Tampere University

ANNA TOLEDO

Effects of Narrow-band Ultraviolet B Radiation on Formation of Neuroendocrine Mediators in the Skin and on Mood, and Findings on Tanning Dependence and Winter Blues among Sunbathers

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to Carlos, Isabella and Emilia

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Anna Toledo

ABSTRACT

Exposure to ultraviolet radiation (UVR) has many effects on skin, including DNA mutation and simultaneous immunosuppression, carcinogenesis and vitamin D (VitD) synthesis. In the skin there exists a local hypothalamus-pituitary-adrenal (HPA) analogue axis that produces numerous neuroendocrine mediators (NECM), including β-endorphin (β-END), which may lead to tanning addiction. A visible portion of sunlight relieves the symptoms of seasonal affective disorder (SAD) through the eyes. SAD has been proposed to be associated with tanning addiction. Many physiological functions, including hormone secretion and cellular functions, as well as psychological and behavioural rhythms are under the control of the human circadian clock. Psychological symptoms such as insomnia and depressed mood are associated with evening chronotype, while exposure to UVR is known to induce a chain of neural and humoral reactions that may involve mood-enhancing effects. The present studies of which this dissertation is composed aimed, by means of immunohistochemical (IHC) staining of skin biopsies, to shed light on the biochemical background of UVR-related tanning dependence in a sample of healthy volunteers (Study I), and through questionnaires the association of tanning dependence and symptomatic seasonality among a cohort of sunbathers in Southern Finland (Study II). We also studied whether the narrowband-UVB (NB-UVB) exposures elicit a change in mood states differently depending on chronotype, and if such change may be associated with skin-borne circulating mediators (Study III).

In study I, the 12 healthy volunteers received whole-body NB-UVB exposures. Seven volunteers received only one standard erythema dose (SED) on day one, whereas five other volunteers received a total cumulative dose of 3 SED (1 SED + 2 SED) on two consecutive days. The skin biopsies were taken 24 hours after the last NB-UVB exposure. The IHC staining showed, as a novel finding, increased epidermal β-END expression in 11 out of 12 samples and also, as expected and previously reported, an increase in nuclear p53 expression in all 12 samples. NB-UVB-induced changes in the expression of pro-opiomelanocortin (POMC) and α-melanocyte stimulating hormone (α-MSH) were only detected in single volunteers. It was summarized that NB-UVB increases β-END expression effectively in epidermal keratinocytes *in vivo*. Similar findings have been reported in animal and cell

culture studies. NB-UVB-induced cutaneous β-END expression may be the key link to tanning dependence.

In study II, the frequency of tanning dependence or abuse and symptoms of SAD among Finnish sunbathers were evaluated using the Structured Interview for Tanning Abuse and Dependence (SITAD) and the Seasonal Pattern Assessment Questionnaire (SPAQ). Out of 229 sunbathers, 18 (7.9%) met the criteria of tanning dependence and 59 (25.8%) tanning abuse. In addition, 60 (26.2%) of respondents had symptoms equivalent to subsyndromal seasonal affective disorder (S-SAD) and 37 (16.2%) equivalent to SAD. It was summarized that sunbathing dependence or abuse and SAD or S-SAD were frequent among Finnish sunbathers, but showed no statistical connection and appeared to be separate phenomena.

In study III, the healthy subjects received a total of four whole body NB-UVB exposures on consecutive days. Six volunteers received a cumulative dose of 8 SED and five volunteers received a cumulative dose of 7 SED. The mean state of mood, assessed with Visual Analogue Scale (VAS), improved significantly over the five days ($p = 0.038$). The mood state improved more in those with circadian preference toward eveningness assessed with a shortened version of Horne and Östberg's Morningness-Eveningness Questionnaire (MEQ), the effect being significant for the change on the VAS-wellbeing dimension ($p = 0.021$). Mean baseline mood state correlated with baseline circulating 25-hydroxyvitamin D [25(OH)D] ($r = -0.54$, 95% CI: −0.86 to −0.09) and with baseline circulating cortisol level ($r = -0.57$, 95% CI: −0.87 to −0.04). NB-UVB exposures significantly improved mean circulating 25(OH)D concentrations of all volunteers from initial mean values of 75.5 ± 28.0 nmol/L to 83.7 ± 27.0 nmol/L, the average increase being 8.3 nmol/L ($p < 0.001$). Additionally, mean circulating interleukin-6 (IL-6) decreased significantly by −0.35 pg/mL from the initial values of 1.12 ± 0.66 pg/mL to 0.76 ± 0.19 pg/mL, the decrease being significant ($p = 0.025$). Mean circulating immunoreactive β-END and cortisol levels showed no significant increase or decrease. It was summarized that four sub-erythematous NB-UVB exposures had a significant impact on perceived mood states, circulating 25(OH)D and IL-6 levels. Improvement in mood was greater among those with preference toward eveningness rather than morningness.

