 100 treatments and for some patients the follow-up time was short. As it usually takes decades to develop skin cancer, a longer follow-up time would be warranted to study the safety of artificial phototherapy (Hearn et al. 2008, Patel

et al. 2009, Archier et al. 2012). On the other hand, no safe limits can be given for phototherapy because of the wide variation in individual responses to UVR (Tijoe et al. 2003, Dawe 2010). Both UVB and UVA radiation cause DNA mutations, and UVA wavelengths promote photoageing. NB-UVB and BB-UVB appear to be similar in their mutagenic potential (Tijoe et al. 2003, Snellman et al. 2003).

Heliotherapy

The Finnish Psoriasis Association and the Finnish Central Organisation for Skin Patients have been arranging heliotherapy (HT) courses for patients with psoriasis and AD in the Canary Islands since the 1980s. The patients are referred by a dermatologist, and trained nurses provide guidance on sunbathing and skin care during the course. Snellman et al. (1993) studied the effects of a four-week course of HT on 361 Finnish psoriasis patients, and found it to alleviate the severity of the disease by at least 75% in 84% of the patients as measured in terms of the Psoriasis Severity Index. HT was also shown to be cost-effective in cases of severe psoriasis (Snellman et al. 1998). The benefits of HT in the Canary Islands with regard to psoriasis symptoms have also been confirmed in three other studies (Mork and Wahl 2002, Wahl et al. 2005, Wahl et al. 2015). Autio et al. (2002) found, that a two-week HT course reduced the SCORAD index in 216 Finnish AD patients by 70% and that the disease severity three months afterwards was still 45% lower than initially. Vähävihi et al. (2008) showed that, in addition to alleviating the severity of AD, a two-week course of HT in winter increased the S-25(OH)D levels of these patients. HT administered in the Dead Sea area has also been found suitable for treating psoriasis (Harari et al. 2007) and AD (Harari et al. 2000, Adler-Cohen et al. 2012) throughout the year.

Heliotherapy raises some safety concerns. The median UVB dose received by a patient during two weeks of HT can be more than 100 SED (Vähävihi et al. 2008), and although a study of over 1000 psoriasis patients who received such a course at the Dead Sea did not show an increased skin cancer risk, they did show more signs of sun damage such as solar elastosis, poikiloderma and facial wrinkles than did the control patients (David et al. 2005). An increased risk of non-melanoma skin cancer was observed in a Danish cohort of 1738 psoriasis patients undergoing Dead Sea climatotherapy (Frentz et al. 1999).

6 Aims of the research

Even though wintertime vitamin D insufficiency has been common in Finland despite national efforts to increase its intake, there are no recent studies of the wintertime vitamin D status in healthy Finnish subjects. S-25(OH)D levels are typically lowest between February and April, but little is known as to whether they can be raised by vernal UVB radiation. The maintaining of an adequate vitamin D status throughout the year and the advancement of skeletal health in the population as a whole is thus a health care priority, and an NB-UVB radiation exposure protocol for maintaining S-25(OH)D levels is warranted. Heliotherapy is known to improve disease severity and HRQoL in dermatological patients, but the contents of heliotherapy courses have been revised and the new courses have not yet been properly studied. The aims of this thesis were:

- I. to study the effect of NB-UVB phototherapy on S-25(OH)D levels in psoriasis patients and healthy subjects receiving oral supplementations of 20 µg cholecalciferol daily,
- II. to examine whether regular NB-UVB exposures suffice to maintain S-25(OH)D levels during the winter in healthy subjects,
- III. to study the effect of vernal solar UV radiation on S-25(OH)D levels in healthy subjects at a high latitude in Finland, and
- IV. to assess the effects of empowering heliotherapy as performed in the Canary Islands on disease severity and the health-related quality of life in patients with psoriasis or atopic dermatitis.

7 Materials and methods

7.1 Patients and healthy subjects

Altogether 139 psoriasis patients, 72 atopic dermatitis patients and 77 healthy subjects participated in the trials reported in *papers I-V* of this thesis (Table II).

Table II. Demographic data for the subjects involved in the five papers making up this thesis (I-V) and the ultraviolet radiation (UVR) exposures to which they were submitted.

Paper	Subjects	Male / Female	Mean age, years (range)	Body mass index, kg m ⁻² , mean ± SD	Ultraviolet radiation modality	Cumulative mean UVB dose, SED (range)
I	Psoriasis (n = 12)	7/5	43 (20–61)	29.6 ± 5.4	NB-UVB Waldmann UV 7001	26 (19–28) ¹
	Healthy subjects (n = 15)	1/14	46 (19–62)	23.4 ± 3.8		92 (77–102) ²
						26 (19–28)
II	Healthy subjects (intervention) (n = 16)	3/13	35 (21–61)	23.0 ± 1.9	NB-UVB Waldmann UV 7002	25
	Healthy subjects (control) (n = 18)	1/17	36 (20–61)	25.2 ± 3.4		none
III	Healthy subjects (n = 25)	8/17	43 (22–71)	23.4 ± 4.0	Solar UVR	12 (2–23)
IV	Psoriasis (n = 22)	8/14	52 (34–68)	28.2 ± 6.2	Solar UVR	30 (22–39)
	Atopic dermatitis (n = 13)	0/13	44 (21–74)	27.1 ± 6.9		43 (3–54)
V	Psoriasis (n = 127)	57/70	51 (20–75)	N.D.	Solar UVR	N.D.
	Atopic dermatitis (n = 59)	9/50	37 (19–74)			

¹After the 9th exposure; ²After the 18th exposure

Paper I focused on a cohort of volunteers who received cholecalciferol supplementation. These included 12 psoriasis patients (mean age 43, range 20–61 years) and 15 healthy hospital employees (mean age 46, range 19–62 years; Table II) as controls. The inclusion criterion was avoidance of phototherapy, solarium or sunny holidays during the 2 preceding months. The patients had been taking 20 µg oral cholecalciferol daily for a mean of 3.3 months before the intervention and the controls for a mean of 3.4 months. Supplementation continued during the intervention and thereafter.

In *paper II*, focusing on maintenance UVB therapy, 37 healthy volunteers were randomized to an intervention group (mean age 35, range 21–61 years; n = 18) or a control group (mean age 36, range 20–61 years; n = 19; Table II). The inclusion criteria were age 18 years or older and avoidance of solarium visits, phototherapy, sunny holidays and vitamin D supplementation during a one-month washout period prior to the trial and during it. The exclusion criteria were pregnancy, skin disease, having had skin cancer, intake of photosensitizing drugs and Fitzpatrick's skin phototype I (Fitzpatrick 1988). Two intervention subjects were excluded for failing to follow the irradiation schedule and one control subject for taking oral cholecalciferol supplements. All three were excluded from the analyses.

Paper III, investigating the effect of vernal UVR, was based on a group of 27 healthy volunteers (mean age 43, range 22–71 years; Table II) who were able to spend time outdoors during working days, or who were going on a holiday in Lapland in spring. The other inclusion and exclusion criteria were as in *paper II*. Two volunteers were disqualified for failing to follow the exposure regimen. Ten subjects had been taking oral cholecalciferol prior to the one-month washout

period, and all of them ceased taking it at that point. The median dose had been 13 µg (range 5–25 µg) daily.

Paper IV is centred on a group of volunteer dermatological patients attending a 2-week EHT course in Puerto Rico, Gran Canaria, Spain. The inclusion criteria were a diagnosis of psoriasis or AD without any minimum severity requirement, age 18 years or older and a referral from a doctor, and the exclusion criteria were photosensitivity, Fitzpatrick's skin phototype I (Fitzpatrick 1988), use of photosensitising drugs, excessive alcohol consumption, drug abuse, severe cardiovascular disease, poorly controlled diabetes and mental disorders. Twenty-two psoriasis patients (mean age 52, range 34–68 years) and 13 atopic dermatitis patients (mean age 44, range 21–74 years; Table II) participated. Fifteen patients had psoriatic arthritis and 5 of them were taking biological drugs, in 3 cases in combination with methotrexate. A further two patients were receiving methotrexate as a monotherapy. These patients continued to use their previously prescribed topical and systemic medication during the EHT course, whereas the nineteen patients (14 psoriasis, 5 AD) who had been receiving oral cholecalciferol supplementation (mean 23 µg daily, range 5–50 µg) before the EHT course did not take it during the course or for the first 3 months after it.

The trial for *paper V* was arranged in the same manner and with the same entry criteria as that for *paper IV*, but instead of one heliotherapy course, twelve courses were provided. A total of 133 psoriasis patients (mean age 51, range 20–75 years) and 60 atopic dermatitis patients (mean age 37, range 19–74 years; Table II) participated, including those involved in *paper IV*. Sixty-seven of the psoriasis patients had psoriatic arthritis. Six patients in the psoriasis group and one in the AD group were excluded from the analyses, six due to inadequately

filled-in questionnaires and one who stayed in Puerto Rico for an additional week. Fifteen psoriasis patients were taking biological drugs, including 10 who were also taking methotrexate. Eighteen patients were receiving methotrexate as a monotherapy. Four patients were taking acitretin and one cyclosporine.

The ethics committee of Tampere University Hospital approved the study protocols (code numbers R11172, R13095, R12266, R12219; I-V) and all the participants gave their informed consent. The authors followed the principles of the Declaration of Helsinki.

7.2 Methods

7.2.1 Ultraviolet radiation exposures (I-V)

Narrow-band ultraviolet B radiation (I, II)

The NB-UVB exposures reported in *paper I* were performed from December 2011 to April 2012. Twelve psoriasis patients and 15 healthy subjects received whole-body NB-UVB exposures three times a week in a Waldmann UV 7001 cabin equipped with 40 TL01 tubes (Schulze & Böhm, Brühl, Germany). The dose was 0.19 J cm^{-2} (1.11 SED) on the first occasion and was increased each time up to the 9th exposure, i.e. to 0.97 J cm^{-2} (5.70 SED). In cases of itching or erythema the next dose was reduced, or at least not increased. This was the case in 6 psoriasis patients and 8 healthy subjects. Thereafter, the exposures were given only to the psoriasis patients, until their symptoms had almost or entirely disappeared. This took a mean of 20.5 (range 11–31) exposures.

The intervention group of 16 subjects monitored in *paper II* received 13 whole-body NB-UVB exposures, given every other week for 24 weeks with a Waldmann UV 7002 cabin equipped with 42 TL01 tubes (Schulze & Böhm, Brühl, Germany). Eighteen subjects acted as controls. The first physical NB-UVB dose was 0.17 J cm^{-2} (1 SED), and doses were then increased to 0.34 J cm^{-2} (2 SED). The cabin was calibrated by the Nuclear Safety Authority of Finland using an Ocean Optics S2000 spectroradiometer. After correction for stray light and other systematic errors, the estimated measurement uncertainty (2σ) of the Ocean Optics S2000 device is 14% (Ylianttila et al. 2005) and the measurements are traceable to the

NIST. Previously measured lamp spectra were used for the dosage calculations (Ylanttilla et al. 2005).

Solar ultraviolet radiation (III-V)

The monitored and scheduled solar UVR exposures for *paper III* were implemented in March and April 2013 and 2014 (Table III) for three groups in Sodankylä (67°N) and one group in Lahti (61°N). The 25 participants were instructed to expose their hands and face without using a sunscreen. Groups I and III were on their winter holiday and performed outdoor activities for several hours per day, while groups II and IV were exposed to solar UVR when walking outdoors for 1 hour daily at noon on working days and were encouraged to perform outdoor activities in their own time.

The sunbathing time in *papers IV* and *V* was divided equally between both sides of the body and varied according to Fitzpatrick's skin phototype (Fitzpatrick 1988), the disease group, the severity and the season. The first solar UVR exposure times ranged from 20–90 min for psoriasis and 15–30 min for AD patients and the time was increased to 90 min within a week, and even to 300 min for psoriasis and 120 min for AD in cases of skin phototype IV. The scheduled exposures took place in the mornings or afternoons without sunscreens. A sunscreen was made liberally available thereafter. Nurses were on hand at all times.

Table III. Ultraviolet radiation exposure locations, time periods, exposure instructions and average daily available doses ultraviolet B radiation.

Group	Location	Dates	Daily available UVB radiation dose (SED), mean (range)	Maximum UV index
I (n = 10)	Sodankylä, Finland (67°N, 26°E)	17.3. – 1.4.2013 ¹	5.5 (4.1 – 7.3) ^{3,4}	1.2
II (n = 5)	Sodankylä, Finland (67°N, 26°E)	7.4. – 17.4.2014 ²	9.6 (4.5 – 13.2) ³	2.3
III (n = 3)	Sodankylä, Finland (67°N, 26°E)	18.4. – 20.4.2014 ¹	13.0 (10.4 – 15.3) ³	2.7
IV (n = 7)	Lahti, Finland (61°N, 25°E)	2.4. – 12.4.2013 ²	12.1 (9.1 – 15.4) ⁵	2.7

¹Maximum exposure during holiday outdoor activities, ²Walking outdoors for 1 hour daily at noon on working days, ³NILU-UV measurements from FMI-ARC, ⁴Local Robertson-Berger meter, ⁵Spectroradiometer measurements from FMI, Jokioinen Observatory.

7.2.2 Empowerment methods (IV, V)

The focus of heliotherapy has traditionally been on sunbathing and topical treatment of psoriasis and AD, and while various empowering methods have been involved, they have not necessarily been systematically reported or analysed (Snellman et al. 1993, Autio et al. 2002, Mork and Wahl 2002, Vähävihi et al. 2008) until recently (Wahl et al. 2015). Patient education has been a recent focus of interest as a means of empowerment for the patients and to help them to cope with their skin disease. A review by de Bes et al. (2011) showed that such education is effective in improving the HRQoL of patients and reducing the perceived severity of the disease. Consequently, the role of adjunctive patient education during HT was strengthened in 2012, so that the current empowering heliotherapy (EHT) protocol consists of meeting peers and sharing experiences, adopting a healthy life-style, engaging in physical and mental exercise and attaining the ability to cope with a chronic skin disease. The staff of the EHT courses consists of nurses, physiotherapists and psychologists. This means that the outcomes may differ from those of traditional HT courses, and some

additional benefits have already been shown in the case of psoriasis patients (Wahl et al. 2015).

The two-week courses for psoriasis and AD patients referred to in *papers IV and V* were arranged by the Finnish Psoriasis Association (Psoriasisliitto) and the Finnish Central Organization for Skin Patients (Iholiitto ry) in Puerto Rico (27°N, 15°W), Canary Islands, Spain, between October 2012 and April 2013. They included an orientation day before EHT and a reunion weekend three months afterwards. The total cost per patient was €2450, of which the patient's own contribution was €400. *Paper IV* reports on a pilot study focusing on one course, while *paper V* assesses twelve courses, including that of *paper IV*.

The supporting programme during the EHT courses included teaching in self-management and a healthy life-style, group discussions and physical exercise for both groups of patient. Two nurses and two physiotherapists experienced in group leadership and rehabilitation were available for the psoriasis patients, and the empowering programme included interactive teaching on themes such as *psoriasis as a chronic disease (2h)*, *safe sunbathing (1h)*, *the benefits of sleep and rest (2h)*, *skin care and pain relief (2h)*, *nutrition and health (1h)* and *weight control (1h)*. Three mentored group discussions (5-6h) were arranged in which the patients could share their thoughts on living with psoriasis and their experiences of different treatment modalities, discuss the achieving of a healthy life-style and exchange tips for stopping smoking and coping with stress. Scheduled physical exercise (24h) included trekking, gymnastics and water sports.

A group leader, a nurse and a psychologist were available for the AD patients during the EHT courses, where the interactive teaching included themes such as *atopic dermatitis as a chronic skin disease (2h)*, *safe sunbathing (1h)*, *skin care and self-management (2h)*, *nutrition and health (1h)* and *information about*

patient organizations (1h). The group discussions with a psychologist (6h) included topics such as *mental well-being*, *psychosomatics* and *acceptance of a chronic skin disease*. The aim was to empower the patients, support self-care and increase general well-being. Physical exercise (11h) consisted of trekking, gymnastics and water sports.

7.2.3 Measurement of UV radiation doses (III, IV)

The vernal solar UVR dose administered to the subjects in *paper III* was measured using personal dosimeters (VioSpor Blue Line Type II, BioSense, Bornheim, Germany) attached to the upper arms or wrists with straps (Quintern et al. 1997, Thieden et al. 2000, Vähävihi et al. 2010b). Blue Line Type II dosimeters can detect UVB radiation doses ranging from 1.0 to 55 SED. Fourteen subjects in groups I-III in *paper III* had a personal dosimeter, and one subject in group IV wore a dosimeter to demonstrate the UV dose acquired by all those in the group (Table III).

The ambient solar UVR data were obtained from NILU-UV multichannel radiometer recordings (Høiskar et al. 2003) made at the Finnish Meteorological Institute's Arctic Research Centre (FMI-ARC) in Sodankylä or were measured locally using a Robertson-Berger meter (Solar Light Model 501 UV-meter s/n 635; Solar Light Co. Inc., Glenside, PA, U.S.A.) (Robertson 1968, Berger 1976). The NILU-UV radiometer was calibrated by Innovation Nilu AS by reference to the NIST (Gaithersburg, MD, U.S.A.) and was placed on the roof of the FMI-ARC sounding station to collect data as one-minute averages. The Robertson-Berger meter is calibrated annually by the Radiation and Nuclear Safety Authority, Helsinki, Finland, also by reference to NIST. Its calibration uncertainty (2σ) is 8%.

The meter was placed on a high roof near the UVR exposure area. As there were no ambient UVR measurements available for Lahti, use was made of UVR data obtained at Jokioinen (61°N) with the FMI's Brewer spectroradiometer (Lakkala et al. 2008), which was calibrated by reference to the MIKES-Aalto National Standards Laboratory. Comparisons with the European reference spectroradiometer have shown that discrepancies are less than $\pm 5\%$ (www.pmodwrc.ch/wcc_uv).

The personal UVB doses received by the patients during the EHT course in *paper IV* were also measured using personal dosimeters (VioSpor Blue Line Type III) (Quintern et al. 1997, Vähävihi et al. 2010b). The dosimeter was replaced after the first week to avoid saturation. Type III dosimeters detect doses ranging from 1.5 to 90 SED. The dosimeters were attached to the patients' upper arms or wrists with straps, and during sunbathing they were placed on towels beside the patients (Thieden et al. 2000). Eighteen psoriasis and 12 AD patients wore dosimeters. The ambient maximum solar UV irradiance was measured as a mean dose for 2 VioSpor Type III dosimeters at a time, the meters being put in an open place and replaced every second day to avoid saturation. The Spanish Agency of Meteorology (Agencia Estatal de Meteorología; www.aemet.es) supplied global solar UV irradiance data from the nearby Maspalomas C. Insular Turismo weather station (distance 15 km). The first EHT week was rainy and cloudy, but the second week was sunny. Maximum UV indices varied between 5 and 8.

7.2.4 Assessment of psoriasis and atopic dermatitis severity (I, IV, V) and health-related quality of life (IV, V)

The severity of psoriasis was assessed in *papers I* and *IV* using the Psoriasis Area and Severity Index (PASI) and in *papers IV* and *V* using the Self-Administered Psoriasis Area and Severity Index (SAPASI). In the case of AD, disease severity was assessed using the Scoring of Atopic Dermatitis (SCORAD) questionnaire in *paper IV*, while the patients in *papers IV* and *V* filled in the Patient-Oriented Scoring of Atopic Dermatitis (PO-SCORAD) questionnaire. Disease severity and pruritus were assessed globally using the Visual Analogue Scale (VAS). All the assessments were made at the beginning and end of the intervention and three months afterwards (*IV, V*). For the HRQoL assessments in *papers IV* and *V* we used a dermatology-specific measure, the Dermatology Life Quality Index (DLQI), and a generic measure, RAND-36. Physical and mental component summary scores were calculated from the eight RAND-36 subscales.

7.2.5 Measurement of S-25(OH)D and estimation of dietary vitamin D intake (I-IV)

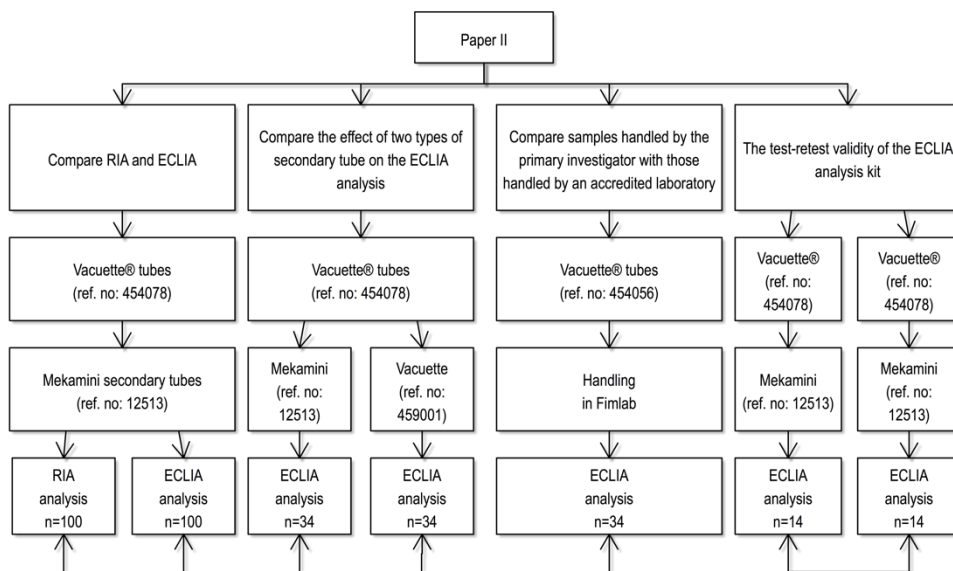
Blood samples for S-25(OH)D measurements were taken before, during and after the interventions as described in *papers I-IV*. Blood was collected into Vacuette® Z Serum Clot Activator Tubes with a Gel Separator (ref. no. 454078, Greiner Bio-One International GmbH, Kremsmünster, Austria). The samples were light-protected and centrifuged. The sera were separated out into light-protected secondary tubes (Mekamini, ref. no. 12513, Mekalasi Oy, Nurmijärvi, Finland), and frozen at -20°C until analysed. The S-25(OH)D concentration was measured in duplicate using IDS RIA (Immunodiagnostic Systems, Boldon, UK)

for *papers I and IV*, and using ECLIA (Elecsys® Total Vitamin D, Roche Diagnostics, Mannheim, Germany) for *papers II and III*.

We evaluated the validity of the S-25(OH)D measurements and compared the IDS RIA and ECLIA analysis methods in *paper II* by taking multiple blood samples (Fig. 2). We also studied the effect of using two types of secondary tube on the S-25(OH)D results. In addition to using Mekamini tubes, one set of samples was separated out into transparent Vacuette® Secondary Tube Multiplex PP tubes (ref. no. 459001), which were light-protected with aluminium foil. We ensured that our sample handling accuracy corresponded to that of an accredited laboratory (Fimlab Laboratoriot Oy, Tampere, Finland) by sending whole blood samples in Vacuette® LH Lithium Heparin Tubes (ref. no: 454056) to Fimlab for centrifuging, separation and ECLIA analysis and comparing the results with samples centrifuged and separated by us. The within-run variation in the ECLIA analyses was assessed by analysing a small proportion of the samples twice.

The plasma parathyroid hormone samples discussed in *paper II* were taken at the onset of the trial and at 14 weeks. Blood was collected into EDTA tubes, centrifuged and analysed by immunochemiluminometric assay. The dietary vitamin D intake was estimated with a Food Frequency Questionnaire (II, III).

Figure 2. Protocol used for analysing the validity of the S-25(OH)D samples. Unpublished data.



7.2.6 Statistical analyses (I-V)

The sample size calculation in *paper I* was based on previous experience (Ala-Houhala et al. 2012a) and suggested that the cohort should include 15 psoriasis patients and 15 healthy subjects. The groups were compared statistically using Student’s t-test, a permutation test or the χ^2 test as appropriate. The changes occurring among the patients with psoriasis and the healthy subjects were analysed by applying a permutation test to the relevant samples. Repeated measures were analysed using generalizing estimating equation (GEE) models with unstructured correlations assessed using bootstrap-type standard error.

The sample size calculation in *paper II* was designed to show an inter-group difference in S-25(OH)D of at least 12 nmol L^{-1} , with an α -value of 0.05 and a β -value of 0.90. An assumed SD of 9 nmol L^{-1} for the S-25(OH)D analyses at 50 nmol L^{-1} was used. It was decided that 12 volunteers per group should complete the trial. Confidence intervals of 95% were obtained by bias-corrected bootstrapping using 5000 replications. An analysis of t-test co-variance (ANCOVA) was used for the statistical comparisons. In the case of violation of the assumptions (e.g. non-normality), a bootstrap-type test was used. Longitudinal measures for continuous outcomes were analysed using a bootstrap-type GEE model. No adjustment was made for multiple testing. When comparing the increases in S-25(OH)D concentrations, the model was adjusted for the baseline value, body mass index (BMI) and Fitzpatrick's skin phototype (Fitzpatrick 1988). Pearson's χ^2 test was used when comparing nominal data. The STATA 13.1 (StataCorp LP, College Station, TX, USA) statistical package was used for the analyses.