To conclude, in the present three studies with healthy volunteers NB-UVB exposures effectively induced β-END in epidermal keratinocytes of human skin. NB-UVB exposures also significantly increased serum 25(OH)D levels of the 12 volunteers, meanwhile their serum IL-6 levels were decreased significantly. At the same time, we found the mean state of mood of volunteers to improve and this improvement was found to be greater in chronotype "eveningness" than in those

presenting with chronotype “morningness”. UVR-related tanning dependence and symptoms of SAD/S-SAD were frequent among Finnish sunbathers, but showed no association.

TIIVISTELMÄ

Ultraviolettisäteily (UV-säteily) vaikuttaa ihoon monin tavoin, kuten vaurioittamalla DNA:ta, vaimentamalla immuunivastetta sekä aiheuttamalla ihosyöpiä. UV-säteilyn tärkeä myönteinen vaikutus on D-vitamiinin muodostuminen ihossa jo vähäisessä UV-altistuksessa. Ihossa toimii paikallinen keskushermoston hypotalamus-aivolisäke-lisämunuais (HPA) -akselia vastaava mekanismi. Iho kykenee tuottamaan monia neuroendokriinisiä välittäjäaineita mukaan lukien β-endorfiinia (β-END), jonka on ajateltu voivan aiheuttaa rusketusriippuvuutta. Näkyvän valon on havaittu hoitavan tehokkaasti kaamosmasennuksen (seasonal affective disorder, SAD) oireita. Kaamosoireilun ja rusketusriippuvuuden välillä on puolestaan ehdotettu olevan yhteys. Sisäinen kello ohjaa monia elimistön toimintoja, kuten psykologisia ja käyttäytymiseen liittyviä rytmejä sekä hormonien eritystä ja solujen toimintaa. Iltavirkut ovat muita alttiimpia psyykkisille oireille, kuten unettomuudelle ja masennukselle. UV-säteily voi aikaansaada hermostollisten ja kudosnesteen mukana kulkevien tekijöiden ketjureaktion, mikä voi johtaa UV-säteilyn mielialaa parantavaan vaikutukseen. Väitösosatyössä I selvitettiin terveiden vapaaehtoisten ihossa mahdollisesti auringonottoriippuvuutta aiheuttavien biokemiallisten vaikuttaja-aineiden muutoksia immunohistokemiallisissa värjäyksissä ihon pintakerroksessa (orvaskedessä) UV-säteilytyksen jälkeen. Osatyössä II selvitimme, voidaanko eteläsuomalaisilla auringonottajilla havaita yhteyttä rusketusriippuvuuden ja kaamosoireiden välillä. Osatyössä III selvitimme, vaikuttaako kapeakaistainen UVB (NB-UVB)-säteily tutkittavien mielialaan eri tavoin kronotyypin mukaan ja onko mielialan muutoksen ja verenkierrosta mitattujen välittäjäaineiden pitoisuuksien välillä yhteyttä.