The sample size calculation in *paper III* was designed to show an increase in S-25(OH)D of at least 15 nmol L^{-1} with an α -value of 0.05 and a β -value of 0.80. An assumed SD of 15.5 nmol L^{-1} for the S-25(OH)D analyses was used. It was decided that 16 volunteers should complete the trial. The strength of the adjusted relationship between the baseline S-25(OH)D concentrations and their changes after the solar UVR exposure period was described by means of a partial correlation coefficient. The significance of the change in S-25(OH)D concentrations was calculated using the paired-samples t-test, and Pearson's χ^2 test was used when comparing nominal data. All the analyses were performed using STATA 14 (StataCorp LP, College Station, TX).

The size of the group in *paper IV*, was decided by the patient associations, and thus no calculations were required. The data are presented as means with SDs or as counts with percentages. Confidence intervals (95% CI) were obtained by bias-corrected bootstrapping using 5000 replications. ANCOVA was used for the statistical comparisons, and longitudinal measures for continuous outcomes were analysed using a bootstrap-type GEE model. No adjustment was made for multiple testing. When comparing increases in S-25(OH)D concentrations, the model was standardized with respect to age, sex and BMI. Pearson's χ^2 test was used when comparing nominal data. The STATA 13.1 (StataCorp LP, College Station, TX, USA) statistical package was used for the analyses.

The size of the group in *paper V* was again decided by the patient associations, and thus no calculations were made. Repeated data were analysed using GEE models, as in *paper III*. Regression analysis was used to model the relationship between the change in global VAS and the QoL measures. Bonferroni adjustments were performed to correct the significance levels for the multiple test. The normality of the variables was evaluated using the Shapiro-Wilk statistics. The Stata 14.0 statistical package of StataCorp LP (College Station, TX, USA) was used for the analyses.

The significances of the differences between mean S-25(OH)D concentrations measured by different analysis methods were calculated using the independent-samples t-test and the correlations using Pearson correlation. The analyses were performed using IBM SPSS Statistics for Macintosh, version 21.0 (IBM Corp., Armonk, NY, U.S.A.).

8 Results

8.1 Effect of NB-UVB exposures on S-25(OH)D levels in psoriasis patients and healthy subjects receiving vitamin D supplementation (I)

The psoriasis patients and healthy control subjects received oral cholecalciferol 20 µg daily before, during and after the NB-UVB exposures, which were given three times a week. The mean cumulative dose received by the 12 psoriasis patients during 9 exposures was $4.49 \pm 0.44 \text{ J cm}^{-2}$ ($26.4 \pm 2.6 \text{ SED}$), while that received by the remaining 9 patients during 18 exposures was $15.63 \pm 1.67 \text{ J cm}^{-2}$ ($91.9 \pm 9.8 \text{ SED}$). The mean total dose received by the 15 healthy subjects in 9 exposures was $4.37 \pm 0.55 \text{ J cm}^{-2}$ ($25.7 \pm 3.2 \text{ SED}$) (Table II, IV). The total doses provided by the 9 exposures did not differ between the groups ($p = 0.57$).

The baseline S-25(OH)D level was $74.1 \pm 22.9 \text{ nmol L}^{-1}$ in the 12 psoriasis patients and $74.3 \pm 14.8 \text{ nmol L}^{-1}$ in the 15 healthy subjects. None of the subjects had a level $< 50 \text{ nmol L}^{-1}$. By the 9th NB-UVB exposure S-25(OH)D had increased by 13.2 nmol L^{-1} (95% CI 7.2–24.9, $p = 0.0029$) in the psoriasis patients and by 17.0 nmol L^{-1} (95% CI 6.7–21.0, $p < 0.001$) in the healthy subjects (Fig. 3, Table IV), and by the 18th exposure it had increased by 49.4 nmol L^{-1} (95% CI 35.9–64.6, $p = 0.0039$) in the 9 psoriasis patients (Table IV). The PASI score improved from 8.7 (range 4.0–16.2) at the baseline to 6.4 (range 2.1–12.8) at the 9th exposure and 4.5 (range 1.1–8.2) at the 18th ($p < 0.001$). One month after exposures, S-25(OH)D was still 29.9 nmol L^{-1} (95% CI 13.6–49.0; $p = 0.0078$) above the baseline

in the 8 psoriasis patients and 17.5 nmol L^{-1} (95% CI 10.1–24.9; $p < 0.001$) above in the 15 healthy subjects.

Baseline levels of CYP27A1 and CYP27B1 mRNA expression were significantly lower ($p < 0.001$) in the psoriasis patients than in the healthy subjects. Baseline mRNA expression of cathelicidin was similar in the psoriasis lesions and in the normal skin of healthy subjects, but human beta defensin 2 mRNA levels were significantly ($p < 0.001$) higher in the psoriasis lesions.

NB-UVB exposure did not alter the CYP27A1, CYP27B1 and cathelicidin mRNA expression levels in the psoriasis patients, but the human beta defensin 2 mRNA expression level decreased significantly ($p = 0.002$). In the healthy subjects NB-UVB exposure significantly reduced the CYP27A1, CYP27B1 and cathelicidin mRNA expression levels, while human beta defensin 2 increased slightly.

Figure 3. S-25(OH)D levels before and after the 9th NB-UVB exposure in 12 patients with psoriasis and 15 healthy subjects, all receiving oral cholecalciferol 20 μg daily.

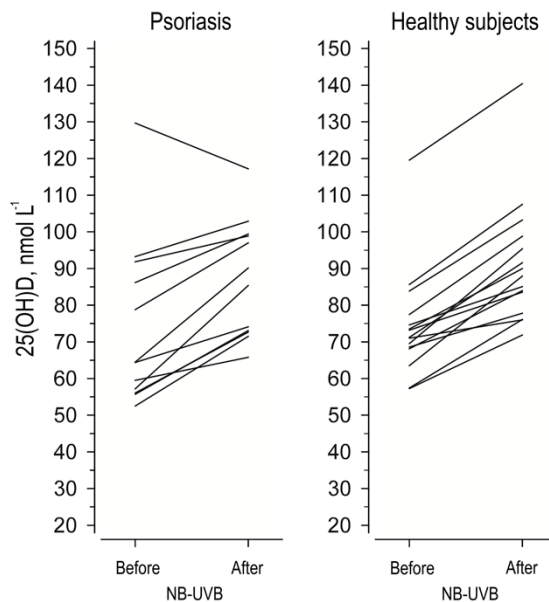


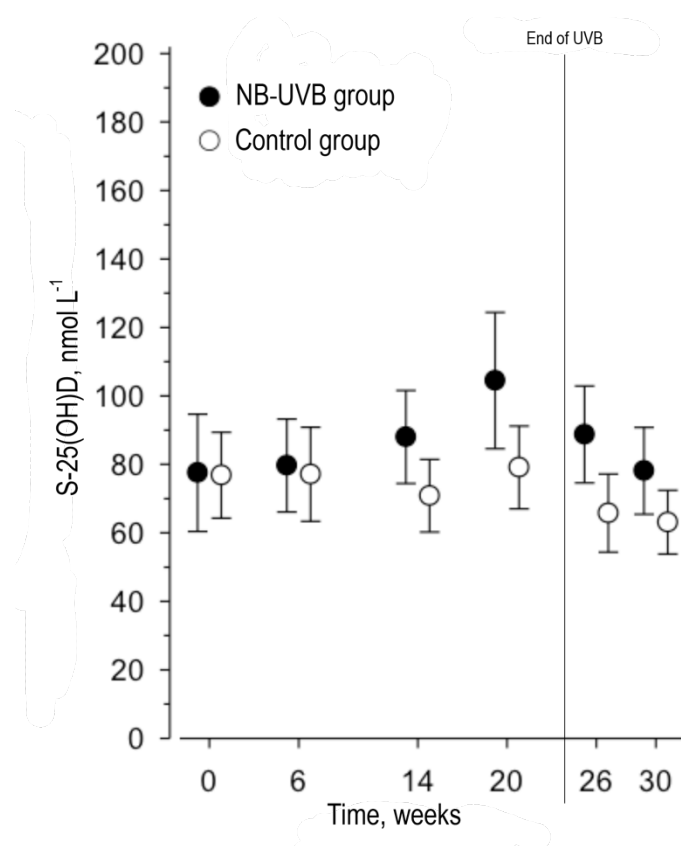
Table IV. Mean S-25(OH)D concentrations at baseline and at the end of the interventions in papers I-IV.

Paper	Group	Mean S-25(OH)D at baseline, nmol L ⁻¹	Mean S-25(OH)D at end, nmol L ⁻¹	Change from baseline, nmol L ⁻¹ (95% CI), p-value	Cumulative mean UVB dose, SED (range)
I NB-UVB exposures, Waldmann 7001	Psoriasis patients (n = 12)	74.1 ± 22.9	87.3 ± 16.0	13.2 (7.2–24.9, p = 0.0029)	9 th exposure 26 (19–28)
	Healthy subjects (n = 15)	74.3 ± 14.8	91.3 ± 17.1	17.0 (6.7–21.0, p < 0.001)	18 th exposure 92 (77–102)
II NB-UVB exposures Waldmann 7002	Healthy subjects (intervention) (n = 16)	78.3 ± 36.1	88.7 ± 29.2	11.7 (1.9–20.0, p = 0.029)	25
	Healthy subjects (control) (n = 18)	76.8 ± 26.6	65.8 ± 23.9	-11.1 (-19.4 to -2.7, p = 0.022)	none
III Vernal solar UV radiation	Healthy subjects (n = 25)	88.6 ± 32.6	88.5 ± 29.4	-0.1 (-3.2 to 3.2, p = 0.971)	12 (2–23)
IV Empowering heliotherapy	Psoriasis patients (n = 22)	86.6 ± 20.0	100.4 ± 17.2	13.8 (8.6–19.0, p < 0.001)	30 (22–39)
	Atopic dermatitis patients (n = 13)	84.1 ± 16.0	104.6 ± 24.4	20.5 (14.0–25.6, p < 0.001)	43 (3–54)

8.2 Effect of NB-UVB exposures on the maintenance of S-25(OH)D levels (II)

The intervention group received 13 NB-UVB exposures over 24 weeks from October to April, totalling 4.25 J cm^{-2} (25 SED). The mean \pm SD daily vitamin D intake at onset was $7.0 \pm 3.7 \mu\text{g}$ in the intervention group and $6.7 \pm 2.2 \mu\text{g}$ in the control group ($p = 0.78$). The mean baseline S-25(OH)D concentration in October was 78.3 nmol L^{-1} in the intervention group and 76.8 nmol L^{-1} in the control group (Table IV, Fig. 4), showing a moderate negative correlation with BMI ($r = -0.43$, $p = 0.012$). Three subjects in each group had baseline vitamin D insufficiency, i.e. S-25(OH)D $< 50 \text{ nmol L}^{-1}$. The mean \pm SD concentrations in the intervention group peaked at $104.5 \pm 40.2 \text{ nmol L}^{-1}$ in February, i.e. in week 20 (Fig. 4), and showed a mean increase of 11.7 nmol L^{-1} ($p = 0.029$) by the end of the intervention period, in April (week 26), at which point the mean for the control group had decreased by 11.1 nmol L^{-1} ($p = 0.022$, Table IV, Fig. 4). One intervention subject and four control subjects had vitamin D insufficiency in week 26. The difference between the groups was statistically significant ($p < 0.001$) when adjusted for the baseline value, BMI and Fitzpatrick's skin phototype (Fitzpatrick 1988). After the 1-month follow-up period i.e. by week 30, the mean S-25(OH)D level in the intervention group had decreased by 10.6 nmol L^{-1} ($p < 0.001$) and that in the control group by 2.7 nmol L^{-1} ($p = 0.18$; Table IV, Fig. 4).

Figure 4. S-25(OH)D concentrations in the NB-UVB-treated and control groups during the intervention (weeks 0–26) and follow-up periods (weeks 26–30).



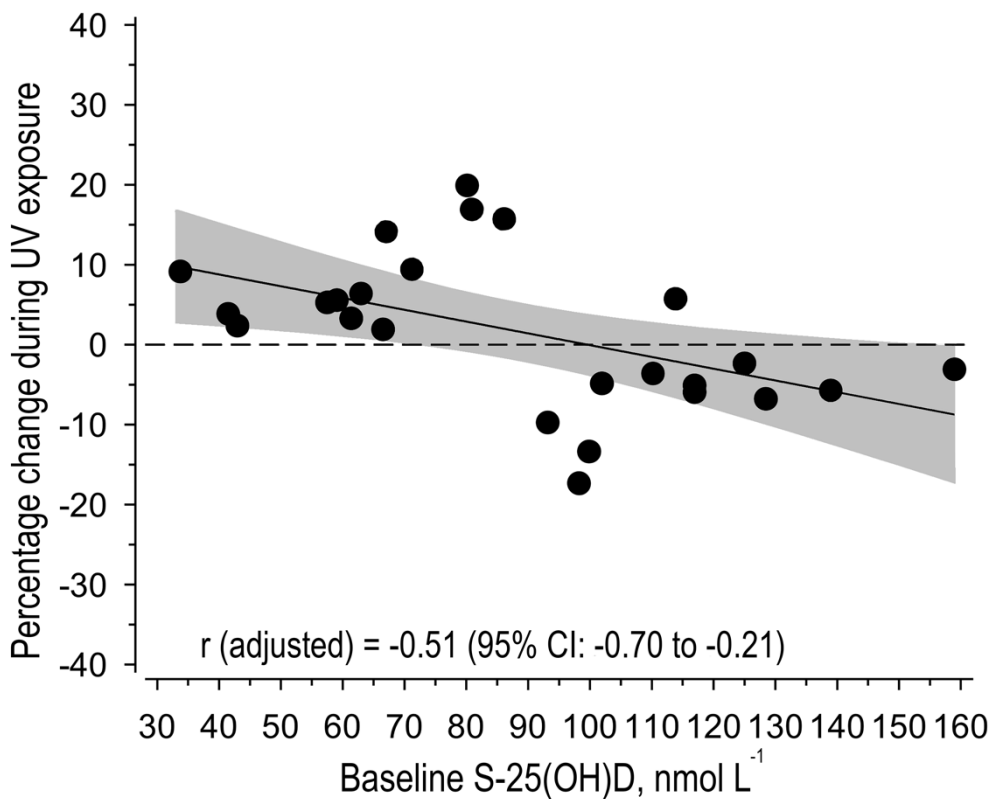
8.3 Effect of vernal solar UVR on S-25(OH)D levels (III)

The mean daily vitamin D intake of the 25 subjects was $8.5 \pm 3.2 \mu\text{g}$ and the mean baseline S-25(OH)D concentration was $88.6 \pm 32.6 \text{ nmol L}^{-1}$. Four subjects with baseline S-25(OH)D below 90 nmol L^{-1} and six subjects with baseline S-25(OH)D above 90 nmol L^{-1} had been receiving a mean daily vitamin D supplementation of $13.3 \pm 7.3 \mu\text{g}$ (range 5–25 μg) prior to the one-month washout period. The mean dose did not differ significantly between the groups ($p = 0.081$). Three subjects had S-25(OH)D $< 50 \text{ nmol L}^{-1}$ at onset and at the end. The mean personal total UVB dose was $11.8 \pm 4.9 \text{ SED}$ over a mean of 12.3 ± 4.3 hours spent out of doors (Table II). The mean available daily UVB dose was 9.4 SED and the maximum UV indices were 1.1–2.7 (Table III). The mean S-25(OH)D concentration showed a non-significant decrease of 0.1 nmol L^{-1} ($p = 0.971$) after the exposures, but the baseline S-25(OH)D levels had an inverse relationship with the percentage change after exposure when adjusted for age, BMI and Fitzpatrick's skin phototype (Fitzpatrick 1988) ($r = -0.51$, $p = 0.011$; Fig. 5).

The baseline S-25(OH)D concentrations $< 90.0 \text{ nmol L}^{-1}$ increased after exposure, whereas concentrations $> 90.0 \text{ nmol L}^{-1}$ decreased in all subjects except one (Fig. 5). The mean S-25(OH)D concentration in the $< 90.0 \text{ nmol L}^{-1}$ group ($n = 13$) increased from 62.4 nmol L^{-1} to 68.4 nmol L^{-1} ($p < 0.001$) and that in the $> 90.0 \text{ nmol L}^{-1}$ group ($n = 12$) decreased from $116.9 \text{ nmol L}^{-1}$ to $110.3 \text{ nmol L}^{-1}$ ($p < 0.01$) (Fig. 5). The mean BMIs were 24.5 and 22.2 kg m^{-2} , respectively ($p = 0.16$). The correlation between BMI and baseline S-25(OH)D was non-significant ($r = -0.272$, $p = 0.19$). The total UVB doses received by the volunteers correlated with the number of hours spent out of doors ($r = 0.612$, $p = 0.02$), whereas no correlation was found between the UVB doses and the changes in

S-25(OH)D concentrations. There were no differences in baseline S-25(OH)D levels, changes in S-25(OH)D levels or UVB doses between the March and April groups, nor were there any differences in demographic variables or UVB doses between the $< 90.0 \text{ nmol L}^{-1}$ and $> 90.0 \text{ nmol L}^{-1}$ subgroups.

Figure 5. Relationship of changes in S-25(OH)D levels after solar ultraviolet radiation exposure to baseline concentrations.



R adjusted for age, body mass index and Fitzpatrick's skin phototype, $p = 0.011$

8.4 Effect of empowering heliotherapy on S-25(OH)D levels, disease severity and quality of life in psoriasis and atopic dermatitis patients (IV, V)

UVR exposures, S-25(OH)D concentrations and disease severity in paper IV

The psoriasis patients studied in *paper IV* received a mean UVB dose of 30 ± 16 SED during EHT and the AD patients 43 ± 16 SED (Table II), i.e. there was no significant difference ($p = 0.062$). No patient had baseline S-25(OH)D < 50 nmol L⁻¹. The correlation between baseline S-25(OH)D and BMI was non-significant ($r = -0.233$, $p = 0.212$). During EHT the S-25(OH)D levels increased by 13.8 nmol L⁻¹ from 86.6 ± 20.0 nmol L⁻¹ ($p < 0.001$) in the psoriasis patients and by 20.5 nmol L⁻¹ ($p < 0.001$) from 84.1 ± 16.0 nmol L⁻¹ in the AD patients. The increase was similar ($p = 0.56$) in both groups. Three months after EHT the levels had decreased in both groups to the pre-EHT values.

The mean baseline PASI score was 3.1 ± 3.2 and that of the SAPASI score 7.4 ± 5.8 , with a close and significant correlation ($r = 0.738$, $p < 0.001$). The mean SCORAD score was 21.6 ± 16.9 and PO-SCORAD was 36.7 ± 15.5 , also with a close, significant correlation ($r = 0.932$, $p < 0.001$). The mean SAPASI decreased significantly by 4.9 units ($p < 0.001$) during EHT and the PO-SCORAD index by 19.5 units ($p < 0.001$).

Health-related quality of life and disease severity at the end of heliotherapy in paper V

The mean DLQI score observed in *paper V* decreased by 5.4 units from 8.2 ± 5.9 ($p < 0.001$) in the psoriasis patients and by 7.7 units from 10.4 ± 5.5 ($p < 0.001$) in the AD patients. All the subscales of RAND-36 as well as the PCS and MCS

scores improved significantly in both groups (Table V, Fig 6). The mean SAPASI decreased by 5.0 units from 7.4 ± 5.8 ($p < 0.001$) and the PO-SCORAD index by 24.0 units from 36.7 ± 15.5 ($p < 0.001$). Twenty psoriasis patients (16%) and one AD patient (2%) experienced complete clearance, and 75% clearance was seen in 59 psoriasis patients (46%) and 27 AD patients (46%). VAS indicated a significant decline in disease severity and pruritus in both groups (Table V).

Health-related quality of life and disease severity after heliotherapy in paper V

Three months after EHT the DLQI scores reported in *paper V* had decreased significantly as compared with the initial values (Table V, Fig. 6), the mean score for the psoriasis patients by 2.6 units ($p < 0.001$) and that for the AD patients by 4.8 units ($p < 0.001$). The DLQI had improved to a clinically significant extent, i.e. by at least 4 points (Basra et al. 2015) in 34/92 (37%) psoriasis patients and 26/45 (58%) AD patients. The decrease in SAPASI was 2.7 units ($p < 0.001$) and that in PO-SCORAD 14.6 units ($p < 0.001$). All but one of the RAND-36 subscales improved in the psoriasis patients and all but two in the AD patients, while the PCS and MCS scores improved in both patient groups (Table V). In VAS terms, global disease severity and pruritus had decreased in both groups (Table V).

Disease severity subgroup outcomes

When the patients were divided by disease severity into *mild* and *moderate-to-severe* subgroups, the cut-off points being a SAPASI score of 10 and a PO-SCORAD index of 25, the patients with *mild* psoriasis ($n = 86$) had a mean age of 51 years (range 23–72), 62% were females and their baseline DLQI was 6.7 ± 4.8 , whereas those with *moderate-to-severe* psoriasis ($n = 33$) had a mean age of 48 years (range 20–71), 52% were females and their baseline DLQI was 11.0 ± 6.0 .

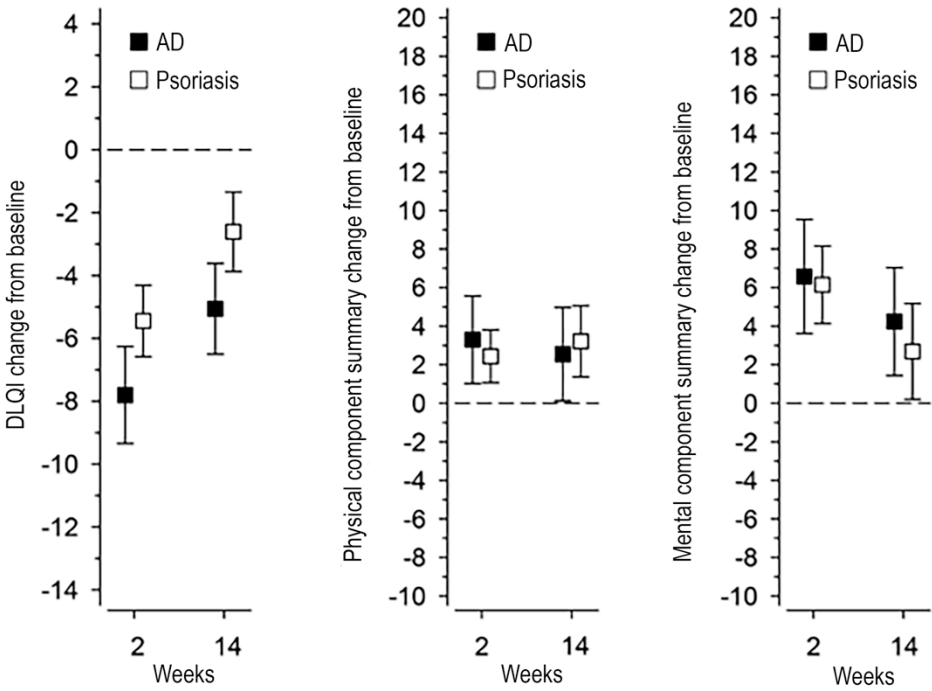
The former had a mean DLQI improvement of 1.7 ± 5.4 and the latter of 5.3 ± 6.0 , the latter improvement being clinically significant. The groups differed only in their baseline DLQI ($p < 0.001$). The patients with *mild* AD ($n = 16$) had a mean age of 39 years (range 20–74), 88% were females and their baseline DLQI was 5.6 ± 3.1 , whereas those with *moderate-to-severe* AD ($n = 43$) had a mean age of 37 years (range 19–65), 84% were females and their baseline DLQI was 12.2 ± 5.2 . The former had a mean DLQI improvement of 1.1 ± 2.9 and the latter 6.3 ± 4.2 , the latter improvement being clinically significant. The groups differed only in their baseline DLQI ($p < 0.001$).

Table V. Improvement in the Dermatology Life Quality Index (DLQI), RAND-36 scores and disease severity measures during the empowering heliotherapy course.