Ensimmäisessä väitöskirjan osatyössä (I) terveet vapaaehtoiset (n=12) saivat NB-UVB-säteilyä koko vartalolle. Seitsemän vapaaehtoista sai yhden standardieryteemayksikön (SED) suuruisen valoannoksen ensimmäisenä päivänä ja viisi vapaaehtoista kolmen SED:n (1 SED + 2 SED) suuruisen kumulatiivisen valoannoksen kahtena peräkkäisenä päivänä. Ihobiopsiat otettiin 24 tuntia NB-UVB-valotuksen jälkeen. Immunohistokemiallisissa värjäyksissä 11/12 näytteestä havaittiin ihon orvaskedessä β-END:n muodostumista ja kaikissa odotetusti p53-proteiinia. NB-UVB-valotuksen aikaansaamat pro-opiomelanokortiinin (POMC) ja

α-melanosyyttiä stimuloivan hormonin (α-MSH) muodostuminen oli havaittavissa vain yksittäisissä näytteissä. Keskeinen uusi löydös oli, että ihmisilläkin kapeakaistainen UVB-säteily *in vivo* johtaa β-END:n muodostumiseen ihon pintakerroksen keratinosyyteissä. Vastaava on aiemmin osoitettu hiirillä ja soluviljelmissä. β-END:n muodostus ihossa saattaa liittyä rusketusriippuvuuteen.

Toisessa väitöskirjan osatyössä (II) suomalaisten auringonottajien rusketusriippuvuuden ja kaamosoireiden yleisyyttä tutkittiin rusketusriippuvuuden seulontaan suunnatulla kyselyllä (Structured Interview for Tanning Abuse and Dependence questionnaire, SITAD) ja kaamosmasennuksen seulontaan tarkoitetulla kyselyllä (Seasonal Pattern Assessment Questionnaire, SPAQ). Kyselyyn vastasi 229 auringonottajaa, joista 18 (7,9 %) luokiteltiin rusketusriippuvaisiksi ja 59 (25,8 %) rusketuksen väärinkäyttäjiksi. Lisäksi vastaajista 60 (26,2 %) kertoi kärsivänsä kaamosoireista siinä määrin, että ne vastasivat kaamosmasennusta lievempää oireyhtymää (subsyndromal seasonal affective disorder, S-SAD). Vastaajista 37 (16,2 %) kertoi kärsivänsä kaamosoireista siinä määrin, että ne vastasivat vakavuudeltaan kaamosmasennusta. Tulosten mukaan rusketusriippuvuus tai ihon ruskettamisen ”väärinkäyttö” sekä SAD tai S-SAD olivat molemmat yleisiä eteläsuomalaisten auringonottajien otoksessa, mutta rusketushakuisuuden ja kaamosoireilun välillä ei kohortissa havaittu tilastollista yhteyttä vaan ne tulkittiin toisistaan erillisiksi ilmiöiksi.

Kolmannessa väitöskirjan osatyössä (III) terveet vapaaehtoiset saivat neljänä peräkkäisenä päivänä NB- UVB-valotuksen koko keholleen. Vapaaehtoisista kuusi sai 8 SED:n kumulatiivisen UVB-annoksen ja viisi 7 SED:n annoksen. Tutkittavien mielialaa arvioitiin tutkittavan 100 mm:n pituisille janoille merkityn mielialaa osoittavan arvion mukaan (Visual Analogue Scales, VAS). VAS:n mukaan tutkittavien mieliala parani tilastollisesti merkitsevästi viiden tutkimuspäivän aikana ($p = 0.038$). Mieliala parani enemmän iltavirkuilla. Kronotyyppi määritettiin erillisellä kronotyypin seulontaan käytetyllä kyselyllä (Morningness-Eveningness Questionnaire, MEQ). Mieliala parani tilastollisesti merkitseväsi hyvinvointia mittaavassa VAS-osiossa ($p = 0.021$). Tutkimuksen alussa ennen valotuksia mieliala korreloi seerumin kalsidioli[25(OH)D]-pitoisuuden kanssa ($r = -0,54$, 95 % CI: $-0,86$ to $-0,09$) ja seerumin kortisolipitoisuuden kanssa ($r = -0,57$, 95 % CI: $-0,87$ to $-0,04$). NB-UVB-valotus nosti seerumin 25(OH)D-pitoisuutta kaikilla tutkittavilla tilastollisesti merkitsevästi lähtötasosta 75,5 ± 28,0 nmol/L tasolle 83,7 ± 27,0 nmol/L, jolloin yksilöllinen muutos oli keskimäärin 8,3 nmol/L ($p < 0,001$). Lisäksi seerumin interleukiini-6:n (IL-6) pitoisuus laski tilastollisesti merkitsevästi $-0,35$ pg/mL yksikön verran lähtötasosta 1,12 ± 0,66 pg/mL tasolle 0,76 ± 0,19 pg/mL ($p = 0,025$). Seerumin β-END- ja kortisolipitoisuudet eivät nousseet tai