Psoriasis	Baseline mean (SD)	Δ Week 2 Mean (95% CI)	Δ Week 14 Mean (95% CI)
SAPASI	7.4 (5.8)	-5.0 (-5.9 to -4.1)***	-2.7 (-3.8 to -1.6)***
VAS pruritus	3.4 (2.4)	-2.0 (-2.4 to -1.7)***	-0.7 (-1.2 to -0.1)*
VAS global	4.1 (2.0)	-2.0 (-2.3 to -1.7)***	-1.2 (-1.7 to -0.8)***
DLQI	8.2 (5.9)	-5.4 (-6.4 to -4.4)***	-2.6 (-3.7 to -1.4)***
Physical function	73.6 (21.7)	5.3 (1.9 to 8.7)***	5.0 (1.6 to 8.5)**
Physical role	46.2 (42.3)	14.4 (6.7 to 22.1)***	15.3 (5.5 to 25.2)**
Bodily pain	58.3 (26.1)	7.5 (3.8 to 11.1)***	6.7 (1.3 to 12.0)**
General health perceptions	48.5 (18.9)	6.1 (3.2 to 9.0)***	5.0 (2.1 to 8.0)***
RAND-36 physical component	41.1 (10.7)	2.7 (1.4 to 4.0)***	3.9 (2.4 to 5.3)***
Social function	68.9 (22.7)	10.8 (6.9 to 14.6)***	6.6 (1.1 to 12.2)*
Emotional role	61.8 (42.3)	12.5 (3.8 to 21.3)**	7.5 (-2.5 to 17.5)
Vitality	50.6 (19.9)	15.9 (12.0 to 19.7)***	9.3 (4.9 to 13.8)***
Mental health	67.1 (18.1)	10.5 (7.1 to 13.8)***	4.6 (0.6 to 8.5)*
RAND-36 mental component	45.7 (11.0)	6.1 (4.3 to 7.9)***	3.0 (1.0 to 4.9)**
Atopic dermatitis			
PO SCORAD	36.8 (15.5)	-24.0 (-27.3 to -20.1)***	-14.6 (-18.7 to -10.6)***
VAS pruritus	4.5 (2.3)	-3.4 (-4.0 to -2.8)***	-2.4 (-3.1 to -1.6)***
VAS global	3.9 (2.1)	-2.8 (-3.3 to -2.3)***	-1.9 (-2.5 to -1.4)***
DLQI	10.4 (5.5)	-7.7 (-9.1 to -6.4)***	-4.8 (-6.2 to -3.5)***
Physical function	84.7 (16.6)	8.1 (3.5 to 12.8)**	6.3 (1.7 to 10.9)**
Physical role	56.6 (42.1)	15.5 (3.2 to 27.7)*	20.7 (5.4 to 36.0)*
Bodily pain	70.0 (24.4)	10.1 (2.7 to 17.4)**	2.7 (-4.8 to 10.3)
General health perceptions	56.0 (21.2)	8.1 (3.5 to 12.8)***	6.3 (1.7 to 10.9)**
RAND-36 physical component	46.6 (8.8)	3.3 (1.5 to 5.2)**	2.8 (0.9 to 4.7)*
Social function	69.1 (24.7)	12.6 (6.2 to 19.0)***	13.2 (5.4 to 21.1)***
Emotional role	63.1 (41.7)	14.8 (1.0 to 28.7)*	16.1 (1.7 to 30.4)*
Vitality	55.5 (20.6)	15.5 (9.8 to 21.1)***	5.8 (-0.3 to 11.8)
Mental health	67.6 (18.1)	11.9 (7.8 to 16.0)***	5.4 (1.1 to 9.7)*
RAND-36 mental component	44.5 (10.5)	6.5 (3.9 to 9.2)***	4.7 (2.4 to 7.1)**

Bonferroni-adjusted p-values *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ compared with day 1 of heliotherapy.

Figure 6. Improvements in mean Dermatology Life Quality Index (DLQI) and RAND-36 physical and mental component summary scores in patients with psoriasis and atopic dermatitis (AD).



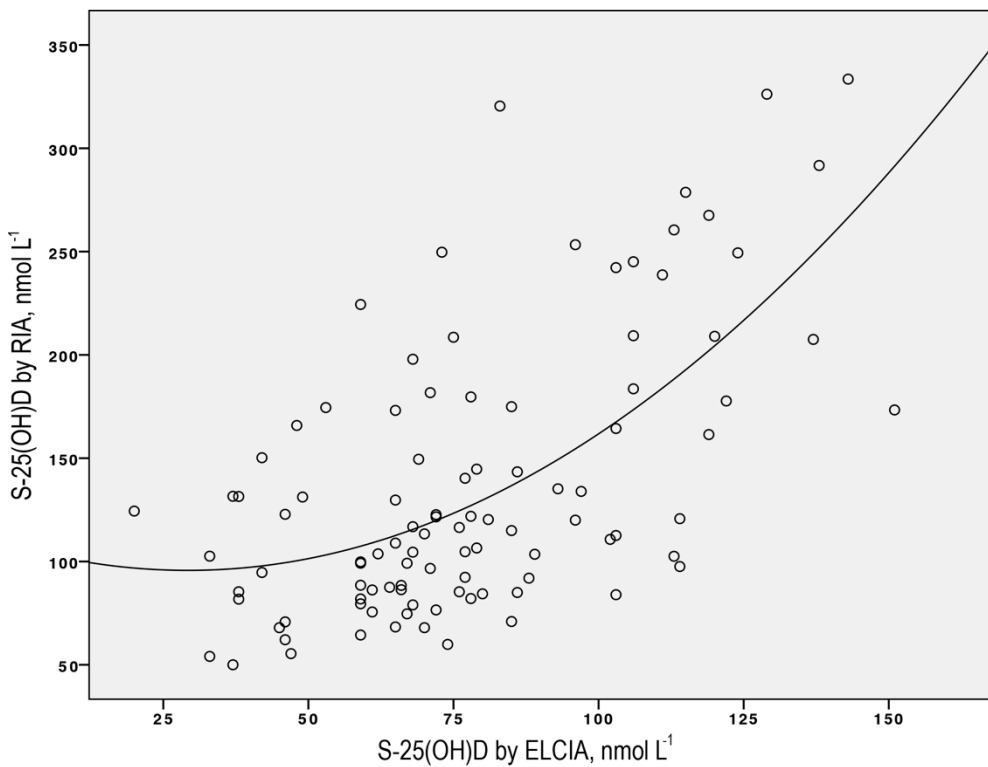
8.5 S-25(OH)D measurement technique comparisons (unpublished data from the work reported in *paper II*)

In *paper II* the S-25(OH)D samples ($n = 100$) were analysed by IDS RIA and ECLIA, the concentrations being $137.0 \pm 67.0 \text{ nmol L}^{-1}$ and $78.1 \pm 27.4 \text{ nmol L}^{-1}$, respectively, with a correlation $r = 0.593$ ($p < 0.001$) (Table VI, Fig. 7). IDS RIA detected values $> 200 \text{ nmol L}^{-1}$ in 18 samples (range 207–333 nmol L^{-1}), while the highest value in ECLIA was 151 nmol L^{-1} . The effect of the choice of Mekamini or Vacuette® secondary tubes was analysed with 34 ECLIA duplicates, the mean S-25(OH)D in the Mekamini tubes being $76.6 \pm 28.6 \text{ nmol L}^{-1}$ and that in the transparent Vacuette® tubes 47.7 ± 24.4 (Table VI), with a correlation $r = 0.856$ ($p < 0.001$). 34 blood samples from the same patients were drawn into Vacuette® LH Lithium Heparin primary tubes and sent to an accredited laboratory (Fimlab) for centrifugation, separation and ECLIA analysis, the mean S-25(OH)D being $67.9 \pm 25.7 \text{ nmol L}^{-1}$ (Table VI). The correlation between the samples in Mekamini tubes processed by us and those in the primary tubes sent to Fimlab was $r = 0.978$ ($p < 0.001$), while that between the samples in Vacuette® tubes processed by us and the Fimlab results was $r = 0.896$ ($p < 0.001$). The within-run analysis of variation in ECLIA performed with 14 duplicates in Mekamini tubes resulted in figures of $85.4 \pm 35.5 \text{ nmol L}^{-1}$ and $86.6 \pm 37.2 \text{ nmol L}^{-1}$, with a correlation $r = 0.992$ ($p < 0.001$) (Table VI).

Table VI. S-25(OH)D concentrations (nmol L⁻¹, mean ± SD) measured with IDS RIA and ECLIA using different sample handling techniques.

	RIA S-25(OH)D in Mekamini tubes (n = 100)	ECLIA S-25(OH)D in Mekamini tubes (n = 100)	ECLIA S-25(OH)D in Mekamini tubes (n = 34)	ECLIA S-25(OH)D in Vacuette® tubes (n = 34)	ECLIA P-25(OH)D handled by Fimlab (n = 34)	ECLIA S-25(OH)D in Mekamini tubes, variation analysis (n = 14)	ECLIA S-25(OH)D in Mekamini tubes, variation analysis (n = 14)
Mean ± SD	137.0 ± 67.0	78.1 ± 27.4	76.6 ± 28.6 ^c	47.7 ± 24.4 ^d	67.9 ± 25.7 ^e	85.4 ± 35.5 ^f	86.6 ± 37.2 ^g
Correlations	a-b: r = 0.593*** c-d: r = 0.856*** c-e: r = 0.978*** d-e: r = 0.896*** f-g: r = 0.992*** ***p < 0.001						

Figure 7. Correlation between S-25(OH)D concentrations (n = 100) measured using IDS RIA and ECLIA.



9 Discussion

9.1 NB-UVB treatment increases S-25(OH)D levels in supplemented psoriasis patients and healthy subjects (I)

It has been shown previously that NB-UVB phototherapy is capable of raising S-25(OH)D levels in psoriasis patients during the winter (Osmancevic et al. 2009b, Vähävihi et al. 2010a, Ryan et al. 2010, Lesiak et al. 2011, Romaní et al. 2012). The baseline S-25(OH)D levels in these studies varied from 36 to 71 nmol L⁻¹, the number of NB-UVB exposures from 15 to 27 and the increase in S-25(OH)D from 66 to 163%. Where baseline S-25(OH)D levels were below 60 nmol L⁻¹ the increases were from 113 to 163%, whereas where baseline levels were over 60 nmol L⁻¹ the increases were smaller, from 66 to 73%. The present subjects had been receiving supplements of 20 µg cholecalciferol daily prior to the study, and their baseline S-25(OH)D concentrations were thus higher, 74 nmol L⁻¹ in both the psoriasis patients and the healthy subjects. Nine NB-UVB exposures raised the S-25(OH)D concentrations by 17% and 23%, respectively, and the patients who received 18 NB-UVB exposures had an increase of 58%. The results are comparable to those of Osmancevic et al. (2009b), who demonstrated a 66% increase in S-25(OH)D levels in psoriasis patients who had a baseline level of 71 nmol L⁻¹.

An inverse relationship between the change in S-25(OH)D level following UVR exposures and its baseline level has been shown previously (Edvardsen et al. 2007, Brustad et al. 2007, Bogh et al. 2010). One explanation may be the

homeostatic S-25(OH)D control system, which ensures stable availability despite high variations in vitamin D supply (Vieth 1999). The highest S-25(OH)D concentration measured in the present subjects was 155 nmol L^{-1} . One patient had an exceptionally high baseline S-25(OH)D concentration of 130 nmol L^{-1} , but was the only one showing a decrease after the NB-UVB exposures, perhaps due to this negative feedback system.

On account of volumetric dilution and possibly sequestration of vitamin D in the adipose tissue, S-25(OH)D levels tend to be lower in obese subjects (Drincic et al. 2012, Pannu et al. 2016). The BMI of the psoriasis patients was significantly higher, and a high BMI is known to be associated with greater prevalence and severity of psoriasis (Herron et al. 2005, Wolk et al. 2009). Interestingly, the baseline S-25(OH)D concentrations and their increase following NB-UVB exposures were similar in our psoriasis and control groups despite the significantly higher BMI of the psoriasis patients. One explanation may be that the distance of the skin from the UV lamp during irradiation is less in obese subjects, who therefore inevitably receive a larger UVB dose (Storan et al. 2014). The calculated total dose obtained from the 9 NB-UVB exposures was 26 SED in both groups. Also, the groups differed in their gender distribution, 42% of psoriasis patients being females as compared with 93% in healthy subjects. This difference may have caused a bias, as given the same BMI, females have 10% more adipose tissue for the dilution of vitamin D (Jackson et al. 2002).

Ala-Houhala et al. (2012a) have shown previously that twelve NB-UVB exposures totalling 48 SED during 4 weeks raised S-25(OH)D by 41 nmol L^{-1} from 53 nmol L^{-1} in healthy subjects, more than did daily oral doses of $20 \mu\text{g}$ cholecalciferol, which raised the mean level by 20 nmol L^{-1} from 54 nmol L^{-1} . Bogh et al. (2012a) showed, that eighteen NB-UVB exposures totalling 53 SED administered to vitamin D-deficient subjects increased their S-25(OH)D by 56

nmol L⁻¹ from 19 nmol L⁻¹, whereas 40 µg oral cholecalciferol daily raised S-25(OH)D by 37 nmol L⁻¹ from 23 nmol L⁻¹. Lagunova et al. (2013), comparing the effects of 50 µg oral cholecalciferol daily and ten UVB exposures totalling 24 SED, showed that both interventions raised S-25(OH)D levels similarly, by 20–25 nmol L⁻¹. Our results are in agreement with these previous observations that UVB radiation is a potent inducer of vitamin D synthesis by comparison with oral cholecalciferol, even in subjects receiving sufficient vitamin D. One month after the NB-UVB exposures the S-25(OH)D levels started to decrease despite vitamin D supplementation, which demonstrates the effectiveness of cutaneous vitamin D synthesis. In all the subjects the S-25(OH)D level remained far below the upper normal limit of 250 nmol L⁻¹ (Holick 2015), and thus 20 µg of cholecalciferol daily can be administered safely during NB-UVB phototherapy. On the other hand, as NB-UVB phototherapy has been shown to increase S-25(OH)D from 66 to 163%, with vitamin D-insufficient patients benefitting most, there seems to be no reason for vitamin D supplementation during phototherapy.

It has been shown previously that the CYP27A1 and CYP27B1 enzymes in keratinocytes are capable of hydroxylating vitamin D to 1,25(OH)₂D (Lehmann 2007). In the present results, the baseline levels of CYP27A1 and CYP27B1 expression were lower in the psoriatic lesions than in the skin of the healthy subjects. Kaukinen et al. (2015) have recently shown that the percentage of CYP27A1-positive mast cells was lower in psoriasis lesions than in the healthy skin of control subjects, which is in agreement with our findings, while CYP27B1 activity has also been demonstrated in both psoriatic lesions and the skin of healthy controls (Karakelides et al. 2006). We found a significant decrease in the expression of CYP27B1 in the skin of healthy subjects following NB-UVB exposures, as has also been demonstrated in HaCat keratinocytes *in vitro* (Seifert et al. 2009). The down-regulation of CYP27B1 gene expression is thought to be

mediated by the end product of CYP27B1 hydroxylation, 1,25(OH)₂D itself (Turunen et al. 2007). Since psoriatic lesions showed no change in the expression of either CYP27A1 or CYP27B1, we can conclude that the enzymatic activity in healthy skin reacts more actively to NB-UVB radiation than does that in psoriatic skin.

Antimicrobial peptides seem to have a proinflammatory role in psoriasis (Hollox et al. 2008, Felton et al. 2012). As expected, we found an increase in baseline human beta defensin 2 mRNA expression in psoriatic lesions and reduced expression after NB-UVB exposures, in agreement with a previous observation (Vähävihi et al. 2010a). Cathelicidin is not typically detected in the keratinocytes of healthy skin (Vähävihi et al. 2010a), but we found similar cathelicidin expression in both psoriasis patients and healthy subjects. This can be explained by the supplementation with 20 µg of oral cholecalciferol, a dose which has been shown to induce cathelicidin production in both healthy and lesional skin (Hata et al. 2008). The cathelicidin levels decreased following NB-UVB exposures in the healthy subjects, but not in the psoriasis patients, evidently on account of the highly significant decrease in CYP27B1 expression in healthy subjects, since inhibition of CYP27B1 expression has been shown to prevent the induction of cathelicidin in human keratinocytes (Schauber et al. 2007).

Despite the observed differences in CYP27A1 and CYP27B1 enzyme activities, our healthy subjects and psoriasis patients showed similar S-25(OH)D increases, and the mean PASI score of the psoriasis patients was improved by 48% during 18 exposures, which is in agreement with results quoted by Vähävihi et al. (2010a).

In summary, we studied the effect of NB-UVB exposures on S-25(OH)D levels in subjects receiving daily supplementations of 20 µg oral cholecalciferol and

showed that these exposures raised S-25(OH)D levels similarly and significantly in both the psoriasis patients and the healthy subjects, all of whom were sufficient in vitamin D at the baseline. Since UVB radiation is very effective in raising S-25(OH)D levels, patients can discontinue vitamin D supplementation during NB-UVB phototherapy. The observed difference in the expression of vitamin D hydroxylating enzymes in the skin of psoriasis patients and healthy subjects and the role of cutaneously synthesized vitamin D in the healing of psoriasis lesions, are both topics that deserve further investigation.

9.2 NB-UVB exposures can be used to maintain S-25(OH)D levels in winter (II)

At latitude 52°N and above the intensity of solar UVR is not capable of inducing cutaneous vitamin D synthesis between October and March (Webb et al. 1988), so that S-25(OH)D levels tend to decrease during this period. We found that post-summer S-25(OH)D levels could be maintained and even increased from October to April by means of sub-erythemal NB-UVB exposures of 2 SED every second week, whereas levels in the control group decreased. No adverse short-term effects were observed. Altogether 13 exposures were administered during 24 weeks, entailing a total UVB dose of 25 SED. Bogh et al. (2012b) have shown previously that a BB-UVB exposure of 1 SED every second week maintained the post-summer S-25(OH)D levels. They gave 9 exposures, totalling 9 SED, over 16 weeks and observed a non-significant decrease of 4.7 nmol L⁻¹ from the baseline S-25(OH)D level of 72.0 nmol L⁻¹. We achieved a significant increase of 11.7 nmol L⁻¹ from a baseline level of 78.3 nmol L⁻¹. The dose (2 SED vs. 1 SED) and the length of exposure (24 vs. 16 weeks) seem to be the reasons for the better response in our subjects. In addition, the fact that the vitamin D action spectrum-weighted irradiance dose (Boullion et al. 2006) for an exposure of 1 SED is higher with NB-UVB (23 mJ cm⁻² CIE) than with BB-UVB (16 mJ cm⁻² CIE) may also have affected the results. NB-UVB is better tolerated than BB-UVB (Picot et al. 1992, Almutawa et al. 2013), and in agreement with this, we did not observe any adverse effects during the present study.

The repetitive administration of UVB irradiation over a long period of time raises a question of the potential risks attached to UV-induced long-term immunosuppression. The human action spectrum for immunosuppression peaks at UVA and UVB wavelengths, as demonstrated by UV-induced suppression of

recall-type immunity (Matthews et al. 2010a and 2010b), and the most sensitive UVA wavelength is 370 nm, where the minimum immunosuppressive physical dose is 409.4 mJ cm^{-2} (Matthews et al. 2010a). We found that a dose of 2 SED was equal to an integrated non-weighted dose of 22 mJ cm^{-2} between 360 and 390 nm from NB-UVB lamps, as also for BB-UVB lamps, i.e. clearly below the immunosuppressive dose. In the UVB range immunosuppression peaks at 300 nm and no immunosuppression has been recorded at 322 nm (Matthews et al. 2010b). Thus, the minimum immunosuppressive dose will not be exceeded with a 1 SED dose of NB-UVB or BB-UVB, but it could be exceeded with a 2 SED dose of either NB-UVB or BB-UVB. These calculations were performed with an exponential triangular model using data from Matthews et al. (2010a and 2010b). Even though an immunosuppressive dose can be reached easily, no association has been observed between NB-UVB treatment and skin cancer during a median follow-up time of 5.5 years (range 0–18 years) (Hearn et al. 2008). The suitability of NB-UVB for the treatment of a skin disease, or for improving vitamin D status, must naturally be assessed individually (Tijoe et al. 2003, Dawe 2010). Repeated NB-UVB exposures cannot directly be recommended as a first-line source of vitamin D for the general population until we have more research data available, but it could be considered if dietary vitamin D or supplementations fail to provide an adequate vitamin D status. It must be kept in mind, however, that tanning beds emitting mainly UVA and less than 5% UVB are still widely used by light-skinned Caucasians solely to improve appearance. Tanning beds are considered responsible for 800 melanoma deaths yearly in Europe (Boniol et al. 2012), and in this context NB-UVB can be seen as a relatively safe modality.

The dietary vitamin D intake of our intervention group was $7.0 \mu\text{g}$ and that of the control group $6.7 \mu\text{g}$. These intakes were lower than in the recent national

survey FINDIET2012 (Helldan et al. 2013). Only a few of the present subjects were receiving the recommended intake of 10 µg dietary vitamin D daily, a dose which is thought to prevent vitamin D insufficiency according to the Nordic Nutrition Recommendations (NORDEN 2012). The BMI of the volunteers correlated negatively with the baseline S-25(OH)D concentration. Three subjects in both groups were vitamin D-insufficient at onset, all of them presenting with BMI > 25 kg m⁻² or vitamin D intake < 10 µg. At week 26 after the irradiations only one intervention subject was still vitamin D-insufficient, implying that NB-UVB maintenance effectively prevented vitamin D insufficiency.

Cyclic NB-UVB exposures could be used for patients with a chronic kidney disease requiring haemodialysis. These patients often present with vitamin D insufficiency and respond slowly to oral cholecalciferol supplementation (Kandula et al. 2011). Ala-Houhala et al. (2012b) have previously shown that a short course of NB-UVB exposures can increase S-25(OH)D levels in haemodialysis patients, but the level decreased again after one month to close to the baseline. Therefore, a cyclic exposure protocol is evidently required for maintaining S-25(OH)D levels. Phototherapy is not a suitable option for immunosuppressed organ transplant patients, however, as they have at least a 65-fold risk of squamous cell cancer (Hofbauer et al. 2010). Thus, long-term maintenance therapy with NB-UVB cannot be considered for patients who have received or are due to receive a kidney transplant. Since vitamin D insufficiency is common in patients with psoriasis or AD (Gisoni et al. 2012, Cheng et al. 2014) and NB-UVB phototherapy alleviates the skin symptoms of both diseases, NB-UVB maintenance therapy could be targeted to these patient groups.

We also used serum samples from the present work to compare two S-25(OH)D measurement techniques and noticed that S-25(OH)D levels measured with IDS RIA were higher than those measured with ECLIA. IDS RIA detected S-

25(OH)D levels above 200 nmol L⁻¹ in 18 samples, which are probably overestimations due to the low UV index season. IDS RIA particularly overestimated values over 100 nmol L⁻¹ (Fig. 7). We were surprised by this, as Roth et al. (2008) reported a bias of -15% when comparing IDS RIA with LC-MS/MS, and Carter et al. (2004) reported a bias of -5% relative to the DEQAS All-Laboratory Trimmed Mean. Exceptionally high S-25(OH)D values had not occurred previously in *papers I* and *IV*, where IDS RIA was used. We performed duplicate ECLIA analyses using red Mekamini and transparent Vacuette® secondary tubes and compared the results with those obtained by Fimlab from whole blood samples in Vacuette® LH Lithium Heparin tubes. S-25(OH)D in red Mekamini tubes correlated better with the Fimlab results than S-25(OH)D in transparent Vacuette® tubes ($r = 0.978$ vs. $r = 0.896$), and thus red Mekamini tubes seem to be more suitable for ECLIA analyses. The results in *papers II* and *III* are based on samples in red Mekamini tubes analysed with ECLIA, which has shown a within-run variation of $\leq 7\%$, a within-laboratory variation of $\leq 9.5\%$, and good correlations with HPLC ($r = 0.91$) and LC-MS/MS ($r = 0.93$) (Emmen et al. 2012, Wielders et al. 2015, Kocak et al. 2015). We found a within-run variation of 8% for ECLIA.

To increase the generalizability of S-25(OH)D measurements, the results can be calibrated to NIST reference material, but within-trial changes can also be assessed without calibration. To our knowledge, no correlation between IDS RIA and ECLIA (Elecsys Vitamin D total) has been published previously. The ECLIA method showed good within-run variation and can be considered reliable on the basis of the present measurements and previous ones (Emmen et al. 2012, Wielders et al. 2015, Kocak et al. 2015).

In summary, the aim here was to examine the ability of sub-erythemal NB-UVB exposures to maintain post-summer S-25(OH)D concentrations during the

winter. The results confirmed that a 2 SED dose given every second week from October to April was enough to maintain S-25(OH)D levels and even increased them. NB-UVB exposures could be given for selected patients such as those with psoriasis or atopic dermatitis or patients undergoing haemodialysis, to maintain their S-25(OH)D levels. These patients might benefit more from NB-UVB radiation than from oral vitamin D supplements. Further investigations into safety issues are warranted, however.

9.3 Vernal solar UV radiation is capable of increasing S-25(OH)D in patients with low baseline levels (III)

The present study has shown that vernal UVB radiation at a high latitude (67°N or 61°N) administered to the face and hands, i.e. 7% of the total body surface area, is capable of raising S-25(OH)D levels significantly in subjects with baseline levels below 90 nmol L⁻¹, whereas concentrations above that will decrease. The fact that the mean baseline level of 88.6 nmol L⁻¹ was higher than expected at high latitudes in early spring could be attributed to recent updates of the national food fortification policy and the general improvement in dietary vitamin D intake (Helldan et al. 2013), the use of vitamin D supplementation or holidays taken at low latitudes prior to the washout period. In the control group used here for *paper II*, where S-25(OH)D levels were monitored from October to May in subjects with no vitamin D supplementation during the winter nor any exposure to UVR, the mean concentration in early April was somewhat lower, 65.8 nmol L⁻¹.

The increase in lower S-25(OH)D levels early in spring is a solid finding, but the decreasing trend in higher levels was unexpected, since all the subjects were exposed to a source of vitamin D. It could be that in those with higher levels the S-25(OH)D gained from previous vitamin D sources kept decreasing due to its half-life, and that this decrease exceeded the input of S-25(OH)D produced by vernal UVB. Interestingly, unpublished data connected with *paper II* show similar kinetics in the control subjects – higher values keep on decreasing from early April to early May, whereas lower values begin to increase. This phenomenon has also been demonstrated elsewhere in the Nordic countries at high latitudes (Edvardsen et al. 2007, Brustad et al. 2007, Bogh et al. 2010).

The UVR exposures were performed in late March and early April and were based on previous UVR measurements on a horizontal surface, suggesting that cutaneous vitamin D photosynthesis is possible from early March onwards (Kazantzidis et al. 2009). Skin areas exposed to the sun, such as the face, are typically in a vertical position during outdoor activities in snow-covered terrain, and thus horizontally measured UVR data are not directly comparable to the doses received by areas of the body exposed to the sun. We assumed when planning the experiment that vernal UVR could raise S-25(OH)D levels because of UVR reflected from the snow. The albedo of snow varies between 0.5 and 0.7 (Meinander et al. 2008 and 2013) and the effect is most pronounced for vertical surfaces (Jokela et al. 1993). All the outdoor activities performed by our subjects took place in snow-covered terrain, which probably substantially increased the measured cumulative UVB radiation dose.

The close correlation between UVB radiation doses and hours spent out of doors reflects the reliability of personal spore film dosimetry (Quintern et al. 1997, Vähävihi et al. 2010b). The UVB radiation doses received by the subjects were only a few SED per day, which is the optimal dose for vitamin D synthesis. The formation of previtamin D₃ in fair skin reaches a plateau after 15 min of exposure to noon equatorial UVR, all the surplus previtamin being isomerized to biologically inactive lumisterol and tachysterol (Holick 1981, MacLaughlin et al. 1982). The present UVR exposure was received on the face and hands, which has been shown to raise S-25(OH)D levels (Vähävihi et al. 2010c, Osancevic et al. 2015).

A previous study performed with healthy subjects in Denmark (56°N) showed a significant increase in S-25(OH)D levels by April 8th when the shoulders and upper body were exposed to the sun at least twice a week (Datta et al. 2012), while at latitude 68°N in Norway (Edwardsen et al. 2007), exposure of only the

facial area to UVR between February 8th and April 12th yielded no significant increase in the mean S-25(OH)D, being in this respect in agreement with our results. Interestingly, the subjects in that cohort with S-25(OH)D < 30 nmol L⁻¹ responded in early March, whereas in our data the cut-off limit was 90 nmol L⁻¹. Our cut-off limit was significantly higher probably because of the larger skin area exposed (4% vs. 7%), the longer minimum daily exposure time (20 min vs. 60 min) and the extension of the exposure period further into the spring (April 20th). Contrary to those of Edvardsen et al. (2007), our subjects showed highly significant increases in S-25(OH)D at levels below 90 nmol L⁻¹, which strengthens the evidence for the effects of vernal UVR on vitamin D. In a comparable experiment reported by Petersen et al. (2014), a one-week skiing holiday in mountains at a lower latitude (47°N) in March increased S-25(OH)D by 8.6 nmol L⁻¹, but here the subjects received a high UVB dose of 109 SED on the face, and a sunscreen was used on 90% of the exposure days.

In summary, we showed that vernal solar UVR is capable of raising S-25(OH)D levels significantly in subjects with a baseline level below 90 nmol L⁻¹ when only the face and hands are exposed, but the intensity of vernal solar UVR is not great enough to increase high baseline S-25(OH)D levels, which seem to keep decreasing in spring due to the half-life of 25(OH)D. The factors lying behind this interesting phenomenon could be elucidated in future field trials.

9.4 Empowering heliotherapy improves vitamin D status and quality of life and alleviates disease severity in psoriasis and atopic dermatitis patients (IV, V)

The two-week EHT course improved the HRQoL and alleviated disease severity in the psoriasis and AD patients for at least 3 months. A larger proportion of the AD patients (58%) than of the psoriasis patients (37%) achieved a clinically significant DLQI improvement at the 3-month evaluation, possibly because of their higher baseline DLQI score. The RAND-36 PCS score improved more in the psoriasis patients, reflecting improved physical health, while the MCS score reflecting mental health improved more in the AD patients. This could be attributed to the fact that the schedule for the psoriasis patients included more physical exercise, with the aim of inspiring them to control their weight (Herron et al. 2005, Wolk et al. 2009), in order to reduce the risk of comorbidities and alleviate symptoms related to psoriatic arthritis (Oliveira et al. 2015). The presence of the same psychologist on all the AD courses might explain the greater improvement in the MCS score among the AD patients. The difference in course content must thus be kept in mind when comparing the results between disease groups.

Although the mean initial SAPASI was low, indicating mild psoriasis, the reduction achieved, 68%, was moderate, and the proportion of patients who achieved 75% clearance was only 46%, probably due to the short duration of the EHT course, or even more, due to the insensitivity of SAPASI with regard to a mild disease state. SAPASI remained reduced by 35% for three months after EHT when compared with the baseline scores. Wahl et al. (2015), studying the effects of a two-week EHT course which was essentially similar to that provided for our patients, showed a SAPASI reduction of 81% from a rather low baseline score of

8.6, and the score remained decreased by 26% three months afterwards, in agreement with our results.

Psoriasis in general requires more than two weeks of phototherapy to achieve alleviation (Nast et al. 2012). Four weeks of traditional HT at the Dead Sea has been shown to result in a PASI score reduction of 75% or more in 76% of patients, from an initial score of 31.7 (Harari et al. 2007). Snellman et al. (1993) studied the effects of a four-week HT course in the Canary Islands on 361 Finnish psoriasis patients, and noted that the disease severity as measured by the Psoriasis Severity Index decreased by at least 75% in 84% of the patients and the mean score was significantly lower 6 months after HT than at the baseline. These results suggest that a 4-week HT course is more effective than the present 2-week model for treating psoriasis, but it has to be kept in mind that the disease severity was mild in the present instance, and that it would be best to adjust the length of the EHT course according to the severity of the patients' disease.

In the AD patients, the initial PO-SCORAD score (mean 36.8) improved by 65% during EHT and remained reduced by 40% relative to the baseline score for three months after EHT, thus being in agreement with results obtained with traditional HT by Autio et al. (2002). They found no differences in efficacy with regard to disease severity between a two-week and a three-week course, suggesting that AD patients receive no additional value from a longer course or a larger UVB dose. Vähävihi et al. (2008) similarly reported that SCORAD improved by 74% in their January group and by 70% in their March group, results that are approximately similar to ours as reported in *papers IV* and *V*. Their patients received a mean UVB dose of 60 SED in January and 109 SED in March, i.e. higher than the November dose of 43 SED reported for our AD patients in *paper IV*. It seems that the alleviation of AD does not require such a high UVB dose, and for safety reasons EHT for AD could be arranged during a lower UV index season or

using a more careful sunbathing protocol at a high UV season. Two weeks seems like an appropriate length of course for AD patients.

We were surprised that our AD patients received a higher UVB dose than the psoriasis patients (43 SED vs. 30 SED), since psoriasis patients can afford to take more risks and are prone to higher UVR doses than AD patients (Bahmer et al. 2007, Sansone R and Sansone L 2010). The AD patients had less outdoor hours in their schedules, however, although they may have received more UVR in several other activities independently (Holman et al. 1983). Since it has been shown that psoriasis patients benefit from a longer HT course than two weeks, and since psoriasis generally requires several weeks of phototherapy to alleviate the symptoms, it might be justified to arrange EHT courses for psoriatics during a higher UV index season than the courses for AD patients.

The larger cumulative UVB dose in the AD patients in *paper IV* resulted in a larger S-25(OH)D increase. In spite of the fact that none of the patients was vitamin D-insufficient at onset, EHT raised the S-25(OH)D statistically significantly in both groups, by 13.8 nmol L⁻¹ for the psoriasis patients and by 20.5 nmol L⁻¹ for the AD patients, which underlines the fact that the solar UVB radiation received in a heliotherapy course is capable of raising S-25(OH)D levels even in individuals with adequate vitamin D, as shown in *paper I* and in some previous studies (Vähävihi et al. 2008, Osancevic et al. 2009a).

The persisting alleviation of disease activity reported three months after the EHT course, as measured by global VAS, had a moderate positive relationship with DLQI, explaining 40% of the improvement in DLQI scores. The relationship with the RAND-36 component summary scores was weak, however, as the VAS improvement explained only 8% of the improvement in PCS scores and 3% of that in MCS scores. This may be attributed to the alleviation of other diseases or to successful empowerment, probably both. It is therefore essential to use a

dermatology-specific assessment for HRQoL, since general HRQoL assessments do not respond well to the alleviation of skin symptoms. General HRQoL assessment tools can be used as a supplement if the therapy is also thought to affect other aspects of the patient's health as well as the skin problems.

Empowerment through patient education as an adjunct to treatment is a novel element in the long-term management of chronic skin diseases. Teaching in self-care and relaxation practices, peer-to-peer support, workshops and multidisciplinary discussion groups have been shown to improve the HRQoL of such patients (de Bes et al. 2011). The role of empowerment has been strengthened in the new EHT courses. The patients are away from their homes and jobs during the course and in a stress-free environment together with peers, which probably affects the short-term results. Thus, the HRQoL scores immediately after EHT have to be interpreted with caution. Indeed, the challenge is how to maintain the effects in the long term after returning to normal life (Wahl et al. 2015).

The primary intention is that the relaxation and self-management practices should be adopted into daily life after the EHT course in order to maintain the results. The patients are also encouraged to make firm decisions to improving their lifestyle by changing their diet, increasing the amount of exercise they undertake or quitting smoking. These intentions could be supported by steering the patients to take part in the regular activities of their local patient organizations, or other motivational groups. Also, medical care should not be forgotten, since patients might be better motivated towards self-management after an EHT course, and additional support from a doctor in the form of a treatment plan and the necessary medications could be effective. It would also be necessary in future research to actually measure empowerment and self-

management, e.g. in terms of the novel 8-section Health Education Impact Questionnaire (HeiQ), as was used by Wahl et al. (2015) in their EHT study.

As over half of our patients failed to achieve a clinically significant 3-month improvement in DLQI, it may be necessary to use stricter inclusion criteria for future EHT courses. Generally, patients with moderate-to-severe symptoms achieve clinically significant improvements in their DLQI scores, whereas those with mild symptoms fail to benefit from EHT. The inclusion of patients with moderate-to-severe symptoms would also improve the cost-effectiveness of the courses (Snellman et al. 1998).

Light-based therapies have traditionally not been provided for patients taking methotrexate or cyclosporine in Finland, because of an increased risk of skin cancer. Scott et al. (2016) showed that methotrexate is associated with an increased risk of non-melanoma skin cancer in rheumatoid arthritis patients with a history of this form of cancer. Rheumatoid arthritis patients taking methotrexate also have a 3-fold melanoma risk as compared with the general population (Buchbinder et al. 2008). In the present work 28 psoriasis patients receiving methotrexate, and one receiving cyclosporine were accepted for the EHT course. Long-term cyclosporine is known to increase the risk of non-melanoma skin cancer, and previous exposure to PUVA therapy further increases the risk significantly (Muellenhoff and Koo 2012). In our opinion, cyclosporine or methotrexate patients should not be accepted for an EHT course that includes sunbathing for up to 300 minutes daily. If the skin symptoms are moderate-to-severe while the patient is taking either of these drugs, the dose should be increased or the drug should be changed. These controversies underline the need for a full dermatological assessment during the patient selection process to ensure that the EHT course is carried out in accordance with guidelines that apply to other light-based therapies as well.

To conclude, the EHT courses improved the HRQoL of psoriasis and AD patients for at least 3 months and alleviated the severity of their disease. Future courses for AD patients could be arranged during a lower UV index season, and the length of the course for psoriasis patients could be adjusted according to their disease severity. A clinically significant improvement in HRQoL was only shown by patients with moderate-to-severe symptoms, suggesting that EHT should be targeted primarily at these cases. Post-EHT care should be developed further to sustain the empowering effects. Future investigations should be focused on comparing domestic empowerment and EHT courses in terms of their short and long-term effects on psoriasis and AD.

10 Conclusions and future prospects

The conclusions and future prospects arising from the present investigations into the effects of NB-UVB and solar UVR on vitamin D synthesis and the effects of empowering heliotherapy on the quality of life and disease activity in dermatological patients, may be set out as follows:

NB-UVB phototherapy increases S-25(OH)D levels in subjects receiving supplementations and can be used to maintain these levels during the winter in subjects not receiving supplementations.

We showed that wintertime NB-UVB exposures increased the S-25(OH)D level significantly in vitamin D-sufficient psoriasis patients and healthy subjects who were receiving 20 µg oral cholecalciferol supplements daily. Levels started to decrease one month after the exposures, however, despite the cholecalciferol supplementation, demonstrating the effectiveness of NB-UVB exposures for improving vitamin D status. This is consistent with reports that NB-UVB exposures have raised S-25(OH)D level more markedly than oral cholecalciferol at doses of 20 µg or 40 µg daily (Ala-Houhala et al. 2012a, Bogh et al. 2012a). An equilibrium between NB-UVB exposures and oral cholecalciferol seems to be reached with a daily dose of the latter of approximately 50 µg (Lagunova et al. 2013). Since psoriasis patients not receiving supplements experience a greater increase in S-25(OH)D levels during phototherapy than did our patients who were receiving supplements (Osmanovic et al. 2009b, Vähävihi et al. 2010a, Ryan et al. 2010, Lesiak et al. 2011, Romaní et al. 2012), there seems to be no need to use supplementation at all during phototherapy.

We also showed that post-summer S-25(OH)D levels can be maintained and even increased during the winter by means of suberythemal NB-UVB exposures of 2 SED given every second week, a dose which can theoretically cause immunosuppression. NB-UVB phototherapy has not been shown clinically to increase the risk of skin cancer (Hearn et al. 2008), unlike the popular tanning beds, which emit mainly UVA radiation (Boniol et al. 2012). More long-term follow-up data would be needed, however, before NB-UVB exposures could with confidence be considered safe.

Millions of citizens in the Nordic countries travel to sunny destinations each year, especially during the winter, a large proportion of them for sunbathing purposes. A one-week holiday in the sun can raise a person's S-25(OH)D level by more than 20 nmol L^{-1} , but the production of vitamin D correlates with the UVR-induced DNA damage (Petersen et al. 2014). Thus, although the increase in S-25(OH)D levels acquired during a 2-week holiday in the sun can last for more than 2 months and prevent wintertime vitamin D insufficiency (Vähävihi et al. 2008), suberythemal NB-UVB exposures resulting in continuous vitamin D synthesis could potentially be a safer method.

Scandinavians have a significantly higher incidence of malignant cutaneous melanomas than other Europeans (Forsea et al. 2012). It is known that intermittent UVR exposures such as sunny holidays are a risk factor for melanoma, but it has recently been speculated that the role of sunburn may not be so straightforward. Interestingly, outdoor workers who are constantly exposed to the sun and experience frequent sunburns (Glanz et al. 2007) have an increased risk of basal cell and squamous cell carcinoma (Trakatelli et al. 2016), but only about half the risk of developing melanoma that indoor workers have (Gandini et al. 2005). Merrill et al. (2015) demonstrated that the increase in the incidence of melanoma since the 1960's correlates linearly with the

decrease of personal annual UV doses, and proposed that the lack of constant cutaneous vitamin D production might be a stronger risk factor for melanoma than sunburn. If this is true, maintaining the cutaneous production of vitamin D in subjects living at high latitudes during the winter by means of NB-UVB exposures offers attractive future prospects. More research is needed, however, to assess the benefits and potential risks of such exposures.

Cyclic suberythemal NB-UVB exposures could be targeted at psoriasis and AD patients, who often present with vitamin D insufficiency (Gisondi et al. 2012, Cheng et al. 2014) and experience alleviation of skin symptoms as a result of NB-UVB phototherapy. Cyclic NB-UVB could also be used for haemodialysis patients, who can suffer from more severe vitamin D deficiency and respond slowly to oral supplementation (Kandula et al. 2011). On the other hand, phototherapy cannot be used for immunosuppressed organ transplant patients, who have at least a 65-fold increase in the risk of squamous cell cancer (Hofbauer et al. 2010). Thus, NB-UVB exposures cannot be contemplated for patients who have received or could potentially receive a kidney transplant.

In summary, our present knowledge of NB-UVB therapy suggests that it is a relatively safe modality with established health benefits such as clearance of psoriasis and AD and improvement of vitamin D status. Maintenance exposures with NB-UVB could be considered for psoriasis, atopic dermatitis and haemodialysis patients in selected cases. The health benefits of small, repeated UVB radiation doses, providing constant cutaneous vitamin D production without sunburn, probably outweigh any harm that they may cause, but the roles of cutaneous vitamin D production and NB-UVB maintenance exposures in skin diseases and skin cancer still need to be assessed further.

Vernal solar UV radiation is capable of raising S-25(OH)D levels in patients with baseline values below 90 nmol L⁻¹.

We demonstrated a highly significant increase in S-25(OH)D as a result of vernal solar UV radiation at a high latitude in patients with baseline levels below 90 nmol L⁻¹, whereas levels above that decreased. The mean total UVB dose was 12 SED during a mean of 12 hours spent out of doors. Petersen et al. (2014) demonstrated an S-25(OH)D increase of 8.6 nmol L⁻¹ in Danish skiers at a lower latitude (47°N) during one week in March. The subjects received a UVB dose of 109 SED on 4% of their total skin area when this was covered with a sunscreen, but even so the high UVB dose resulted in elevated urinary thymine dimers as a sign of DNA damage. At a higher latitude in Norway (68°N) vernal exposure of the facial area yielded no significant increase in the mean S-25(OH)D level (Edwardsen et al. 2007), although the subjects with S-25(OH)D below 30 nmol L⁻¹ were shown to respond to vernal UVR, which agrees with our findings in subjects with levels below 90 nmol L⁻¹.

The vernal increase in S-25(OH)D in subjects with lower baseline levels is a solid finding and should be examined more thoroughly in both snow-covered and non-snow-covered terrain. Subjects who engage in frequent outdoor activities can benefit from vernal solar UVB in the form of improved vitamin D status. In light-skinned subjects, a sufficiency of vitamin D seems to reflect a life-style that favours physical exercise, weight-control and outdoor activities. An association between vitamin D and lifestyle factors has indeed been shown recently in a large sample of French adults by Touvier et al. (2015), who noted that vitamin D deficiency and insufficiency were more frequent in obese, underweight, less physically active and sun-avoiding subjects, and it was also the case that high latitude increased the risk of vitamin D deficiency. Most importantly, vitamin D

status showed an improvement even in the second quartile of the patients in terms of the amount of exposure to sun, being stable over the third and fourth quartiles, suggesting the benefits of low-dose daily exposure. This finding should encourage people to aim at sensible rates of exposure. Physical activity was correlated with better vitamin D status independently of sun exposure, and a substantial impact on vitamin D status (+33%) was observed for normal weight subjects as compared with obese ones. These findings are in line with a suggestion by Pannu et al. (2016) that weight-loss can improve vitamin D status. It would be interesting to study in a randomized setting how vitamin D status would change in response to regular exercise and dietary instructions as compared with oral supplements.

To conclude, a low dose of vernal solar UVR seems to induce cutaneous vitamin D synthesis in subjects with low baseline levels according to present work and an earlier Norwegian study (Edvardsen et al. 2007). The face and hands, or face alone, will receive enough UVR, but whether reflections from the snow constitute an extra factor needed for this effect is at present unknown.

Empowering heliotherapy improves quality of life and alleviates disease severity in psoriasis and atopic dermatitis patients.

The two-week EHT course improved the HRQoL and alleviated disease severity in the psoriasis and AD patients for at least 3 months, but clinically significant DLQI improvement was achieved by less than half of the patients. The SAPASI score remained reduced by 35% relative to the baseline for 3 months after EHT, which was in agreement with results presented by Wahl et al. (2015). Since better results have been achieved through traditional four-week HT courses

(Snellman et al. 1993, Harari et al. 2007), it would be best to adjust the length of the course for psoriasis patients according to the severity of their disease.

The PO-SCORAD index was still 40% below the baseline score 3 months after the EHT course, in agreement with results presented by Autio et al. (2002), and the patients reported on by Vähävihiu et al. (2008) who received a much higher UVB dose in January and March than ours did in November had an essentially similar PO-SCORAD outcome. Thus, EHT courses for AD patients could be arranged during a lower UV index season or using a more careful sunbathing protocol. Two weeks seems to be an appropriate length of course for AD patients (Autio et al. 2002).

Empowerment methods have been shown to improve the HRQoL of patients with a chronic skin disease (de Bes et al. 2011), but the challenge is how to maintain the effects in the long term (Wahl et al. 2015). Post-EHT care should be further developed by the patient organizations with the aim of sustaining the empowering effects of EHT. As over half of our patients failed to achieve a clinically significant 3-month improvement in DLQI, it may be necessary to use stricter inclusion criteria for future EHT courses. Generally, patients with moderate-to-severe symptoms achieved clinically significant improvements in their DLQI scores, whereas patients with mild symptoms failed to benefit from EHT. Patients with mild symptoms should primarily be steered to domestic empowerment courses, the effects of which should be assessed in future studies. For patients with moderate-to-severe symptoms, EHT is a useful treatment modality which combines patient education, empowerment methods and treatment of the skin symptoms with phototherapy. Even in this group, however, the costs of a 2-week EHT course combined with the costs of 2 weeks of absence from work, might result in a weak cost-efficacy ratio. A

dermatologist's assessment of the potential risks and benefits of the therapy in individual cases should be sought during patient selection.

To sum up, NB-UVB exposure is effective in raising and maintaining S-25(OH)D levels in psoriasis patients and healthy subjects. Vernal solar UVR can induce vitamin D synthesis and raise S-25(OH)D levels in subjects with a baseline level below 90 nmol L⁻¹. Empowering heliotherapy improves the HRQoL to a clinically significant extent in patients with moderate-to-severe psoriasis or AD. The risks and benefits of repeated NB-UVB exposures, and the role of cutaneously produced vitamin D in the development of dermatoses and skin cancer, should be investigated further.

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ORIGINAL PUBLICATIONS

INVESTIGATIVE REPORT

Narrow-band Ultraviolet B Treatment Boosts Serum 25-hydroxyvitamin D in Patients with Psoriasis on Oral Vitamin D Supplementation

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A course of treatment with narrow-band ultraviolet B (NB-UVB) improves psoriasis and increases serum 25-hydroxyvitamin D (25(OH)D). In this study 12 patients with psoriasis who were supplemented with oral cholecalciferol, 20 µg daily, were given a course of NB-UVB and their response measured. At baseline, serum 25(OH)D was 74.14 ± 22.9 nmol/l. At the 9th exposure to NB-UVB 25(OH)D had increased by 13.2 nmol/l (95% confidence interval (95% CI) 7.2–18.4) and at the 18th exposure by 49.4 nmol/l (95% CI 35.9–64.6) above baseline. Psoriasis Area Severity Index score improved from 8.7 ± 3.5 to 4.5 ± 2.0 ($p < 0.001$). At baseline, psoriasis lesions showed low vitamin D metabolizing enzyme (CYP27A1, CYP27B1) and high human β -defensin-2 mRNA expression levels compared with those of the healthy subjects. In conclusion, NB-UVB treatment significantly increases serum 25(OH)D in patients with psoriasis who are taking oral vitamin D supplementation, and the concentrations remain far from the toxicity level. Healing psoriasis lesions show similar mRNA expression of vitamin D metabolizing enzymes, but higher antimicrobial peptide levels than NB-UVB-treated skin in healthy subjects. Key words: psoriasis; ultraviolet B radiation; vitamin D; CYP27A1; CYP27B1; cathelicidin; human β -defensin.

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Vitamin D insufficiency is common in Europe and North America, especially during the winter when vitamin D synthesis induced by sunlight is zero (1, 2). The desirable concentration of serum 25-hydroxyvitamin D (25(OH)D), which is the best indicator of vitamin D status, is still under debate. A concentration below 75 nmol/l (30 ng/ml) is considered to be insufficient for bone fracture prevention (3). In addition to osteoporosis, low serum 25(OH)D concentration has recently been associated with risk of colorectal cancer and cardiovascular disease (4,

5). Vitamin D is also known to affect skin inflammation and innate or adaptive immune responses (6, 7).

Recent studies suggest that vitamin D insufficiency is also common in patients with psoriasis (8–10). Gisondi et al. (8) found that, in Italy in winter, serum 25(OH)D was below 50 nmol/l in 81% of patients with psoriasis compared with 30% of healthy controls. They also showed that vitamin D insufficiency was associated with psoriasis independently of age, sex and body mass index (BMI). Romani et al. (10) concluded that the insufficiency was also common in patients with psoriasis and controls in Spain, but in their study carefully matched controls had a higher insufficiency rate than patients with psoriasis.