laskeneet tilastollisesti merkitsevästi. Neljän NB-UVB-valotuksen vaikutus tutkittavien mielialaan, seerumin 25(OH)D- ja IL-6-pitoisuuksiin havaittiin merkittäväksi, ja lisäksi totesimme mielialan parantuneen enemmän iltavirkuilla kuin aamuvirkuilla.

Yhteenvetona, NB-UVB-valotus lisäsi β-END:n määrää terveiden vapaaehtoisten ihon pintakerroksen keratinosyyteissä ihonäytteiden immunohistokemiallisten värjäysten perusteella arvioituna. Valotus nosti merkitsevästi samojen tutkittavien seerumin 25(OH)D-pitoisuutta ja laski merkitsevästi seerumin IL-6-pitoisuuksia. Lisäksi tutkittavien mieliala parani tutkimuksen aikana ja muutos oli suurempi iltavirkuilla kuin aamuvirkuilla. UV-säteilyyn liittyvä rusketusriippuvuus ja kaamosoireilu näyttivät olevan yleisiä kohortin eteläsuomalaisilla auringonottajilla. Rusketusriippuvuusoireiden ja kaamosoireiden välillä ei havaittu yhteyttä.

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ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
α-MSH	Melanocyte stimulating hormone
β-END	β-endorphin
BAITS	Behavioral Addiction Indoor Tanning Screener
BLT	Bright light therapy
CAGE	Cut down, Annoyed, Guilty, Eye-opener
cAMP	Cyclic adenosine monophosphate
CAR	Cortisol-awakening-response
CBT	Cognitive Behavioral Therapy
CDI	Children's Depression Inventory
CIDI	Composite International Diagnostic Interview.
CIE	Commission Internationale de l'Éclairage
CNS	Central nervous system
CORT	Corticosterone
CRH	Corticotropin-releasing hormone
CSM	Composite Scale of Morningness
DSM	Diagnostic and Statistical Manual of Mental Disorders
GSS	The Global Seasonality Score
HPA	Hypothalamus-pituitary-adrenal
ICH	Immunohistochemical
IL-6	Interleukin-6
MC	Melanocortin
MCTQ	Munich ChronoType Questionnare
MDD	Major depressive disorder
MEQ	Morningness-eveningness questionnaire
MES	Morningness-eveningness sum score
MESC,	Morningness/Eveningness Scale in Children
NB-UVB	Narrow-band ultraviolet B
NECM	Neuroendocrine mediators
25(OH)D	25-hydroxyvitamin D

p53	Tumor Protein P53
POMC	Pro-opiomelanocortin
QIDS-SR	Quick Inventory of Depressive Symptomatology – Self-Rated
SAD	Seasonal affective disorder
SED	Standard erythema dose
S-SAD	Subsyndromal Seasonal affective disorder
SCN	Suprachiasmatic nucleus
SITAD	Structured Interview for Tanning Abuse and Dependence
SPAQ	Seasonal Pattern Assessment Questionnaire
SSRI	Selective Serotonin Reuptake Inhibitor
UVR	Ultraviolet radiation
VAS	Visual Analogue Scale
VitD	Vitamin D

ORIGINAL PUBLICATIONS

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

I Jussila A, Huotari-Orava R, Ylianttila L, Partonen T, Snellman E. Narrow-band Ultraviolet B Radiation Induces the Expression of β-endorphin in Human Skin *in vivo*. J Photochem Photobiol B 2016; 155:104-8.

II Toledo A, Yli-Uotila E, Kautiainen H, Pirkola S, Partonen T, Snellman E. Tanning dependence and seasonal affective disorder are frequent among sunbathers but are not associated. Psychiatry Res 2019; 272:387-91.