Narrow-band UVB (NB-UVB) phototherapy, a widely used effective treatment for psoriasis (11), suppresses interferon-gamma (IFN- γ) and interleukin (IL)-17 signalling pathways to resolve psoriatic inflammation (12). NB-UVB light emitting at 311–313 nm is also capable of activating vitamin D synthesis in cultured keratinocytes (13). Moreover, several studies have shown that, in addition to healing of psoriasis, NB-UVB treatment significantly increases serum 25(OH)D (14–17), and this increase correlates with the activation of circulating regulatory T cells (18).

Interestingly, the expression of cathelicidin, which is one of the most important antimicrobial peptides in human skin, is dependent on 1,25-dihydroxyvitamin D (1,25(OH)₂D) and is triggered by UVB-induced vitamin D metabolism (6, 19). Cathelicidin and another inducible cutaneous antimicrobial peptide, human β -defensin-2 (HBD2), can act as immune-regulating effectors or “alarmins” and link adaptive and innate immune responses (20). In addition, these antimicrobial peptides seem to have a role in the control of skin inflammation in psoriasis (21, 22).

The present study examined whether NB-UVB treatment can increase serum 25(OH)D in patients with psoriasis who are already supplemented with oral vitamin D. In addition, we investigated the effects of NB-UVB exposure on cutaneous mRNA expression of vitamin D-metabolizing enzymes and antimicrobial peptides.

METHODS

Patients with psoriasis and healthy subjects

A total of 12 patients with psoriasis (mean age 42.8 years; Table I) participated in the study. Four patients had also psoriatic arthritis, but none of them received any systemic drug treatment because their arthritis was not active during the study. Inclusion criteria were no phototherapy, solarium or sunny holidays during the 2 preceding months. Before the NB-UVB course the patients had used cholecalciferol 20 µg (800 IU) daily for a mean of 3.3 months (Table I). Fifteen nurses and other hospital employees (mean age 46.1 years; Table I) volunteered as controls in the study. These subjects had used oral cholecalciferol for a mean of 3.4 months (Table I). The patients with psoriasis and the healthy subjects continued to use oral cholecalciferol during the NB-UVB course and subsequent to it.

The study protocol was approved by the ethics committee of Tampere University Hospital and all subjects gave their informed consent to participate. The study protocol followed the principles of the Declaration of Helsinki.

Narrow-band UVB exposure

The study was performed in winter from December 2011 to April 2012 in order to exclude the effect of the sun. The study subjects received NB-UVB exposure 3 times a week on the whole body area with a Waldmann UV 7001 cabin equipped with 40 TL01 tubes (Schulze & Böhm, Brühl, Germany). The first NB-UVB dose was 0.19 J/cm² (1.11 standard erythema dose (SED)) and it was gradually increased each time, according to a fixed protocol, up to 9 exposures, i.e. to 0.97 J/cm² (5.70 SED). If the subjects experienced mild itching or erythema, the next NB-UVB dose was either not increased or was reduced. This was the case in 6 patients with psoriasis and 8 healthy subjects. Thereafter, the NB-UVB treatment was given only to patients with psoriasis until the rash was almost or totally cleared. This took a mean of 20.5 (range 11–31) NB-UVB exposures. Clinical improvement was measured with the Psoriasis Area Severity Index (PASI) score.

The mean cumulative dose of NB-UVB given to the 12 patients with psoriasis during 9 exposures was 4.49 ± 0.44 J/cm² and to the 9 patients during 18 exposures 15.63 ± 1.67 J/cm². These doses are equivalent to 26.4 ± 2.6 SED and to 91.9 ± 9.8 SED, respectively. One SED is equivalent to 10 mJ/cm² Commission Internationale de l'Eclairage (CIE) erythema-weighted irradiance. In the 15 healthy subjects the mean cumulative dose of 9 NB-UVB exposures was 4.37 ± 0.55 J/cm², which is equivalent to 25.7 ± 3.2 SED. The cumulative NB-UVB doses given up to 9 exposures to the patients with psoriasis and healthy subjects did not differ ($p=0.57$).

Measurement of serum 25-hydroxyvitamin D concentrations

Blood samples for serum 25(OH)D measurements were taken at baseline, and at 9th and 18th NB-UVB exposures. Follow-up

Table I. Demography and use of oral cholecalciferol before narrow-band ultraviolet B (NB-UVB) course in 12 patients with psoriasis and 15 healthy subjects

	Psoriasis patients	Healthy subjects	<i>p</i> -value
Male/female, <i>n</i>	7/5	1/14	0.008
Age, years, mean ± SD	42.8 ± 14	46.1 ± 11	0.47
Body mass index, kg/m ² , mean ± SD	29.6 ± 5.4	23.4 ± 3.8	0.002
Fitzpatrick skin type II/III/IV, <i>n</i>	3/6/3	3/10/2	0.66
Oral cholecalciferol, 20 µg daily before NB-UVB course, months, mean (range)	3.3 (1–24)	3.4 (1–24)	0.75

SD: standard deviation.

samples were taken one month after the NB-UVB course. The samples were protected from light, centrifuged and then stored at –70°C. Serum 25(OH)D concentration was analysed in duplicates using radioimmunoassay (Immunodiagnostic Systems, Boldon, UK), as described previously (23).

Skin biopsies and quantitative real-time PCR

Punch biopsies were taken from skin lesions of 12 patients with psoriasis (8 from the buttocks, 2 from elbows, and 2 from lower back) and from the buttocks of 13 healthy subjects at baseline and at the 9th NB-UVB exposure. The biopsies were immediately frozen and stored at –70°C. 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 expression levels of CYP27A1 and CYP27B1 enzymes, and antimicrobial peptides cathelicidin and HBD2 were evaluated using a LightCycler® 2.0 system and the corresponding human Universal Probe Library Set (Roche), as described previously (15).

Statistical analysis

Statistical comparison between the groups was performed by Student's *t*-test, permutation test or χ^2 test, when appropriate. The changes within patients with psoriasis and healthy subjects were analysed by applying a permutation test to related samples. Repeated measures were analysed using generalizing estimating equation models with the unstructured correlation structure using bootstrap-type standard error.

RESULTS

Serum 25(OH)D concentrations at baseline, during and after NB-UVB course

At baseline, serum 25(OH)D concentration was 74.14 ± 22.9 nmol/l (mean ± SD) in the 12 patients with psoriasis and 74.30 ± 14.8 nmol/l in the 15 healthy subjects. At 9th NB-UVB exposure serum 25(OH)D had increased by 13.2 nmol/l (95% CI 7.2–24.9, $p=0.0029$) in the patients with psoriasis and by 17.0 nmol/l (95% CI 6.7–21.0, $p<0.001$) in the healthy subjects (Fig. 1, Table II).

At 18th NB-UVB exposure 25(OH)D had increased by 49.4 nmol/l (95% CI 35.9–64.6, $p=0.0039$) in the 9 patients with psoriasis (Table II). PASI score improved in the patients with psoriasis from 8.7 (range 4.0–16.2) at baseline to 6.4 (range 2.1–12.8) at 9th and to 4.5 (range 1.1–8.2) at 18th exposure ($p<0.001$; Table II).

One month after NB-UVB exposure, serum 25(OH)D was still increased from baseline by 29.9 nmol/l (95% CI 13.6–49.0; $p=0.0078$) in the 8 patients with psoriasis and by 17.5 nmol/l (95% CI 10.1–24.9; $p<0.001$) in the 15 healthy subjects (Table II).

Antimicrobial peptide and enzyme mRNA expression in skin biopsy specimens

At baseline, the mRNA expression levels of CYP27A1 and CYP27B1 were significantly lower ($p<0.001$) in

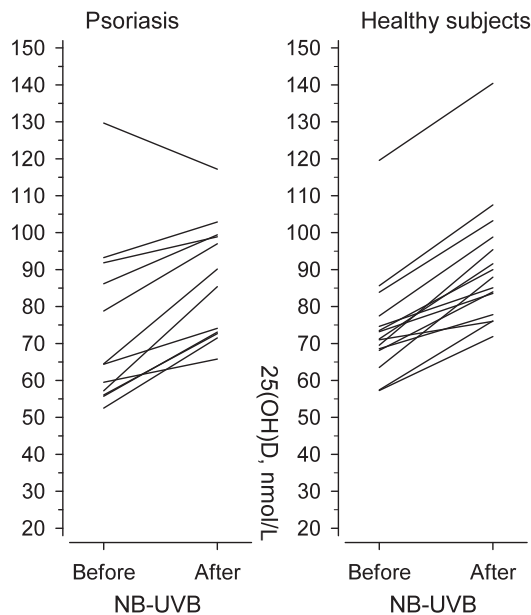


Fig. 1. Serum 25-hydroxyvitamin D (25(OH)D) concentrations before and at 9th (after) narrow-band ultraviolet B (NB-UVB) exposure in 12 patients with psoriasis and 15 healthy subjects. The patients with psoriasis and healthy subjects received oral cholecalciferol, 20 µg daily. The increase is significant in the patients with psoriasis ($p=0.003$) and healthy subjects ($p<0.001$).

the patients with psoriasis than in healthy subjects (Fig. 2A and B, Table III). At baseline cathelicidin mRNA expression levels were similar in the psoriasis lesions and in the normal skin of healthy subjects, whereas HBD2 mRNA levels were significantly ($p<0.001$) higher in the psoriasis lesions (Fig. 2C, Table III).

NB-UVB exposure did not change CYP27A1, CYP27B1 and cathelicidin mRNA expression levels in the patients with psoriasis, but a significant ($p=0.002$) decrease was seen in the HBD2 mRNA expression level (Fig. 2 and Table III). In the healthy subjects NB-UVB exposure significantly decreased CYP27A1, CYP27B1 and cathelicidin mRNA expression levels, while HBD2 increased slightly (Fig. 2, Table III).

DISCUSSION

Several recent studies have demonstrated that NB-UVB, a widely used treatment for psoriasis (11), significantly improves serum 25(OH)D concentrations, especially

during the winter (10, 14–17). The number of NB-UVB exposures given in these psoriasis studies varied from 15 to 27 and the increase in serum 25(OH)D ranged from 66% to 163% (Table IV). In contrast to these previous studies, the present patients with psoriasis were additionally supplemented with oral 20 µg of cholecalciferol daily for a mean of 3.3 months before entry into the trial. Due to this pre-treatment their mean serum 25(OH)D was 74 nmol/l at baseline, which is twice as high as in our previous study (14). Nevertheless, UVB treatment further increased serum 25(OH)D, by 17% at the 9th and by 58% at the 18th NB-UVB exposure. Although the BMI was significantly lower in the healthy subjects than in the patients with psoriasis, the baseline 25(OH)D concentration and the increase at 9th NB-UVB exposure was of approximately the same magnitude. This finding is somewhat unexpected because obese subjects are more prone to vitamin D insufficiency due to the deposition of vitamin D precursors in fat tissue (2, 24). In a recent study (25) in which subjects were supplemented with 15 µg oral cholecalciferol daily, BMI in older, but not in younger, adults was shown to be negatively associated with the change in serum 25(OH)D. These results indicate that BMI is important when performing vitamin D studies, and vitamin D insufficiency reported in some psoriasis studies could be attributed to obesity and comorbidities associated with severe psoriasis. The limitation of the present study is that the patients with psoriasis and the healthy subjects were not matched for BMI. However, the similar and significant increases in serum 25(OH)D levels in both groups who were continuously supplemented with a rather high dose of oral vitamin D indicate that NB-UVB exposure is an efficient way to improve vitamin D balance. In agreement with this, 2 recent studies in healthy subjects have documented the superiority of NB-UVB exposure over oral supplementation of cholecalciferol, 20 µg and 40 µg daily, to improve serum 25(OH)D concentration (23, 26).

In the present study the mean increase in serum 25(OH)D at the 18th NB-UVB exposure was 58% and the highest individual concentration measured 155 nmol/l. Overall, the increase observed was not as marked as in previous psoriasis studies (Table IV). This is not unexpected, because it appears to be evident that the lower the starting 25(OH)D concentration the higher

Table II. Narrow-band UVB (NB-UVB) doses, serum 25-hydroxyvitamin D (25(OH)D) concentrations and Psoriasis Area Severity Index (PASI) scores in 12 patients with psoriasis and 15 healthy subjects

	At baseline	At 9 th NB-UVB exposure	At 18 th NB-UVB exposure	1 month after NB-UVB course
Patients with psoriasis, <i>n</i>	12	12	9	8
Total NB-UVB dose, J/cm ² , mean ± SD	–	4.5 ± 0.4	15.6 ± 1.7	–
25(OH)D; nmol/l, mean ± SD	74.1 ± 22.9	87.3 ± 16.0	117.3 ± 28.9	115.0 ± 26.5
PASI score, mean ± SD	8.7 ± 3.5	6.4 ± 3.1	4.5 ± 2.0	–
Healthy subjects, <i>n</i>	15	15	–	15
Total NB-UVB dose, J/cm ²	–	4.37 ± 0.6	–	–
25(OH)D, nmol/l, mean ± SD	74.3 ± 14.8	91.3 ± 17.1	–	88.3 ± 19.9

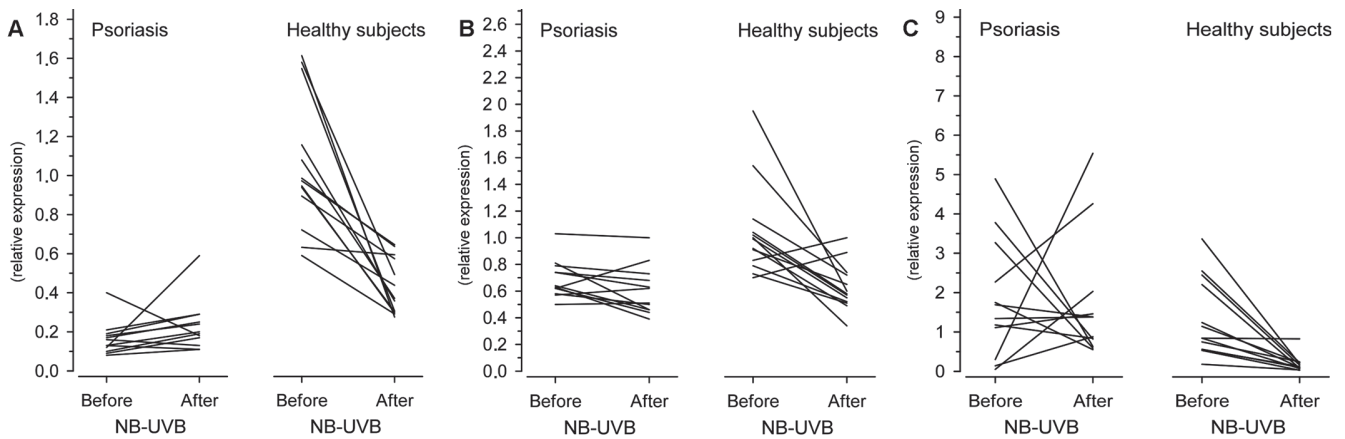


Fig. 2. (A) CYP27A1 mRNA, (B) CYP27B1 mRNA and (C) cathelicidin mRNA expression levels in skin lesions of patients with psoriasis ($n=12$) and normal skin of healthy subjects ($n=13$) before and at 9th (after) narrow-band ultraviolet B (NB-UVB) exposure. Before NB-UVB course the CYP27A1 and CYP27B1 levels are significantly lower ($p<0.001$), but the cathelicidin levels do not differ ($p=0.34$) in the patients with psoriasis compared with the healthy subjects. NB-UVB exposure did not change CYP27A1 mRNA, CYP27B1 mRNA or cathelicidin mRNA levels in the patients with psoriasis (A: $p=0.17$; B: $p=0.070$; C: $p=0.88$) but the decrease is significant (A: $p<0.001$; B: $p=0.002$; C: $p<0.001$) in the healthy subjects.

the response to UVB treatment (27). Moreover, there is evidence that during UVB exposure to the skin, but not during oral supplementation, a negative feedback mechanism controls vitamin D synthesis to prevent overdosing and vitamin D toxicity (28). It is noteworthy in this context, that 1 of the present patients had an exceptionally high level of serum 25(OH)D, i.e. 130 nmol/l at baseline and she was the only one to respond to NB-UVB exposure with decreased serum 25(OH)D.

In addition, although the patients and healthy subjects continued with oral cholecalciferol supplementation, serum 25(OH)D concentrations started to decrease one month after NB-UVB exposure. This is in agreement with our previous NB-UVB studies (15, 23) and demonstrates that even rather high continuous oral vitamin D supplementation is not sufficient to maintain serum 25(OH)D levels achieved by a short course of NB-UVB exposure. It has been reported that NB-UVB exposure given twice a month maintains the levels of 25(OH)D achieved in the summer (29). Therefore, a similar schedule could be applicable to the NB-UVB-treated patients with psoriasis during the winter in order to maintain sufficient vitamin D.

A previous study in UVB-treated organ cultures showed that CYP27A1 and CYP27B1 in the keratinocytes are capable of hydroxylating precursors into the active form of vitamin D, i.e. 1,25(OH)₂D (13). Thus, both

enzymes could be regarded as surrogate markers for vitamin D metabolism. In the present study the expression levels of CYP27A1 and CYP27B1 at baseline were low in the psoriatic lesions compared with healthy skin and did not change during NB-UVB treatment. At first, the low baseline level of CYP27B1 in psoriasis lesions seems difficult to explain. However, the patients with psoriasis were supplemented with oral cholecalciferol, and it could be that the metabolism of vitamin D in the psoriasis lesions is far more active than in the normal skin of healthy subjects. Due to this, the low CYP27A1 and CYP27B1 activities in the psoriasis lesions at baseline and after NB-UVB exposure, and also in the normal skin after NB-UVB exposure, could be due to a very sensitive natural feedback controlling mechanism in cutaneous vitamin D synthesis (6, 7).

Antimicrobial peptides seem to have a role in the pathogenesis of skin inflammation in psoriasis (21, 30). In agreement with our previous study (15), we found in psoriasis lesions at baseline significantly increased mRNA expression of HBD2. We could also show that repeated NB-UVB exposure reduced HBD2 expression in healing psoriasis lesions. In contrast to HBD2, we found no increased mRNA expression of cathelicidin in the psoriasis lesions, either at baseline or after NB-UVB exposure. This is surprising with regard to the findings of our previous study (15). The continuous

Table III. Vitamin D metabolizing enzyme and antimicrobial peptide mRNA expression levels in the psoriasis lesions of 12 patients and normal skin of 13 healthy subjects at baseline and at 9th narrow-band UVB (NB-UVB) exposure

	Psoriasis			Healthy subjects		
	Baseline Mean \pm SD	At 9 th NB-UVB Mean \pm SD	<i>p</i> -value	Baseline Mean \pm SD	At 9 th NB-UVB Mean \pm SD	<i>p</i> -value
CYP27A1	0.16 \pm 0.85	0.23 \pm 0.13	0.17	1.05 \pm 0.34	0.43 \pm 0.14	<0.001
CYP27B1	0.69 \pm 0.14	0.60 \pm 0.18	0.070	1.04 \pm 0.35	0.63 \pm 0.18	0.0017
Cathelicidin	1.81 \pm 1.51	1.70 \pm 1.58	0.88	1.32 \pm 0.99	0.12 \pm 0.08	<0.001
Human β -defensin-2	41,739 \pm 65,700	1,497 \pm 1,037	0.002	159.65 \pm 302.49	471.84 \pm 328.71	0.041

Table IV. Narrow-band UVB (NB-UVB) treatments given in different studies to patients with psoriasis in winter significantly increase serum 25-hydroxyvitamin D (25(OH)D) concentration

	Patients <i>n</i>	NB-UVB treatments <i>n</i>	25(OH)D at baseline nmol/l Mean ± SD	25(OH)D at the end nmol/l Mean ± SD	Increase %
Present study, Finland ^a	12	9	74.1 ± 22.9	87.3 ± 16.0	17
	9	18		117.3 ± 28.9	58
Romani et al., 2012 (10), Spain	50	27 ^b	36.0 ± 26.3	77.0 ± 33.5	113
Lesiak et al., 2011 (17), Poland	17	10	60.5	101.8	68
	17	20		106.8	73
Ryan et al., 2010 (16), Ireland	29	18 ^b	57.5	147.5	155
Vähävihi et al., 2010 (15), Finland	18	15	36.8 ± 12.5	96.7 ± 13.4	163
Osmancevic et al., 2009 (14), Sweden	18	26 ^b	71 ± 17	118 ± 39	66

^aPatients supplemented with oral cholecalciferol, 20 µg daily.

^bMean.

oral supplementation with vitamin D before the study, with possible accumulation of vitamin D precursors and 1,25(OH)₂D in the psoriasis lesions and activation of negative feedback mechanisms, could again be a reason for this finding. Overall, the present cathelicidin and vitamin D-metabolizing enzyme gene expression results suggest that the normal skin of healthy subjects reacts more actively to NB-UVB exposure than the inflamed skin of psoriasis lesions. It is, however, noteworthy that in spite of these gene expression differences the patients with psoriasis and healthy subjects showed similar NB-UVB responses in the serum 25(OH)D concentration.

Knowledge of the risk of vitamin D insufficiency is well-accepted. In Finland, for example, there are recommendations for children and pregnant women to use up to 10 µg and for elderly people up to 20 µg daily of oral vitamin D supplementation (31). Moreover, vitamin D products are actively marketed and voluntary supplementation especially during winter months is now also common among dermatological patients. Many patients with psoriasis will receive NB-UVB treatment, which is effective and, in the short-term, is considered safe with regard to risk of skin malignancy (32, 33). The present NB-UVB study documented that even though the patients with psoriasis received oral cholecalciferol 20 µg daily their serum 25(OH)D concentrations remained far from 250 nmol/l. Levels below this are considered safe with regard to toxicity (34) and, therefore, we conclude that there is no need to stop voluntary oral vitamin D supplementation whenever patients with psoriasis need to start NB-UVB treatment. In addition, in the present study patients with psoriasis supplemented with oral cholecalciferol showed a similar significant decrease in PASI score, as did the patients with no oral vitamin D supplementation in our previous study (15). This suggests that the response to NB-UVB treatment in psoriasis is not dependent on whether the patient is supplemented with oral vitamin D.

In conclusion, this study showed that, although the patients with psoriasis received continuous oral cholecalciferol supplementation, NB-UVB exposure increased serum 25(OH)D concentration by 58%. In

healing psoriasis lesions NB-UVB treatment did not alter the expression of vitamin D-metabolizing enzyme and cathelicidin mRNA, but decreased the expression of HBD2 mRNA.

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The authors declare no conflicts of interest.

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INVESTIGATIVE REPORT

Narrowband Ultraviolet B Exposures Maintain Vitamin D Levels During Winter: A Randomized Controlled Trial

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Exposure to solar ultraviolet B radiation during the summer months is the main source of vitamin D (VD) for people living in northern latitudes. The aim of this study was to determine whether artificial narrowband ultraviolet B (NB-UVB) whole-body exposures could maintain VD levels in winter. The intervention group received 2 standard erythema doses (SEDs) of NB-UVB exposures every second week from October 2013 to April 2014. In October 2013 serum 25-hydroxyvitamin D concentrations were 78.3 nmol/l in the intervention group ($n=16$) and 76.8 nmol/l in the control group ($n=18$). By April 2014 the concentrations had increased by 11.7 nmol/l ($p=0.029$) in the intervention group and decreased by 11.1 nmol/l ($p=0.022$) in the control group. The baseline VD concentration showed a negative correlation ($p=0.012$) with body mass index (BMI). In conclusion, a suberythema NB-UVB dose of 2 SED every second week maintains and even increases serum VD concentrations during the winter. A high BMI seems to predispose subjects to low levels of VD. *Key words: 25-hydroxyvitamin D; ultraviolet B; narrow-band ultraviolet B; body mass index.*

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Vitamin D (VD) insufficiency is a worldwide issue (1). VD is synthesized from 7-dehydrocholesterol in response to ultraviolet B (UVB) radiation and its key role is in adjusting the serum calcium level to enable metabolic functions, signal transduction and neuromuscular activity (2). VD insufficiency has been linked to chronic skeletal (3) and extra-skeletal diseases, such as obesity and type 2 diabetes mellitus (4, 5). The best indicator of VD status is its circulating form, 25-hydroxyvitamin D [25(OH)D] (2). Levels above 50 nmol/l are thought to be sufficient for calcium and bone homeostasis, but the optimal level for extra-skeletal effects is unclear (6). The Institute of Medicine (Washington DC, USA) recommends a dietary intake of VD supplements of 15

µg daily for people aged 1–70 years and 20 µg daily for those older than 70 years (7). In addition to VD supplements, artificial ultraviolet B (UVB) light treatments increase VD concentrations (8). Narrowband ultraviolet B (NB-UVB) exposures given 3 times a week increase serum 25(OH)D concentrations even more than does 20 µg or 40 µg oral cholecalciferol daily (9, 10). Bogh et al. (11) showed that 1 standard erythema dose (SED) of broadband ultraviolet B (BB-UVB) every second week can be used to maintain serum 25(OH)D concentrations during the winter (11). On the other hand, as NB-UVB is better tolerated (12), widely used (13), and provides a higher vitamin D action spectrum-weighted irradiance dose (14), we examined its ability to maintain summer levels of vitamin D throughout the winter period.