III Toledo A, Karppinen T, Miettinen ME, Leppäluoto J, Vuolteenaho O, Ylianttila L, Kautiainen H, Snellman E, Partonen T. Narrow-band ultraviolet B (NB UV-B) exposures improve mood in healthy individuals differently depending on chronotype. Chronobiol Int 2019; 36:1570-80.

1 INTRODUCTION

Excessive exposure to ultraviolet radiation (UVR), whether from sunlight or tanning devices, is considered a predominant environmental risk factor for skin cancers, and the incidence of melanoma and non-melanoma skin cancers rises despite public education campaigns intended to reduce public exposure to natural and artificial UVR (IARC 2012). Despite awareness of the harmful effects of UVR, sunbathing and indoor tanning continue to be prevalent (Nolan et al. 2009). A growing body of literature provides evidence that the need to acquire a tan could be a consequence of tanning dependence, with a physiological, opioid-related background mediated through UVR exposure of the skin (Wintzen et al. 2001a; Feldman et al. 2004; Fell et al. 2014; Skobowiat and Slominski 2015). Earlier animal and cell culture studies have confirmed the role of cutaneously produced β-endorphin (β-END) in the development of UVR addiction (Slominski et al. 2012; Fell et al. 2014; Skobowiat and Slominski 2015). Frequent indoor tanning could also be considered as an attempt to self-treat seasonal affective disorder (SAD) or its milder form subsyndromal SAD (S-SAD) (Hillhouse et al. 2005; Petit et al. 2014; Heckman et al. 2016).

Tanners may find the experience of tanning relaxing and some earlier studies have reported the mood-enhancing effect of UVR (Gambichler et al. 2002b; Taylor et al. 2009; Veleva et al. 2018). Sunlight is the most important source of vitamin D (VitD) for most humans. A possible mood-elevating effect of UVR via the skin may be through the VitD pathway. Also, other UVR-induced neural or humoral reactions may be involved. Psychological symptoms, such as depressed mood, are associated with evening chronotype (Taylor and Hasler 2018). The human circadian clock genes have been shown to be associated with mood disorders (e.g., depressive episodes, see Kovanen et al. 2013), as well as to regulate the sensitivity of the skin to UVR-induced damage (Gaddameedhi et al. 2015). Therefore, the interactions of UVR exposure with circadian clock proteins may affect individual skin cancer risk as well as mood.

Our study aimed to examine whether NB-UVB is capable of inducing β-END formation in skin biopsies *in vivo* as well as in the circulation, and whether the findings

may offer a plausible explanation for the UVR-related tanning addiction. We studied the effect of NB-UVB on mood by chronotype and the effect of NB-UVB on skin-borne circulating mediators, such as VitD. We also aimed to study the prevalence of tanning dependence and symptomatic seasonality of a sample of sunbathers in Southern Finland, and whether the tanning dependence and seasonality showed a connection.

Author's Contribution:

In studies I and III the author participated to design the study and recruited the volunteers for the study. The author gave the NB-UVB phototerapy to the participants and took the skin biopsies from the volunteers' skin. The author participated in collecting the blood samples. In study I the author examined the skin biopsies under the microscope with the dermatopathologist Riitta Huotari-Orava. In study III the author participated to analyse the data with the help of statistician Hannu Kautiainen. In studies I and III the author wrote the paper with the support of supervisors Erna Snellman and Timo Partonen and with the input from all the authors of the paper. The author submitted the paper to the journal and contributed to the final version of the paper with the help of supervisors and other authors of the paper.

In study II the author participated to design the study. The author participated to analyse the data with the help of statistician Hannu Kautiainen. The author wrote the paper with the support of supervisors Erna Snellman and Timo Partonen and with the input from all the authors of the paper. The author submitted the paper to the journal and contributed to the final version of the paper with the help of the supervisors and other authors of the paper.