MATERIALS AND METHODS

Subjects

Thirty-seven healthy volunteers were randomized to an intervention group ($n=18$) or a control group ($n=19$). Inclusion criteria were: age 18 years or older; and avoidance of solarium visits, phototherapy, sunny holidays and vitamin D supplementation during a 1-month washout period prior to the trial and during it. Exclusion criteria were: pregnancy, skin disease, previous skin cancer, intake of photosensitizing drugs; and Fitzpatrick's skin reactive type 1 (15). Recruitment began on 1 September 2013 and the trial was carried out at the Department of Dermatology of Tampere University Hospital from 7 October 2013 to 5 May 2014. The principal investigator assessed the skin types of the volunteers. VD intake at the onset was estimated by means of a 3-day food frequency questionnaire. Altogether 34 subjects completed the trial (Table I). Two intervention subjects were disqualified for failing to follow the irradiation schedule and one control subject was disqualified for taking VD supplements. All 3 were excluded from the analyses. The protocol was approved by the ethics committee of Tampere University Hospital, and all the volunteers gave their informed consent in advance.

Randomization and sample size calculation

Volunteers were randomized to the intervention and control groups in blocks of 2 using a web-based validated program (Research Randomizer (<http://www.randomizer.org>)). The primary investigator randomized and enrolled all the participants. The trial was designed to show an inter-group difference in 25(OH)D of at least 12 nmol/l, with an α -value of 0.05 and a β -value of 0.90. An assumed standard deviation (SD) of 9 nmol/l for the

Table I. Demographics, vitamin D intake and plasma parathyroid hormone concentrations at baseline in the narrow-band ultraviolet B (NB-UVB)-treated and control groups

	NB-UVB n=16	Control n=18	p-value
Males/females, n	3/13	1/17	0.32
Age, years, mean (range)	35 (21–61)	36 (20–61)	0.76
BMI, kg/m ² , mean ± SD	23.0 ± 1.9	25.2 ± 3.4	0.029
Fitzpatrick's skin type II/III/IV, n	8/7/1	8/10/0	0.61
Vitamin D intake, µg/day, mean ± SD	7.0 ± 3.7	6.7 ± 2.2	0.78
Parathyroid hormone, pmol/l, mean ± SD	3.8 ± 1.1	4.2 ± 1.2	0.32

BMI: body mass index; SD: standard deviation.

25(OH)D analyses at 50 nmol/l was used. Thus, it was considered necessary that 12 volunteers per group should complete the trial.

Narrowband ultraviolet B treatment

The intervention group received a total of 13 NB-UVB whole-body exposures, given every other week for 24 weeks with a Waldmann UV 7002 cabin equipped with 42 TL01 tubes (Schulze & Böhm, Brühl, Germany). The first NB-UVB non-weighted total UV dose was 170 mJ/cm² (1 SED), which was subsequently increased to 340 mJ/cm² (2 SED). One SED is equivalent to an erythemal effective radiant exposure of 10 mJ/cm² CIE (16). The cabin was calibrated by the Nuclear Safety Authority of Finland using an Ocean Optics S2000 spectroradiometer. After correction for stray light and other systematic errors, the estimated measurement uncertainty (2σ) of the Ocean Optics S2000 is 14% (17) and the measurements are traceable to the National Institute of Standards and Technology, USA. Previously measured lamp spectra were used for the NB-UVB (TL01) and BB-UVB (Waldmann UV6) dose calculations (18).

Serum 25-hydroxyvitamin D and parathyroid hormone measurements

Blood samples for 25(OH)D analyses were drawn at the onset, and at weeks 6, 14, 20, 26 and 30. During the intervention period the samples of the intervention group were taken just before the scheduled exposure to UVB. The samples were centrifuged and plasma was stored at -20°C and analysed for 25(OH)D by enzyme immunoassay (Roche Diagnostics, Mannheim, Germany). Plasma parathyroid hormone (PTH) samples were taken at the onset of the trial and at 14 weeks. Blood was collected into ethylenediaminetetraacetic acid (EDTA) tubes, centrifuged and analysed by immunochemiluminometric assay.

Statistical analysis

Confidence intervals (95% CI) were obtained by bias-corrected bootstrapping (5,000 replications). Statistical comparisons were made using the analysis of t-test co-variance (ANCOVA). In

the case of violation of the assumptions (e.g. non-normality) a bootstrap-type test was used. Longitudinal measures for continuous outcomes were analysed using a bootstrap-type generalized estimating equations (GEE) model, the GEE having been developed as an extension of the general linear model for analysing longitudinal and other correlated data. GEE models take into account the correlation between repeated measurements in the same subject, they do not require complete data, and a fit can be achieved even when observations for some individuals are lacking at certain time-points. No adjustment was made for multiple testing. When comparing the increases in VD concentrations, the model was adjusted for the baseline value, body mass index (BMI) and Fitzpatrick's skin type. Pearson's χ^2 -test was used when comparing nominal data. The STATA 13.1, StataCorp LP (College Station, TX, USA) statistical package was used for the analyses.

RESULTS

Vitamin D intake and NB-UVB exposures

The mean ± SD daily VD intake at onset was 7.0 ± 3.7 µg in the intervention group and 6.7 ± 2.2 µg in the control group ($p=0.78$) (Table I). The intervention group received 13 NB-UVB exposures over 24 weeks, implying a cumulative NB-UVB dose of 25 SED, which corresponds to a physical dose of 4.25 J/cm². No adverse effects were detected.

Serum 25-hydroxyvitamin D concentrations

The mean baseline serum VD concentration in October was 78.3 nmol/l in the intervention group and 76.8 nmol/l in the control group (Table II, Fig. 1) showing a moderate negative correlation with BMI ($r=-0.43$, $p=0.012$). The mean ± SD concentrations in the intervention group peaked at 104.5 ± 40.2 nmol/l in February, i.e. in week 20 (Fig. 1), and had a mean increase of 11.7 nmol/l ($p=0.029$) by the end of the intervention period, in April (week 26), at which point the mean for the control group had decreased by 11.1 nmol/l ($p=0.022$, Fig. 1, Table II). The difference between the groups was statistically highly significant ($p<0.001$) when adjusted for the baseline value, BMI and Fitzpatrick's skin type. During the 1-month follow-up period the mean concentration of VD in the intervention group decreased by 10.6 nmol/l ($p<0.001$) and that in the control group by 2.7 nmol/l ($p=0.18$; Fig. 1, Table II).

Table II. Serum 25-hydroxyvitamin D concentrations in the narrow-band ultraviolet B (NB-UVB)-treated and control groups at baseline and at the end of the intervention period (week 26)

	Serum 25-hydroxyvitamin D (nmol/l)		
	NB-UVB group n=16	Control group n=18	p-value
Baseline (October 2013), mean ± SD	78.3 ± 36.1	76.8 ± 26.6	0.90 ^a
Week 26 (April 2014), mean ± SD	88.7 ± 29.2	65.8 ± 23.9	0.019 ^a
Change from baseline to week 26, mean (95% CI)	11.7 (1.9–20.0) ^b	-11.1 (-19.4 to -2.7) ^c	0.0017 ^a
Change from week 26 to 30, mean (95% CI)	-10.6 (-15.1 to -5.9) ^d	-2.7 (-6.1–0.8) ^e	0.015 ^a

^aNot adjusted; ^b $p=0.029$; ^c $p=0.022$; ^d $p<0.001$; ^e $p=0.18$.

SD: standard deviation; 95% CI: 95% confidence interval.

Parathyroid hormone concentrations

The mean ± SD initial PTH levels were 3.8 ± 1.1 pmol/l in the intervention group and 4.2 ± 1.2 pmol/l in the control group ($p=0.32$), while those at week 14 were 3.7 ± 1.4 pmol/l and 4.7 ± 1.8 pmol/l, respectively ($p=0.11$) (Table I).

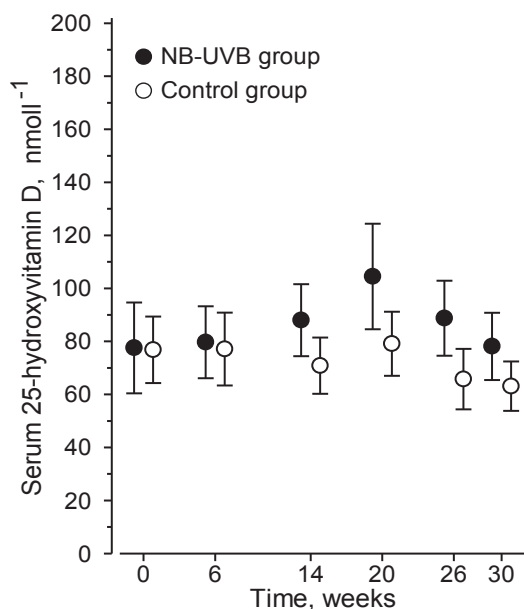


Fig. 1. 25-Hydroxyvitamin D concentrations in the narrow-band ultraviolet B (NB-UVB)-treated and control groups during the intervention (weeks 0–26) and follow-up periods (weeks 26–30).

DISCUSSION

The results of this study show that an artificial NB-UVB exposure of 2 SED every second week maintained VD concentrations throughout the winter, whereas levels in the control group decreased. No adverse effects were observed. The NB-UVB dose was small, given that an average Dane receives 1.5 SED of solar UV radiation daily in July (19). Bogh et al. (11) have shown that a BB-UVB exposure of 1 SED every second week will maintain summer levels of VD. They gave 9 BB-UVB exposures over 16 weeks and observed a non-significant decrease of 4.7 nmol/l from the baseline concentration of VD of 72.0 nmol/l. In the present trial we gave 13 NB-UVB exposures over 24 weeks and achieved a significant increase of 11.7 nmol/l over a baseline concentration of VD of 78.3 nmol/l. The dose (2 SED vs. 1 SED) and the length of exposure (24 vs. 16 weeks) seem to be the major reasons for the better VD response in our volunteers, although the fact that the vitamin D action spectrum-weighted irradiance dose (14) for an exposure of 1 SED is higher with NB-UVB (23 mJ/cm² CIE) than with BB-UVB (16 mJ/cm² CIE) may also have had an effect. It should be noted that the highest concentration of VD was observed at week 20 (Fig. 1), before the last 3 NB-UVB exposures were given. The subsequent decrease in concentration may have been due to negative feedback in the 25(OH)D system (20).

The administration of UVB irradiation over a long period of time raises a question regarding the potential risks related to UV-induced immunosuppression. The human action spectrum for immunosuppression peaks at UVA and UVB wavelengths, as measured by UV-

induced suppression of elicitation of delayed or contact type hypersensitivity (CHS) to nickel (21, 22). The most sensitive UVA wavelength is 370 nm, where the minimum immunosuppressive physical dose is 409.4 mJ/cm² (21). In the present trial a 1 SED dose was equal to an integrated non-weighted dose of 11 mJ/cm² between 360 and 390 nm for both NB-UVB and BB-UVB lamps, which is below the immunosuppressive dose. In the UVB range immunosuppression peaks at 300 nm and no immunosuppression has been recorded at 322 nm (22). With a 1 SED dose of NB-UVB or BB-UVB the minimum immunosuppressive dose is not exceeded, but it could be exceeded with a 2 SED dose of either NB-UVB or BB-UVB. However, suppression of the recall type (efferent) immunity, such as the patch-testing existing nickel allergy, is just one possible end-point of the immune response. Other potentially more relevant end-points are the suppression of the induction of either local (CHS) or systemic delayed type hypersensitivity (the afferent immunity) (23, 24). Even though no association between NB-UVB treatment and skin cancer has been observed (25), there are no safe limits for phototherapy. What is “safe” for one individual is not safe for another (26, 27). Both UVB and UVA cause signature mutations in DNA, and UVA wavelengths promote photoaging. As regards the mutagenic potential of NB-UVB and BB-UVB, they appear to be equal (28).

We found a moderate negative correlation between BMI and baseline VD status in our volunteers. A meta-analysis has confirmed the occurrence of low VD concentrations among obese subjects, suggesting that the reason for this may be volumetric dilution of 25(OH)D in the fat tissue (29). A high BMI therefore seems to predispose subjects to VD insufficiency, which in turn increases the risk of contracting VD-related diseases (3–5). The dietary VD intake was 7.0 µg in the intervention group and 6.7 µg in the control group. These intakes were approximately the same as in our previous study with healthy subjects (8), but remained lower than in the recent national survey carried out in Finland (30). Only a few of the present volunteers were receiving the estimated average requirement of 10 µg dietary VD daily, and none had reached the recommended dietary allowance of 15 µg (7). It seems that an additional 10 µg VD supplement is needed to ensure adequate VD status in the adult population (7, 31).

We have shown previously that regular NB-UVB exposures increase serum VD concentrations more than 20 µg of oral cholecalciferol daily (9). In addition, NB-UVB exposures increased the mean VD concentration by as much as 58% in patients with psoriasis who were receiving a 20 µg oral cholecalciferol supplement daily (32). Lagunova et al. (33) compared the effect of VD supplementation (50 µg oral cholecalciferol daily) and 10 UVB exposures to a total dose of 23.8 SED on VD concentration in a 1-month study. Both interventions increased serum 25(OH)D concentrations similarly, by

20–25 nmol/l. The total UVB dose was comparable to the 25 SEDs given in our study, but the intervention period was only 5 weeks compared with our 24 weeks. In the following commentary, UV dose-response studies with more careful and possibly safer exposure protocol were warranted (34). The strengths of our study are the randomized and controlled design, the long time-frame covering all winter, and the similarity of the groups. A limitation of our study is the need to standardize further the analytical methods for 25(OH)D, as suggested by Volmer et al. (35).

Our goal was to examine the capacity of low-dose NB-UVB exposures to maintain VD concentrations during the winter. The results confirmed that the 2 SED dose given every second week from October to April was enough to maintain the baseline concentrations of VD and even to increase them, suggesting that a NB-UVB dose of 1 SED might be appropriate for this purpose. A parallel comparison of continuous NB-UVB exposures and the recommended oral VD supplementation of 10 µg daily during the winter should be carried out (7, 31).

In conclusion, a suberythemal dose of NB-UVB of 2 SED given to healthy subjects every second week over the winter months can maintain and even increase post-summer VD concentrations.

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The effect of vernal solar UV radiation on serum 25-hydroxyvitamin D concentration depends on the baseline level: observations from a high latitude in Finland

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Running title: Vernal solar UV radiation has an impact on serum 25-hydroxyvitamin D concentration

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ABSTRACT

Background: Humans obtain vitamin D either from conversion of 7-dehydrocholesterol in the skin by ultraviolet B (UVB) radiation, or from dietary sources. As the radiation level is insufficient in winter, vitamin D deficiency is common at higher latitudes.

Objective: To assess whether vernal solar UVB radiation at latitudes 61°N and 67°N in Finland has an impact on serum 25-hydroxyvitamin D [S-25(OH)D] concentrations in healthy subjects.

Design: Twenty-seven volunteers participated in outdoor activities in snow-covered terrain for 4-10 days in March or April, with their face and hands exposed to sunlight. The UVB doses were monitored with dosimeters and S-25(OH)D levels were measured before and after the exposure period.

Results: A mean total UVB dose of 11.8 standard erythema doses (SED) was received during an average of 12.3 hours spent outdoors. The mean S-25(OH)D concentration in subjects with a baseline concentration below 90.0 nmol L⁻¹ (n=13) increased significantly, by 6.0 nmol L⁻¹ from an initial mean of 62.4 nmol L⁻¹ (p<0.001), whereas in those with a basal concentration above 90.0 nmol L⁻¹ (n=12) it decreased significantly, by 6.7 nmol L⁻¹ from a mean of 116.9 nmol L⁻¹ (p<0.01).

Conclusion: Only 7% of total body surface area was exposed to vernal solar UVB radiation and this was capable of increasing S-25(OH)D levels in subjects with a baseline level below 90 nmol L⁻¹ but not in those with higher levels.

Keywords: 25(OH)D, vitamin D, ultraviolet, UVB, spring

INTRODUCTION

Humans obtain vitamin D either from conversion of 7-dehydrocholesterol in the skin by ultraviolet B (UVB) radiation, or from dietary sources. Depending on the latitude, time of the year and the mode of subsistence, dietary sources can be the major form, especially for Northern peoples (1). Vitamin D is hydroxylated in the liver to the circulating form, 25-hydroxyvitamin D [25(OH)D], which is the best indicator of vitamin D status. Vitamin D insufficiency has been linked to chronic skeletal (2) and extra-skeletal diseases such as obesity and type 2 diabetes mellitus (3,4). Low S-25(OH)D levels are common in Scandinavians in winter, because the available sunlight is not capable of inducing vitamin D synthesis and their dietary intake of vitamin D is often suboptimal (5,6). Although recent national policies have succeeded in increasing vitamin D intake in Finland, roughly fourth of men and half of women fail to reach the recommended dietary intake (7). It seems, however, that native populations that have lived at high latitudes for hundreds of generations, have adapted to low S-25(OH)D levels by developing compensating mechanisms (1,8).

The lowest S-25(O)D levels in subjects living at high latitudes are typically measured between February and April (9-12). In theory, there is enough sunlight for vitamin D synthesis from early March onwards at a latitude of 61°N (13). A previous Norwegian study at latitude 68°N supported the theoretical calculations, since individual subjects with low S-25(OH)D responded to solar UV radiation already in early March when just the face was exposed, but the study cohort was very small (10). Another Danish study at latitude 56°N showed an increase in S-25(OH)D by April 8th when more skin than the face and hands were exposed (11). At higher latitudes, cold weather prevents people from exposing more skin than the face and hands during March and April, but exposure of these areas would seem to suffice to increase S-25(OH)D levels (10,14). Reflections from snow-covered terrain can substantially increase the UVB dose received by the skin during outdoor activities (15), which can result in a higher UVB dose than measured previously (13). Since previous studies are scarce, our goal was to determine whether the solar UVB radiation level at high latitudes in Finland in March and April is capable of raising S-25(OH)D concentrations when only the face and hands are exposed.

MATERIAL AND METHODS

Subjects

Twenty-seven healthy volunteers were enrolled, the inclusion criteria being age 18 years or older and avoidance of solarium visits, phototherapy, holidays in low latitudes and vitamin D supplementation during a one-month washout period prior to the trial and during it. Further exclusion criteria were pregnancy, previous skin cancer, intake of photosensitizing drugs and Fitzpatrick's skin phototype I (16). Recruitment began on February 1st 2013 and the trial was coordinated from the Department of Dermatology at Päijät-Häme Central Hospital, terminating in data collection on April 22nd 2014. Vitamin D intake at the onset was estimated by means of a three-day food frequency questionnaire. Twenty-five subjects completed the trial (Table 1), two having been disqualified for failing to follow the exposure regimen. The protocol was approved by the Ethics Committee of Tampere University Hospital (Reg. No. R12266), and all the volunteers gave their informed consent in advance.

Sample size calculation

The trial was designed to show an increase in S-25(OH)D of at least 15 nmol L⁻¹ with an α -value of 0.05 and a β -value of 0.80. An assumed SD of 15.5 nmol L⁻¹ for the S-25(OH)D analyses was used. Accordingly, it was considered necessary that 16 volunteers should complete the trial.

Ultraviolet radiation exposures

The scheduled monitored ultraviolet radiation (UVR) exposures were implemented in Sodankylä (67°N) and Lahti (61°N) in March and April 2013 and 2014 in snow-covered terrain (Table 2). The participants were instructed to expose their hands and face without using a sunscreen. In addition to the scheduled exposures, they were encouraged to perform outdoor activities in their own time.

Ultraviolet radiation measurements

The participants wore UVB dosimeters (VioSpor blue line Type II, BioSense, Bornheim, Germany) attached to their upper arms or wrists with straps to depict the dose received by the skin (17,18). These dosimeters detect radiation ranging from 1.0 to 55 standard erythema doses (SED). The ambient solar UVR data were obtained from NILU-UV multichannel radiometer (19) recordings made at the Finnish

Meteorological Institute's Arctic Research Centre (FMI-ARC) in Sodankylä, or were measured locally using a Robertson-Berger-type broadband UV meter (Solar Light Model 501 UV-meter s/n 635; Solar Light Co. Inc., Glenside, PA, U.S.A.). The NILU-UV radiometer was calibrated by Innovation Nilu AS by reference to the National Institute of Standards and Technology (NIST) (Gaithersburg, MD, U.S.A.) and was placed on the roof of the FMI-ARC sounding station to collect data in the form of one-minute averages. The Robertson-Berger meter is calibrated annually by the Radiation and Nuclear Safety Authority, Helsinki, Finland, also by reference to NIST. Its calibration uncertainty (2σ) is 8%. The meter was placed on a high roof near the UV exposure area. As there were no ambient UVR measurements available for Lahti, use was made of UVR data obtained at Jokioinen (61°N) with the FMI's Brewer spectroradiometer (20), which was calibrated by reference to the MIKES-Aalto National Standards Laboratory. Comparisons with the European reference spectroradiometer have shown that discrepancies are less than $\pm 5\%$ (http://www.pmodwrc.ch/wcc_uv/).

Serum 25-hydroxyvitamin D measurements

The blood samples for 25(OH)D analyses were taken in general in the morning on the first exposure day (range 0-5 days before), and 1-4 days after the last day. The samples were centrifuged and serum was stored at -20°C and analysed for 25(OH)D by electro-chemiluminescence binding assay (Roche Diagnostics, Mannheim, Germany) with a coefficient of variation $\leq 7\%$.

Statistics

The strength of the adjusted relationship between baseline S-25(OH)D concentrations and their change after the solar UVR exposure period was described by means of a partial correlation coefficient. The significance of the change was calculated using the paired-samples t-test, and Pearson's chi-square test was used when comparing nominal data. All the analyses were performed using STATA 14 (StataCorp LP, College Station, TX).

RESULTS

The mean daily vitamin D intake of the 25 subjects was $8.5 \pm 3.2 \mu\text{g}$ (Table 1) and the mean baseline S-25(OH)D concentration was $88.6 \pm 32.6 \text{ nmol L}^{-1}$. The mean personal total UVB dose was 11.8 ± 4.9 SED during a mean of 12.3 ± 4.3 hours spent outdoors (Table 1,2). The mean available ambient daily UVB dose was 9.4 SED and the maximum UV indices were 1.1 – 2.7 depending on the period. The mean S-25(OH)D concentration showed a slight non-significant decrease of 0.1 nmol L^{-1} ($p=0.971$) after the exposures, but the baseline concentrations had an inverse relationship with the percentage change after exposure when adjusted for age, body mass index and Fitzpatrick's skin phototype ($r=-0.51$, $p=0.011$) (Fig. 1).

The baseline S-25(OH)D concentrations below 90.0 nmol L^{-1} increased after exposure, whereas those that were above 90.0 nmol L^{-1} decreased, in all subjects except one (Fig. 2, Table 3). In the $< 90 \text{ nmol L}^{-1}$ group ($n=13$) the S-25(OH)D increased by 6.0 nmol L^{-1} (95% CI 2.8 to 9.2, $p<0.001$) from an initial mean of 62.4 nmol L^{-1} and that in the $> 90 \text{ nmol L}^{-1}$ group ($n=12$) decreased by 6.7 nmol L^{-1} (95% CI -10.3 to -3.0, $p<0.01$) from $116.9 \text{ nmol L}^{-1}$ (Fig. 2, Table 3). The total UVB doses received by the volunteers correlated with the numbers of hours spent outdoors ($r=0.612$, $p=0.02$), but no correlation was found between the UVB doses and the change in S-25(OH)D concentrations. There were no differences in demographic data or UVB doses detected between the subjects with a S-25(OH)D value below or above 90 nmol L^{-1} (Table 3). Four subjects with baseline S-25(OH)D below 90 nmol L^{-1} and six subjects with baseline S-25(OH)D above 90 nmol L^{-1} had used vitamin D supplementation prior the one-month washout period with a mean daily dose of $13.3 \pm 7.3 \mu\text{g}$ (range 5 – 25 μg). The mean dose did not differ significantly between the groups ($p=0.081$). The baseline S-25(OH)D levels in males (83.3 nmol L^{-1}) and females (91.0 nmol L^{-1}) did not show a significant difference ($p=0.593$).

DISCUSSION

Our results indicate that vernal UVB radiation at a high latitude (61° or 67°) between 17th March and 20th April is capable of raising S-25(OH)D levels in subjects with baseline levels below 90 nmol L^{-1} , whereas levels above that decrease. The mean baseline level 88.6 nmol L^{-1} was higher than expected at high latitudes in early spring. This could be attributed to national food fortification policy and improved dietary vitamin D intake (7), use of vitamin D supplementation or holidays in low latitudes prior the washout period. In our previous study (21), where S-25(OH)D levels were monitored from October to May in subjects with no vitamin D supplementation during the winter, nor any exposure to UVR, the mean concentration in early April was lower, 65.8 nmol L^{-1} .

The increase of lower S-25(OH)D levels early in spring is a solid finding, but the decreasing trend in higher levels is unexpected, since all subjects were exposed to a source of vitamin D. It could be that in subjects with higher levels, the S-25(OH)D gained from previous vitamin D sources keeps decreasing due to the half-life of S-25(OH)D, and this decrease exceeds the amount of S-25(OH)D produced by vernal UVB. Interestingly, we further analysed data from our previous study (21) and observed similar kinetics in the control subjects – higher values kept on decreasing from early April to early May, whereas lower values began to increase. In fact, this phenomenon could also be due to an effective homeostatic S-25(OH)D control system for ensuring its stable availability. The points at which this regulation takes place evidently include i) the liver concentration of 25-hydroxylase, which converts vitamin D to 25(OH)D, and ii) catabolism of 25(OH)D to breakdown products in the liver and in other tissues (22). The inverse relationship between the change in S-25(OH)D concentration and its baseline concentration in our subjects might in part be caused by this regulation. Similar phenomenon has been demonstrated also in other high latitude studies in the Nordic countries (10,12,23).

The homeostatic control system exists because of the health risks of having a S-25(OH)D concentration too low or high. It is generally agreed upon, that a U-shaped response curve exists between the S-25(OH)D concentration and various disease risks. In a Swedish study, an approximately 50% higher total mortality rate was observed among men in the lowest 10% ($<46 \text{ nmol L}^{-1}$) and the highest 5% ($>98 \text{ nmol L}^{-1}$) of plasma 25(OH)D concentrations compared with intermediate concentrations (24). S-25(OH)D below 40 nmol L^{-1} and above 60 nmol L^{-1} have shown to increase the risk of prostate cancer in Finnish population, and both high and low levels seem to promote premature

aging (25). A transnational study in women reported increased mortality for 7 types of cancer (endometrial, esophageal, gastric, kidney, non-Hodgkin's lymphoma, pancreatic, ovarian) in subjects with S-25(OH)D below 45 nmol L⁻¹ and above 124 nmol L⁻¹ (26). A pooled meta-analysis including 8 case-control studies reported an increased pancreatic cancer risk in subjects with S-25(OH)D above 100 nmol L⁻¹ (27). The Framingham Heart Study concluded that cardiovascular disease risk increases with S-25(OH)D below 50 nmol L⁻¹ and above 62.5 nmol L⁻¹, and the NHANES III study found a higher all-cause mortality rate for S-25(OH)D above 122.5 nmol L⁻¹ (28).

Like most heritable characteristics, also vitamin D metabolism varies among human populations. Populations that have lived at high latitudes for hundreds of generations, have had time to adapt to limited opportunities for vitamin D synthesis. This kind of adaptation has been shown in the Inuit of Northern Canada and Greenland. Despite low S-25(OH)D levels and a calcium-deficient diet, Inuit have normal blood levels of calcium. They seem to absorb calcium more effectively, perhaps due to their different vitamin D receptor genotype prevalence (8). They also seem to convert vitamin D at a higher rate to its most active form (1). These metabolic differences may explain why Amerindian women have lower S-25(OH)D levels than do Euro-American women, while having higher bone mass density until menopause (29,30).

There is some worrisome evidence, that native Northern people can experience effects of vitamin D toxicity at relatively low levels. A recent study of Greenland Inuit found increasing S-25(OH)D levels to be positively associated with increased fasting- and 2-hour plasma glucose and HbA1c, and decreased beta-cell function (31). This dose-response curve was very different from the one seen in European populations. Although a homeostatic mechanism seems to keep S-25(OH)D levels within a zone of minimal health risks (22), this homeostasis could be circumvented through daily ingestion of high vitamin D supplementation doses. This is a relatively novel situation for our species, and the risks could be higher for those who have adapted to low S-25(OH)D levels.

As cutaneous vitamin D synthesis depends on the UVB dose received in the skin, factors that affect UVB wavelength and irradiance have a direct influence on vitamin D synthesis. The most relevant factor for Northern inhabitants is the solar zenith angle, which depends on the time of day, season of the year and latitude. As the zenith angle increases, the UVB radiation has to travel a longer distance through the atmosphere and has more chance of being absorbed or scattered, which reduces the amount reaching the skin (32). At latitude 52°N no cutaneous vitamin D synthesis is detectable from October to

March, a phenomenon described as the 'vitamin D winter' (32). The length of the 'winter' is not constant, however, but varies with levels of ozone, cloudiness and aerosols (33). Ozone levels can reduce or increase the latitude of the 'vitamin D winter' by 10 degrees, and extend or shorten its duration by up to 2 months (33). The lowest seasonal S-25(OH)D concentrations in subjects living at high latitudes are typically measured between February and April (9-12). In theory, cutaneous vitamin D production is possible at latitude 61°N from early March (13), an estimate based on UV spectroradiometer irradiance measurements on a horizontal surface, but a snowy surface can further increase the subject's UVB dose due to reflections between the ground and sky and direct reflections from the ground to vertical surfaces (14). The albedo of snow varies between 0.5 – 0.7 (34,35) and the effect is most pronounced for vertical surfaces such as the face (15). All the outdoor activities performed by the present subjects took place in snow-covered fields, which probably increased the measured personal UVB doses significantly.

The UV exposures were received on the face and hands, the exposure of which has been shown to suffice to increase S-25(OH)D levels (10,14). A previous study performed in Denmark (56°N) showed an increase in S-25(OH)D levels by April 8th, when more skin than the face and hands was exposed (11). In a Norwegian study (68°N) exposure of the facial area to UVR between February 8th and April 12th yielded no increase in mean S-25(OH)D levels (9), being in agreement with our findings. Interestingly, subjects with S-25(OH)D < 30 nmol L⁻¹ responded in early March (10), whereas in our study the cut-off limit was 90 nmol L⁻¹. Our cut-off limit was higher probably because of a larger exposed skin area (4% vs. 7%), longer minimum daily exposure time (20 min vs. 60 min) and study period that extended further towards the spring (April 20th). Contrary to the previous study (10), in our subjects the increase of S-25(OH)D levels below 90 nmol L⁻¹ was highly significant and strengthens the evidence of the vitamin D effects of vernal UVR. However, the optimal S-25(OH)D level can differ between populations, and thus the findings of the present study are not directly generalizable.

To conclude, vernal solar UVR at high latitudes seems to increase S-25(OH)D concentrations in subjects with a baseline level below 90 nmol L⁻¹ when only the face and hands, i.e. 7% of total body surface area, are exposed. The declining trend seen in volunteers with a high baseline level above 90 nmol L⁻¹ is interesting, and could reflect an especially active homeostatic S-25(OH)D control system in caucasoid people living at high latitudes. This phenomenon should be studied in more detail in future trials.

CONFLICT OF INTEREST AND FUNDING

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TABLES AND FIGURES

Table 1. Demographic data, daily vitamin D intake, baseline S-25(OH)D concentration and personal ultraviolet B radiation dose.

	N=25
Male/Female	8/17
Mean age, years (range)	43 (22 – 71)
Mean body mass index, kg m ⁻² , mean ± SD (range)	23.9 ± 4.5
Fitzpatrick's skin type II/III	7/18
Vitamin D intake, µg/d, mean ± SD	8.5 ± 3.2
Baseline S-25(OH)D (nmol L ⁻¹), mean ± SD	88.6 ± 32.6
UV dosimeter (SED), mean ± SD (range)	11.8 ± 4.9 (2.4 – 23.2)

Table 2. Ultraviolet radiation exposure locations, time periods, exposure instructions and average daily available ultraviolet B radiation doses.

Group	Location	Dates	Daily available UVB radiation dose (SED), mean (range)	Maximum UV index	Personal UVB radiation dose (SED), mean ± SD N=21	Total hours spent outdoors between 10AM and 3PM, mean ± SD
I (N=4)	Sodankylä, Finland (67°N, 26°E)	17.3. – 26.3.2013 ¹	5.0 (4.1 – 6.2) ³	1.2	20.5 ⁶	19.3 ± 3.0
II (N=6)	Sodankylä, Finland (67°N, 26°E)	29.3. – 1.4.2013 ¹	7.1 (6.8 – 7.3) ³	1.2	11.1 ± 7.6	12.2 ± 3.8
III (N=5)	Sodankylä, Finland (67°N, 26°E)	7.4. – 17.4.2014 ²	9.6 (4.5 – 13.2) ³	2.3	10.9 ± 1.7	11.0 ± 3.2
IV (N=3)	Sodankylä, Finland (67°N, 26°E)	18.4. – 20.4.2014 ¹	13.0 (10.4 – 15.3) ³	2.7	11.2 ± 6.9	9.0 ± 1.7
V (N=7)	Lahti, Finland (61°N, 25°E)	2.4. – 12.4.2013 ²	12.1 (9.1 – 15.4) ⁴	2.7	11.9 ⁷	10.7 ± 2.5
Mean					11.8 ± 4.9	12.3 ± 4.3

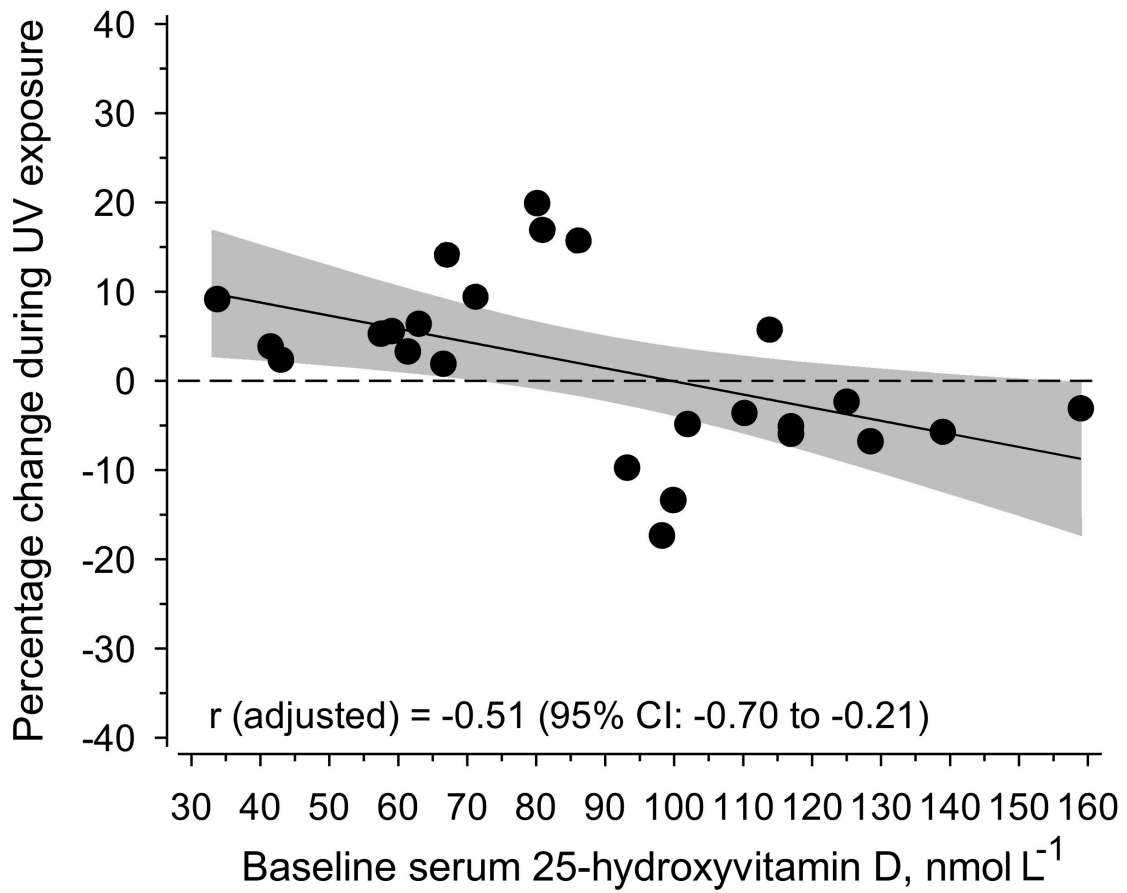
¹Maximum exposure during holiday outdoor activities, ²Walking outdoors for 1 hour daily at noon during working days, ³NILU-UV measurements from FMI-ARC, ⁴Spectroradiometer measurements from FMI, Jokioinen Observatory. ⁵Local Robertson-Berger meter, ⁶Only one subject had a dosimeter, ⁷One dosimeter for the whole group

Table 3. Demographic data and changes in S-25(OH)D concentration after solar exposure in subjects with low (<90.0 nmol L⁻¹) and high (>90.0 nmol L⁻¹) baseline S-25(OH)D concentrations.

	Low S-25(OH)D (N=13)	High S-25(OH)D (N=12)	p-value
Male/Female	5/8	3/9	0.637
Mean age, years (range)	43 (22 – 63)	44 (24 – 71)	0.888
Mean body mass index, kg m ⁻² , mean ± SD (range)	25.0 ± 5.2	22.3 ± 3.1	0.204
Fitzpatrick's skin type II/III	2/11	5/7	0.202
Vitamin D intake, µg/d, mean ± SD	7.6 ± 1.8	9.4 ± 4.1	0.182
Baseline S-25(OH)D (nmol L ⁻¹), mean ± SD	62.4 ± 15.8*	116.9 ± 18.9**	
Change of the S-25(OH)D (nmol L ⁻¹), mean (95% CI)	6.0 (2.8 to 9.2)*	-6.7 (-10.3 to -3.0)**	
S-25(OH)D after the exposure (nmol L ⁻¹), mean ± SD	68.4 ± 20.3*	110.3 ± 21.2**	
UV dosimeter (SED), mean ± SD	11.4 ± 5.0	12.4 ± 5.0	0.642
Hours spent outdoors between 10AM and 3PM, mean ± SD	11.4 ± 3.3	13.2 ± 5.1	0.303

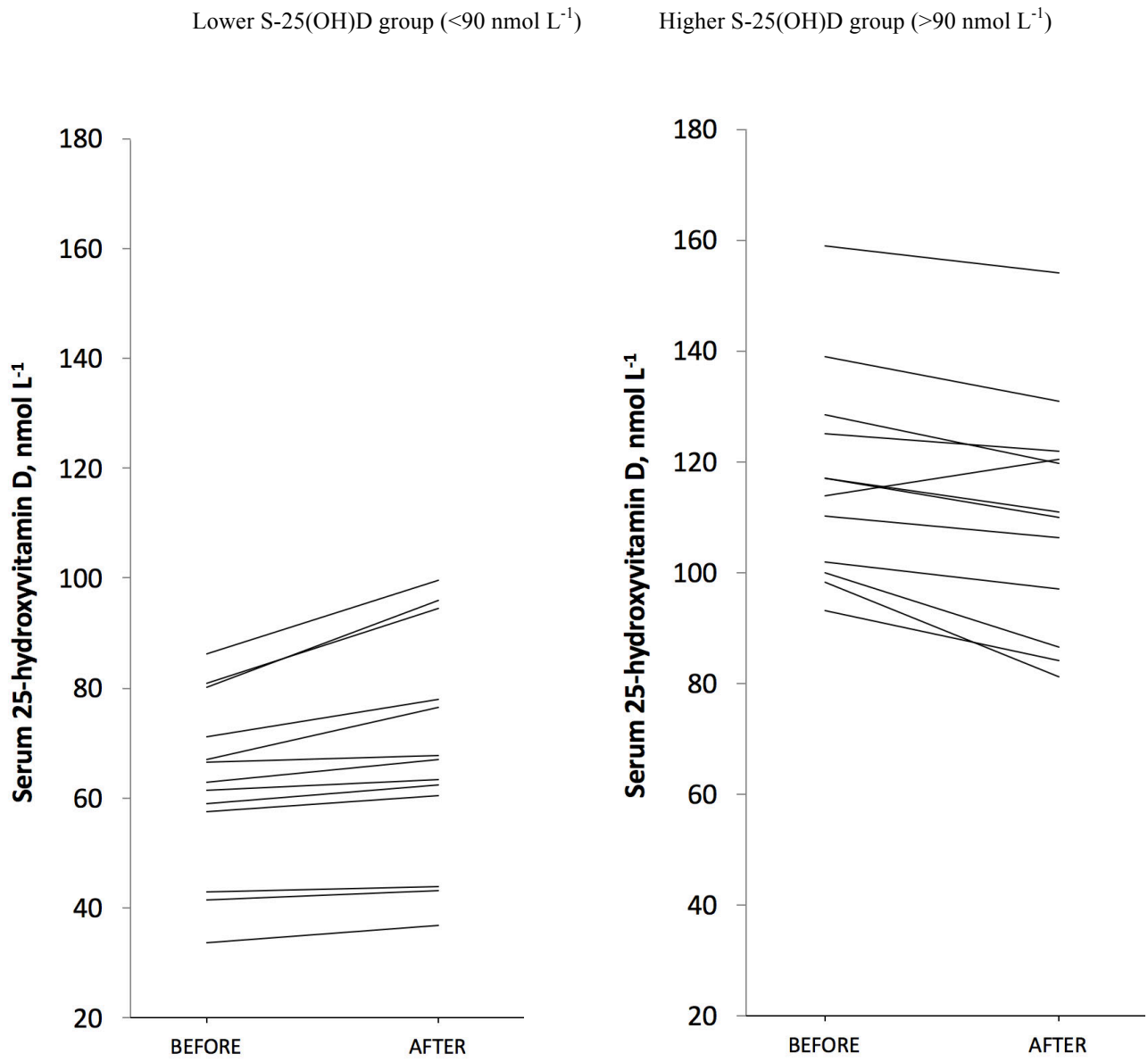
*significant increase (p<0.001) **significant decrease (p<0.01)

Figure 1. Relationship of changes in S-25(OH)D concentrations after the solar ultraviolet radiation exposure period to baseline concentrations.



R adjusted for age, body mass index and Fitzpatrick's skin type, $p=0.011$

Figure 2. Changes in S-25(OH)D concentrations in the lower S-25(OH)D group ($< 90 \text{ nmol L}^{-1}$) and in the higher S-25(OH)D group ($> 90 \text{ nmol L}^{-1}$).



CLINICAL REPORT

Empowering Heliotherapy Improves Clinical Outcome and Quality of Life of Psoriasis and Atopic Dermatitis Patients

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Empowering heliotherapy aims at clinical healing and improved coping with psoriasis and atopic dermatitis, but evidence of long-term effects is scarce. We studied the effect of 2-week empowering heliotherapy in the Canary Islands on clinical outcome and quality of life in 22 psoriasis and 13 atopic dermatitis patients. Empowerment consisted of meeting peers, sharing experiences and performing physical and mental practices. Using the self-administered PASI (SAPASI) psoriasis was alleviated statistically significantly during heliotherapy ($p < 0.001$), and the treatment effect was still detectable 3 months later ($p < 0.001$). Atopic dermatitis was improved ($p < 0.001$) when assessed with the patient-oriented SCORAD (PO-SCORAD), and the effect was still obvious 3 months later ($p = 0.002$). During heliotherapy the dermatology life quality index (DLQI) improved in both groups ($p < 0.001$), persisting in atopic patients for up to 3 months ($p = 0.002$), but not in psoriasis patients. In conclusion, a 2-week empowered heliotherapy showed a long-lasting improvement in psoriasis and atopic dermatitis disease activity, and also in the quality of life of atopic patients. *Key words: vitamin D; ultraviolet B radiation; SAPASI; PO-SCORAD; DLQI.*

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Psoriasis (PS) and atopic dermatitis (AD) are chronic inflammatory skin diseases with a negative impact on quality of life (QoL) (1, 2). PS and AD can be treated with sunbathing, i.e. heliotherapy (HT), alleviating the physical and psychological fatigue of patients (3–5). The Nordic health authorities have funded HT courses since the 1970s (5, 6). Recently, emphasis has moved from HT to empowerment of patients and coping with the disease. Therefore, HT was renovated to meet these goals. The present HT model consists of meeting peers, sharing experiences, adopting a healthy life style, exercising practices, improving physical,

psychological and social wellbeing and coping with the disease guided by health care experts. The staff consists of experienced nurses, a physiotherapist and a psychologist. Because less emphasis is put on HT, the treatment results could differ from those of traditional HT.

To our knowledge, no earlier study has assessed sunbathing habits, QoL or vitamin D (VD) changes in parallel for PS and AD patients receiving HT. The aim of the study was to monitor the efficacy of a 2-week empowered HT on personal UV exposure, clinical outcome, QoL and VD balance in PS and AD patients attending the same course. We also performed a follow-up 3 months after the HT course.

MATERIAL AND METHODS

Patients and heliotherapy course

The Finnish Psoriasis Association and the Finnish Central Organisation for Skin Patients together arranged a 2-week HT course for PS and AD patients in Puerto Rico (27°N, 15°W), the Canary Islands, Spain from 27th October to 10th November in 2012. Inclusion criteria were psoriasis or atopic dermatitis without demanding any minimum severity scorings, subjects had to be aged 18 or older and have a referral from a doctor. Exclusion criteria were photosensitivity, Fitzpatrick's skin photo-type I, photosensitising drugs, excessive alcohol use, drug abuse, severe cardiovascular diseases, unbalanced diabetes or mental disorders (7). The course included an education day before HT and a reunion weekend 3 months afterwards. The amount to be paid by the participants was €400, the total cost per patient being €2,450. Twenty-two PS and 13 AD patients took part in the study (Table I). Fifteen patients had psoriatic arthritis and 5 of them used biologic drugs, 3 of these patients also used methotrexate. Two patients had methotrexate as a monotherapy. During HT the patients were allowed to use their routine topical medication. Nineteen patients (14 PS, 5 AD) used VD supplementation before HT, on mean 23 µg (range 5–50 µg) daily, but not during HT or 3 months after it.

The patients had their sunbathing plans adjusted for their Fitzpatrick's skin types. The first solar exposure times ranged

Table I. Demographics of patients with psoriasis and atopic dermatitis (AD), and UV radiation doses received during a two-week heliotherapy course

	Psoriasis (n=22)	AD (n=13)	p-value
Male/Female, n	8/14	0/13	0.013
Age, years, mean ± SD (range)	52 ± 10 (34–68)	44 ± 17 (21–74)	0.069
Body mass index, mean ± SD (range)	28.2 ± 6.2 (17.1–41.9)	27.1 ± 6.9 (19.1–39.8)	0.643
Fitzpatrick's skin type, II/III/IV	6/15/1	5/8/0	0.615
UV dosimeter (SED), mean ± SD (range)	30 ± 16 (22.4–38.5) ^a	43 ± 16 (32.1–53.9) ^b	0.062

^aFrom 18 patients, ^bFrom 12 patients. SED: standard erythema dose.

from 20–90 min for PS and 15–30 min for AD patients. Both sides of the body were exposed during sunbathing. The time was increased within a week to 90–300 min for PS and to 120 min for AD patients. The scheduled sunbathing treatments were done without sunscreen in the mornings or afternoons. Sunscreen was applied liberally thereafter. The supporting program included teaching self-management and a healthy life style as well as group conversations with a psychologist, altogether for 14 h for both groups. Physical exercise included water sports, trekking and gymnastics for 24 h for the PS group and 11 h for the AD group.

The Ethics Committee of the Tampere University Hospital approved the study protocol. All patients gave their informed consent before the study.

UV exposure measurements

To measure the personal UVB dose received by the skin during HT the patients wore personal UV dosimeters (VioSpor blue line Type III, BioSense, Bornheim, Germany), one meter was used for week one and another for week two (8, 9). The meters detect a dose ranging from 1.5 to 90 Standard Erythema Dose (SED). The dosimeters were attached to the patients' upper arms or wrists with straps and during sunbathing they were placed on towels beside the patients (10). Eighteen PS and 12 AD patients wore dosimeters. The ambient maximum solar UV-irradiance was measured as a mean dose from 2 VioSpor Type III dosimeters at a time. The meters were put in an open place and replaced every other day to avoid overexposure. The Spanish Agency of Meteorology (Agencia Estatal de Meteorología; www.aemet.es) supplied the global solar UV irradiance data from the nearby (distance 15 km) Maspalomas C. Insular Turismo weather station. The first HT week was rainy and cloudy and the second HT week was sunny. During the HT maximum UV index varied between 5 and 8.

Assessment of disease activity

The PS patients filled out the Self-Administered Psoriasis Area and Severity Index (SAPASI) and AD patients the Patient Oriented Scoring of Atopic Dermatitis (PO-SCORAD) to follow the disease activity (11, 12). Disease severity and pruritus were assessed globally using the Visual Analogue Scale (VAS) (13). The Dermatology Life Quality Index (DLQI) was used to assess the change in the QoL (14). All measures were filled out 3 times: at the onset, at the end and 3 months after HT.

Serum 25-hydroxyvitamin D measurements

VD samples were taken immediately before, at the end and 3 months after HT. The sera were deep-frozen and stored at

–20°C. Analysis of 25-hydroxyVD was performed in duplicates using radioimmunoassay (Immunodiagnostic Systems, Boldon, UK), as described earlier (15).