2 REVIEW OF THE LITERATURE

2.1 Ultraviolet radiation

2.1.1 Solar UV radiation

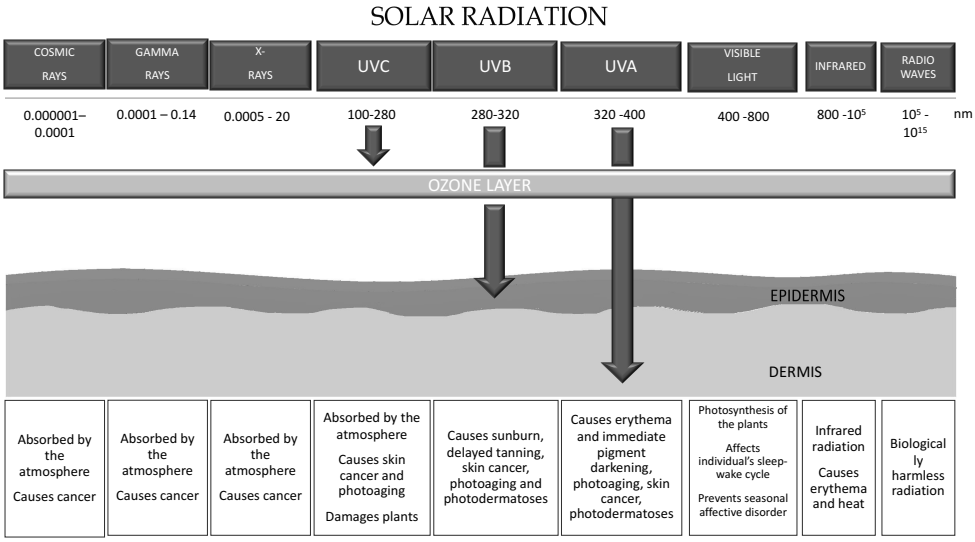
Ultraviolet radiation (UVR) represents the electromagnetic energy, and the spectrum of UVR is divided into three wavelengths including UVC (100–280 nm), UVB (280–315 nm) and UVA (315–400 nm), which have different biological effects (Diffey 2002). Environmental and dermatological photobiologists usually tend to define the wavelength range in different ways: UVC (200–290 nm), UVB (290–320 nm) and UVA (320–400nm) (Diffey 2002). Most of the shortwave and high-energy spectrum of UV is absorbed by the ozone layer and cannot reach the earth's surface. The ozone layer absorbs wavelengths up to approximately 310 nm (Reichrath 2006). In consequence the high energy UVC radiation is almost completely absorbed, UVB radiation is partly absorbed and UVA radiation is absorbed by the ozone layer only minimally or not at all. The UV spectrum of solar radiation reaching the earth's surface is represented by UVB (5–10%) and UVA (90–95%) wavelengths. (D'Orazio et al. 2013.) (Figure 1)

The external factors affecting the intensity of UVR include the amount of ozone, cloud cover, aerosol, the reflectivity (albedo) and altitude of the surface and the solar zenith angle, which depends on the latitude and time of the year and day. The solar zenith angle represents the angle between the local vertical axis and the position of the sun. A small solar zenith angle is a typical finding at low latitudes, in the summer and in the afternoon, while a large solar zenith angle is a typical finding at high latitudes, in the winter and in the early morning/late afternoon. The internal factors affecting the intensity of UVR include skin phototype, use of sunscreen and clothing and individual's age. (Webb 2006.)

The biological effects of UVR are dependent on the wavelength. UVB penetrates the level of dermal stratum papillare and is almost completely absorbed by the epidermis. In contrast, UVA penetrates deep into the dermis (D'Orazio et al. 2013). Both UVB and UVA can damage DNA by inducing structural modifications in

DNA (Reichrath 2006). UVB has cutaneous mutagenic and carcinogenic effects including DNA molecular rearrangements, which result the formation of specific photoproducts such as cyclobutene-type pyrimidine dimers and 6–4 pyrimidime photoproducts (Dakup and Gaddameedhi 2017). UVA is less carcinogenic but creates reactive oxygen species damaging DNA and has effects on photoaging (D'Orazio et al. 2013) (Figure 1).

Figure 1. Electromagnetic spectrum of solar radiation and its effects on biological systems.