Statistics

The data are presented as means with standard deviations (SD) or as counts with percentages. Confidence intervals (95% CI) were obtained by bias-corrected bootstrapping (5,000 replications). Statistical comparisons were made by using analysis of *t*-test, covariance (ANCOVA). In the case of violation of the assumptions (e.g. non-normality), a bootstrap type test was used. Longitudinal measures for continuous outcomes were analysed using a bootstrap type generalised estimating equations (GEE) model. GEE were developed as an extension of the general linear model to analyse longitudinal and other correlated data. GEE 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 there are not observations at all time-points for individuals. No adjustment was made for multiple testing. When comparing increases in VD concentrations, the model was standardised by age, sex and body mass index (BMI). Pearson's χ^2 test was used when comparing nominal data. The STATA 13.1, StataCorp LP (College Station, TX, USA) statistical package was used for the analyses.

RESULTS

UV exposures during heliotherapy

According to personal dosimeter measurements the PS patients received a mean UV dose of 30 ± 16 SED and the AD patients 43 ± 16 SED during HT (Table I) showing no significant difference ($p = 0.062$). The respective cumulative ambient two-week UV irradiance was 244 SED measured by VioSpor III dosimeters and 303 SED using the UV records obtained from the Maspalomas C. Insular Turismo station.

Disease activity and quality of life at the end of heliotherapy

HT was statistically equally effective in PS and AD when disease activity was scored (Table II). Mean SAPASI decreased from 6.7 by 4.9 units ($p < 0.001$) and PO-SCORAD from 30.6 by 19.5 units ($p < 0.001$). Four

Table II. Clinical outcome of a two-week heliotherapy course in patients with psoriasis and atopic dermatitis (AD) measured by Self-Administered Psoriasis Area and Severity Index (SAPASI) or PO SCORAD scores, and by visual analogue scores of severity and pruritus. Vitamin D concentrations were measured at the same time points as the clinical outcome scores. Mean \pm SD and change (Δ) of mean value compared to Day 0

	Psoriasis (n=22)			AD (n=13)		
	Day 0	Δ Week 2	Δ Week 14	Day 0	Δ Week 2	Δ Week 14
SAPASI/PO SCORAD (95% CI)	6.7 \pm 5.6 (4.2–9.2)	–4.9*** (–6.8 to –3.0)	–3.1*** (–4.7 to –1.4)	30.6 \pm 17.6 (20.0–41.3)	–19.5*** (–26.2 to –12.9)	–10.0** (–16.3 to –3.8)
Visual analogue score global (95% CI)	3.9 \pm 2.2 (2.9–4.9)	–2.0*** (–2.8 to –1.0)	–1.4** (–2.3 to –0.4)	3.4 \pm 2.2 (2.1–4.7)	–1.8*** (–2.4 to –1.2)	–0.9 (–2.0–0.2)
Visual analogue score pruritus (95% CI)	3.1 \pm 2.2 (2.1–4.1)	–2.2*** (–3.1 to –1.4)	–0.9 (–1.9–0.1)	3.7 \pm 2.5 (2.2–5.3)	–2.2*** (–3.1 to –1.4)	–0.9 (–2.3–0.4)
Dermatology Life Quality Index (95% CI)	6.1 \pm 3.3 (4.7–7.6)	–4.3*** (–5.5 to –3.1)	–0.8 (–2.8–1.2)	7.2 \pm 4.8 (4.3–10.0)	–5.3*** (–7.3 to –3.4)	–3.2** (–5.2 to –1.2)
Vitamin D, nmol l ^{–1} (95% CI)	86.6 \pm 20.0 (77.8–95.5)	13.8*** (8.6–19.0)	0.4 ^a (–5.2–6.8)	84.1 \pm 16.0 ^b (73.4–94.9)	20.5*** (14.0–25.6)	–3.1 ^c (–10.0–4.0)

** $p < 0.01$, *** $p < 0.001$ compared to heliotherapy day 0 values.

^a20 patients; ^b11 patients; ^c10 patients.

PS but none of the AD patients experienced complete clearance, and 75% clearance was seen in 13 (59%) PS and 2 (15%) AD patients. Using the VAS scales there was significant improvement in disease severity and pruritus in both groups (Table II). QoL was improved showing a decrease in mean DLQI score from 6.1 by 4.3 units ($p < 0.001$) in PS patients and from 7.2 by 5.3 units ($p < 0.001$) in AD patients (Table II). At onset of HT no PS or AD patient was VD-insufficient, defined as $25(\text{OH})\text{D}_3 < 50 \text{ nmol l}^{-1}$. During HT the VD concentrations increased significantly ($p < 0.001$, Table II) and equally ($p = 0.56$) in both patient groups.

Disease activity and quality of life after heliotherapy

At the follow-up 3 months after HT, the treatment effect significantly persisted when compared to initial scores (Table II). The decrease in SAPASI was 3.1 ($p < 0.001$) and in PO-SCORAD 10.0 ($p = 0.002$). Using the VAS the global disease severity also remained decreased ($p = 0.004$) in PS patients but not ($p = 0.11$) in AD patients. The VAS scores depicting pruritus had returned close to baseline levels both in PS ($p = 0.058$) and AD ($p = 0.17$) patients (Table II). In PS patients the 3-month follow-up DLQI scores had dropped to baseline ($p = 0.43$), but in the AD patients it remained improved ($p = 0.002$) (Table II). The VD concentrations had decreased in both the PS and AD patients close to the pre-HT values (Table II).

DISCUSSION

The results showed PS to improve statistically highly significantly during empowering HT, the mean SAPASI reducing by 73%. Complete clearance was reached in 18% and 75% SAPASI clearance in 59% of the patients. The mean initial SAPASI score of 6.7 was markedly lower compared to the PASI or SAPASI scores of previous studies (16–19) indicating a mild disease, but 7 patients were using systemic drugs. Use of methotrexate or biologic drugs should however not dampen the effect of HT since both have synergistic effects with UVB irradiation (20, 21). HT reduced the PS scorings only slightly, which could be due to the short duration of HT, or the insensitivity of SAPASI as regards a mild disease state. In a study by Wahl et al. (17) the mean SAPASI remained decreased by 21.1% 4 months after HT, whereas in our study the reduction was 46.3% at the 3-month follow-up visit. It is not known whether the persisting improvement of the SAPASI in our patients was due to the enhanced educative contents of the course.

In AD patients the mean PO-SCORAD score improved statistically significantly from 30.6 to 11.1 but did not show complete clearance in any of the patients, and only 15% reached 75% clearance. SAPASI and PO-SCORAD are not comparable with each other, because PO-SCORAD includes subjective parameters in addition

to visible signs. At the end of HT there were patients with no visible eczema, but due to pruritus or sleep disturbances the PO-SCORAD did not show complete clearance.

We used the DLQI measure to make a parallel assessment for the QoL of both PS and AD patients. The improvement of QoL in the AD patients seemed to be more long-lasting than in the PS patients, but direct between-groups comparisons are not justified due to limited sample size in this study (22, 23). The PS and AD patient groups also differed significantly for gender ($p = 0.013$), and there were only females in the AD group. This could have influenced the results, because women have been shown to comply better with topical treatments than men (24). In this study, the size of the group as well as inclusion and severity of the patients were in the hands of the patient associations depicting the real life situation, rather than a strict experimental research protocol.

The persistent long-term (up to 3-month) statistically significant improvement of DLQI among AD patients surprised us, because this contradicted the VAS scores measuring disease severity and pruritus. The VAS scores had returned to baseline. This discrepancy could be due to pruritus affecting the QoL of atopic patients more than the DLQI scores can show. It is important to use more than one measure in parallel to increase reliability. An interesting measure, which we unfortunately were not aware of earlier, is the Health Education Impact Questionnaire (25). This was used in a recently published study of Wahl et al. (25), however also in this study the educational impact of empowering HT was a challenge (25).

PS patients could be more risk-taking and prone to higher UV doses than AD patients (26, 27), but it turned out that AD patients received a higher dose in fewer hours. This could be explained by the different outdoor activities of the groups (28). Ambient irradiance is also highly dependent on the season. This became obvious in our earlier study where the personal UV dosimeter exposures of AD patients on two-week HT were 75 SED in January and 131 SED in March (9). The 30 SED and 43 SED UV doses of our PS and AD patients reflect both the lower UV index of November season and unfortunate weather conditions of the first HT week, which probably affected the clearance of the skin diseases.

No patient was VD insufficient at the onset (Table II). Despite this, HT improved the VD status statistically significantly in both patient groups, 13.8 nmol l⁻¹ for PS and 20.5 nmol l⁻¹ for AD having received on mean 30 SED and 43 SED respectively. Sunlight seems to be a very potent VD inductor even in subjects who showed no VD deficiency.

The empowering HT model is a response to public pressures stressing patients' own responsibility and self-management for their care. New courses run by the patient organisations focus more on empowerment than clearance of the disease. Wahl et al. (25) studied the effect of climate therapy on self-management in PS patients, and our study focused also on AD pa-

tients showing that both PS and AD were statistically significantly improved (25). In our past study HT was regarded cost-effective for the high indirect costs only for patients with severe psoriasis (29). Similar to the Norwegian study (25) we were unable to confirm long-term improvement of QoL in PS patients (25).

To conclude, UV doses received by PS and AD patients were comparable showing no obvious differences. The empowering HT cleared the skin symptoms statistically significantly, but in the long run did not improve the QoL of PS patients. In PS patients the decrease in disease severity expressed using the VAS seemed more long-lasting than in AD patients. A two-week HT improved VD status statistically significantly even in non-VD deficient and substituted individuals.

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The authors declare no conflict of interest.

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Empowering heliotherapy improves clinical outcome and quality of life in psoriasis and atopic dermatitis patients: an observational study of 186 subjects

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Keywords: psoriasis, AD, heliotherapy, DLQI, RAND-36, QoL

INTRODUCTION

Psoriasis and atopic dermatitis (AD) have a negative impact on patients' health-related quality of life (HRQoL) (1, 2). There is a long tradition of heliotherapy (HT) in the Canary Islands for the treatment of Finnish patients with psoriasis and AD (3, 4), and the courses have recently been updated to include more empowering elements. The present model of empowering heliotherapy

(EHT) consists of meeting peers, adopting a healthy life-style, performing exercises and gaining increased autonomy. The benefits of the new EHT have been shown for psoriasis and AD patients (5, 6). This study is a sequel to our pilot study (5) and provides more evidence of the effects of EHT.

METHODS

Patients and the heliotherapy course

The two-week EHT courses referred to here were arranged by the Finnish Psoriasis Association and the Finnish Central Organization for Skin Patients in Puerto Rico (27°N, 15°W), Canary Islands, Spain, between October 2012 and April 2013. The impacts of eight psoriasis and four AD courses were studied. Inclusion criteria were psoriasis or AD without demanding any minimum severity scorings, age 18 or older and a referral from a doctor. Exclusion criteria were as reported previously (5). The course included an education day before EHT and a reunion weekend three months later. Of patients attending the courses, 133/168 of those with psoriasis and 60/72 of those with AD volunteered to participate in the study, but six psoriasis patients and one AD patient were excluded from the analyses on account of incomplete questionnaires and one because of remaining in Puerto Rico for an extra week. During the EHT the patients were allowed to use their systemic and topical medication as prescribed and previously used. Of the patients with psoriasis, 15 were using a biological drug with or without methotrexate, five were taking methotrexate as a monotherapy, four acitretin and one cyclosporine. Thirteen of the psoriasis patients were using potent or very potent topical corticosteroids and 30 of the AD patients were using mild-to-potent topical corticosteroids. Adjustments in the drugs were allowed during the study period.

The sunbathing time was divided equally between both sides of the body and varied according to the Fitzpatrick's skin phototype (7), season, disease group and severity, being initially 20–90 min for psoriasis patients and 15–30 min for AD patients. The daily solar exposure time was increased to 90 min within a week and up to 300 min for psoriasis and 120 min for AD in cases of skin phototype IV. The scheduled exposure time was spent in the mornings or afternoons without sunscreens. Thereafter a sunscreen was applied liberally. Nurses were available at any time.

The EHT staff for the psoriasis groups consisted of two nurses and two physiotherapists experienced in rehabilitation and group leading. The supporting education for the psoriasis patients included themes such as *psoriasis as a chronic disease* (2h), *sunbathing* (1h), *the role of sleep and resting* (2h), *skin care and pain relief* (2h), *nutrition* (1h) and *weight control* (1h). Three group

discussions were arranged (5-6h) focusing on sharing experiences of life with psoriasis, treatment experiences, achieving a healthy life-style, coping with stress and cessation of smoking. Physical exercise (24h) included water sports, trekking and gymnastics.

The staff for the AD groups consisted of a group leader, a nurse and a psychologist. The supporting education included themes such as *atopic dermatitis* (2h), *sunbathing* (1h), *skin care* (2h), *nutrition* (1h) and *patient organizations* (1h). Four group discussions with a psychologist were arranged (6h), on topics that included mental well-being, psychosomatics and acceptance of a chronic disease. The objective was to increase empowerment and support self-care of AD and general well-being. Physical exercise (11h) included water sports, trekking and gymnastics.

The protocol was approved by the Ethics Committee of Tampere University Hospital (N:o R12219). All the participants gave their informed consent.

Assessment of health-related quality of life and disease severity

HRQoL was assessed with the Dermatology Life Quality Index (DLQI) (8) and RAND-36 (4-week version), for which physical component summary (PCS) and mental component summary (MCS) scores were calculated (9). Disease severity was assessed with the Self-Administered Psoriasis Area and Severity Index (SAPASI) (10) and Patient-Oriented Scoring of Atopic Dermatitis (PO-SCORAD) (11). Disease severity and pruritus were assessed using a Visual Analogue Scale (VAS) (12). The self-assessments were performed during the education day prior to departure (T1), immediately after returning home from EHT (T2), and 3 months after T2 (T3). Data were available for 127, 125 and 92 psoriasis patients and 59, 57 and 45 AD patients, respectively.

Statistics

Repeated data were analysed using generalising estimating equation (GEE) models with an unstructured correlation structure and bootstrap-type standard error. The GEE models, which were developed as extensions of the general linear model for analysing longitudinal and other correlated data, take into account the correlation between repeated measurements in the same subject, do not require complete data, and allow a fit to be achieved even when observations for some individuals are lacking at certain time-points. Bonferroni adjustments were performed to correct the significance levels for the multiple tests. The normality of the variables was evaluated using the Shapiro-Wilk statistics. The Stata 14.0 statistical package of StataCorp LP (College Station, TX, USA) was used for the analyses.

RESULTS

Demographic data

The mean age of the psoriasis patients was 51 years (range 20 – 75 years), 70 (55%) being females and 67 (53%) having psoriatic arthritis. The Fitzpatrick's skin phototype (II/III/IV) distribution was 21/97/9. Thirty-eight patients (30%) were taking a systemic drug for psoriasis. The mean baseline SAPASI score was 7.4 (95% CI 6.4 – 8.5).

The mean age of the AD patients was 37 years (range 19 – 74 years), and 50 (85%) were females. The Fitzpatrick's skin phototype (II/III/IV) distribution was 11/44/4. The mean baseline PO-SCORAD score was 36.8 (95% CI 32.7 – 40.9).

Health-related quality of life

The DLQI score and the RAND-36 summary scores (PCS and MCS) decreased significantly for both patient groups from the baseline (T1) to the end of the EHT (T2) ($p < 0.001$, Table I, Table IIs). The DLQI scores remained significantly decreased relative to T1 in both patient groups ($p < 0.001$) three months after the EHT (T3). The minimal clinically important difference (MCID) of 4 points (13) was achieved by 34/92 psoriasis patients (37%) and 26/45 AD patients (58%). The RAND-36 summary scores (PCS and MCS) remained significantly improved relative to the baseline (T1) for 3 months after the EHT (T3) in both patient groups (Table I, Table IIs). The change in global VAS had a moderate positive correlation with the DLQI scores ($r^2 = 0.40$), but its correlations with the PCS and MCS scores were weak ($r^2 = 0.08$ and $r^2 = 0.03$) (Fig. 1s).

Disease severity

The SAPASI score was decreased by 5.0 from 7.4 ± 5.8 between T1 and T2 ($p < 0.001$) and the PO-SCORAD score by 24.0 from 36.8 ± 15.5 ($p < 0.001$). A 75% clearance was seen in 59 psoriasis patients (46%) and 27 AD patients (46%). VAS showed a significant decline in disease severity and pruritus in both groups ($p < 0.001$) (Table I). The baseline disease severity and its improvement were independent of the time of year and sunbathing time in both disease groups. Three months after the EHT (T3) the SAPASI and PO-SCORAD scores were still significantly lower than at T1 ($p < 0.001$), and the VAS scores were also significantly lower in both groups (Table I).

Subgroup outcomes

The patients were divided in terms of severity into *mild* and *moderate-to-severe* subgroups, the cut-off points being a SAPASI score of 10 and a PO-SCORAD score of 25.

Patients with *mild* psoriasis (n=86) had a mean age of 51 years (range 23–72), 62% were females and their baseline DLQI was 6.7 ± 4.8 , whereas patients with *moderate-to-severe* psoriasis (n=33) had a mean age of 48 years (range 20–71), 52% were females and their baseline DLQI was 11.0 ± 6.0 . The former had a mean DLQI improvement of 1.7 ± 5.4 and the latter of 5.3 ± 6.0 , the latter also being clinically significant (13). The groups differed significantly only in their baseline DLQI ($p < 0.001$).

The patients with *mild* AD (n=16) had a mean age of 39 years (range 20–74), 88% were females and their baseline DLQI was 5.6 ± 3.1 , whereas those with *moderate-to-severe* AD (n=43) had a mean age of 37 years (range 19–65), 84% were females and their baseline DLQI was 12.2 ± 5.2 . The former had a mean DLQI improvement of 1.1 ± 2.9 and the latter 6.3 ± 4.2 , the latter being clinically significant (13). The groups differed significantly only in their baseline DLQI ($p < 0.001$).

DISCUSSION

The EHT improved the HRQoL and alleviated disease severity in the psoriasis and AD patients for at least 3 months. A larger proportion of the AD patients (58%) than of the psoriasis patients (37%) achieved a clinically significant DLQI improvement, possibly because of their initially more severe disease. The RAND-36 PCS score improved more in the psoriasis patients, and the MCS score in the AD patients. The schedule for the psoriasis patients included more physical exercise, with the aim of inspiring them to reduce their risk of comorbidities and alleviating symptoms related to psoriatic arthritis. The difference in course content must be kept in mind when comparing the results between the groups.

Although the initial SAPASI was low (7.4), indicating a mild disease, the proportion of psoriasis patients who achieved 75% clearance was only moderate (46%), which could have been due to the short duration of the EHT or to the insensitivity of SAPASI with regard to a mild disease state. Psoriasis in general requires more than two weeks of any phototherapy to achieve alleviation. As reported by Wahl et al. (6) in a similar setting, the SAPASI was still significantly reduced after 3

months. The initial PO-SCORAD score reported here (36.8) and its improvement after 3 months are in agreement with the results presented earlier by Autio et al. (4).

Patient education as an adjunct to treatment is a novel element in the long-term treatment of chronic skin diseases. Teaching in self-care, peer-to-peer support, workshops, relaxation practice and multidisciplinary discussion groups have been shown to improve the HRQoL of such patients (14). The new EHT combines these methods with traditional heliotherapy. During an EHT course the patients are away from their homes and jobs and in a stress-free environment together with peers. Since at least five of the questions in DLQI are related to home environment, work and personal relationships, DLQI scores immediately after returning home have to be interpreted with caution. The challenge of EHT is how to maintain the effects in the long term after returning to normal life (6).

There are certain limitations in the study design, such as the lack of a control group. To demonstrate superiority of EHT compared to HT, the patients should be randomized to receive either traditional HT or EHT. This is difficult, since the new EHT model has become a routine practice. There is also no long-term DLQI data from past traditional HT courses arranged in the Canary Islands to compare these new results. Empowerment can be assessed with novel tools, such as the Health Education Impact Questionnaire (heiQ) measuring illness perception, self-management and coping (6), but unfortunately these were not commonly used in dermatology when we were planning the present study. Our primary focus was to demonstrate how empowerment methods and alleviation of disease severity improve HRQoL, which we consider the most important end-point. Season of EHT or sun exposure time did not seem to affect the outcomes during EHT, but drug adjustments were allowed during the 3-month follow-up, which might have had an effect on the outcomes.

To conclude, the EHT courses improved HRQoL of psoriasis and AD patients for at least 3 months and alleviated the severity of their disease. Clinically significant improvements were shown by patients with moderate-to-severe symptoms, suggesting that EHT should be targeted primarily at these cases.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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Table I. *Improvement in the Dermatology Life Quality Index (DLQI) and alleviation of disease severity during the empowering heliotherapy course.*

	Baseline	Change from baseline	
	Mean (SD)	Δ Week 2 Mean (95% CI)	Δ Week 14 Mean (95% CI)
Psoriasis			
DLQI	8.2 (5.9)	-5.4 (-6.4 to -4.4)***	-2.6 (-3.7 to -1.4)***
RAND-36 physical component	41.1 (10.7)	2.7 (1.4 to 4.0)***	3.9 (2.4 to 5.3)***
RAND-36 mental component	45.7 (11.0)	6.1 (4.3 to 7.9)***	3.0 (1.0 to 4.9)**
SAPASI	7.4 (5.8)	-5.0 (-5.9 to -4.1)***	-2.7 (-3.8 to -1.6)***
VAS pruritus	3.4 (2.4)	-2.0 (-2.4 to -1.7)***	-0.7 (-1.2 to -0.1)*
VAS global	4.1 (2.0)	-2.0 (-2.3 to -1.7)***	-1.2 (-1.7 to -0.8)***
Atopic dermatitis			
DLQI	10.4 (5.5)	-7.7 (-9.1 to -6.4)***	-4.8 (-6.2 to -3.5)***
RAND-36 physical component	46.6 (8.8)	3.3 (1.5 to 5.2)**	2.8 (0.9 to 4.7)*
RAND-36 mental component	44.5 (10.5)	6.5 (3.9 to 9.2)***	4.7 (2.4 to 7.1)**
PO SCORAD	36.8 (15.5)	-24.0 (-27.3 to -20.1)***	-14.6 (-18.7 to -10.6)***
VAS pruritus	4.5 (2.3)	-3.4 (-4.0 to -2.8)***	-2.4 (-3.1 to -1.6)***
VAS global	3.9 (2.1)	-2.8 (-3.3 to -2.3)***	-1.9 (-2.5 to -1.4)***

Bonferroni-adjusted p-values *** p<0.001, **p<0.01, *p<0.05 compared with day 1 of heliotherapy.

Table IIs (eSupplement). *Improvement in quality of life as measured by the RAND-36 questionnaire during the empowering heliotherapy course.*

	Baseline	Change from baseline	
	Mean (SD)	Δ Week 2 Mean (95% CI) ¹	Δ Week 14 Mean (95% CI) ¹
Psoriasis			
Physical function	73.6 (21.7)	5.3 (1.9 to 8.7)***	5.0 (1.6 to 8.5)**
Physical role	46.2 (42.3)	14.4 (6.7 to 22.1)***	15.3 (5.5 to 25.2)**
Bodily pain	58.3 (26.1)	7.5 (3.8 to 11.1)***	6.7 (1.3 to 12.0)**
General health	48.5 (18.9)	6.1 (3.2 to 9.0)***	5.0 (2.1 to 8.0)***
Social function	68.9 (22.7)	10.8 (6.9 to 14.6)***	6.6 (1.1 to 12.2)*
Emotional role	61.8 (42.3)	12.5 (3.8 to 21.3)**	7.5 (-2.5 to 17.5)
Vitality	50.6 (19.9)	15.9 (12.0 to 19.7)***	9.3 (4.9 to 13.8)***
Mental health	67.1 (18.1)	10.5 (7.1 to 13.8)***	4.6 (0.6 to 8.5)*
Atopic dermatitis			
Physical function	84.7 (16.6)	8.1 (3.5 to 12.8)**	6.3 (1.7 to 10.9)**
Physical role	56.6 (42.1)	15.5 (3.2 to 27.7)*	20.7 (5.4 to 36.0)*
Bodily pain	70.0 (24.4)	10.1 (2.7 to 17.4)**	2.7 (-4.8 to 10.3)
General health	56.0 (21.2)	8.1 (3.5 to 12.8)***	6.3 (1.7 to 10.9)**
Social function	69.1 (24.7)	12.6 (6.2 to 19.0)***	13.2 (5.4 to 21.1)***
Emotional role	63.1 (41.7)	14.8 (1.0 to 28.7)*	16.1 (1.7 to 30.4)*
Vitality	55.5 (20.6)	15.5 (9.8 to 21.1)***	5.8 (-0.3 to 11.8)
Mental health	67.6 (18.1)	11.9 (7.8 to 16.0)***	5.4 (1.1 to 9.7)*

¹Bonferroni confidence intervals

Bonferroni-adjusted p-values *** p<0.001, **p<0.01, *p<0.05 compared with values on day 1 of heliotherapy.

Figure 1s (eSupplement). Relationship of change from the baseline global VAS score to improvements in a) the DLQI and RAND-36 findings and the b) physical and c) mental component summary scores in patients with psoriasis or atopic dermatitis ($n = 186$).