The spectrum of solar radiation reaches from cosmic rays to radio waves. Most solar radiation is filtered by the atmosphere. The energy of sunlight that reaches the earth's surface consists of UV, visible light and infrared. The ozone layer absorbs UVC when ambient sunlight consists predominantly of UVA (90-95%) and UVB (5-10%). UVA penetrates the epidermis deep into dermis, while UVB penetrates the epidermis. UVA contributes to skin cancer through oxidative injury. UVB has high energy and damages cell DNA in epidermis.

2.1.2 Measurement of UV radiation doses

Radiometric terminology exists to describe the quantities of UVR: the terms radiant energy (J) and radiant flux (W) describe the beam of radiation and terms radiation intensity (W/sr) and radiance (W/m2 sr) describe the source of radiation. The term irradiance (W/m2) refers to the amount of UV arriving on the surface. For measuring the doses of UVR the unit used is joules per square metre or joules per

square centimetre (J/m² or J/cm², where J = Ws), which is the energy received per unit area in a given time. (Diffey 2002.)

Three different units are used to express erythema weighted dose. Different action spectra have been established by the Commission Internationale de l'Éclairage (e.g. erythema reference action spectrum) (CIE 1999). The CIE action spectrum refers to the erythemal effect of UV radiation and CIE-weighted irradiance is represented as mJ/cm2 or J/m2 (CIE 1999). Minimal erythemal dose (MED) is considered a threshold dose that can produce minimal erythema, for example sunburn, on an individual's skin within 24 hours of exposure (Diffey 2002). In phototesting the MED is usually defined as a dose capable inducing faint just perceptible erythema on the skin 24 hours from exposure (Heckman et al. 2013). The MED unit is not a standard measure and every individual has subjective MED that causes noticable erythema of the skin (Diffey 2002). Standard erythema dose (SED) is the standardized value to sun intensity and its effect on skin, regardless of skin phototype. SED as a standardized value equals 100 J/m2 and 10 mJ/cm² CIE erythema weighted irradiance (Diffey et al. 1997; CIE 1999). SED is used to refer to UVR exposures from natural and artificial sources. (Diffey et al. 1997.)

2.2 Cutaneous hypothalamic-pituitary-adrenal axis

2.2.1 Cutaneous elements of the hypothalamic-pituitary-adrenal axis

Skin and cutaneous cells have their own local hypothalamus-pituitary-adrenal (HPA) analogue axis regulated by biological, chemical and physical stressors. Skin and the central nervous system (CNS) have a common embryogenetic origin and the HPA-axes of the skin and CNS communicate in two directions (Slominski et al. 2007; 2012; 2013). The elements including hormones, neurotransmitters, neuropeptides and functional receptors are produced and released in both epidermal, dermal and adnexal cells and in dermal nerve endings (Slominski and Wortsman 2000; Slominski et al. 2007).

External and internal stressors cause the skin to activate either systemic HPA via neural transmission or local pituitary gland or adrenal cortex via skin-derived factors. The activation may be rapid through neural route or slow through humoral or immunological systems. In addition to the neuroendocrine system of the HPA axis, the regulation between the local and systemic responses involves the

hypothalamopituitary-thyroid (HPT) axis and catecholaminergic, melatoninergic, serotoninergic, cholinergic, steroidogenic and secosteroidogenic systems. The cutaneous HPA-axis includes a wide range of neuroendocrine mediators (NECM) including corticotropin-releasing hormone (CRH), the corticotropin-releasing hormone receptor-1 (CRH-R1), pro-opiomelanocortin (POMC) derived β-endorphin (β-END), adrenocorticotropic hormone (ACTH) and melanocyte stimulating hormone (α-MSH), melanocortin (MC), opiate receptors and enzymes needed for corticosteroid synthesis. (Slominski et al. 2012.)

UVR has a profound role among the physical stressors attacking the skin (Slominski et al. 2012). The effect of UVR on cutaneous the HPA axis is wavelength-dependent, and in general the more energetic and shorter the wavelength the stronger is the effect (Skobowiat et al. 2011). UVB radiation is capable of activating the HPA axis of human and mouse skin organ cultures *in vitro* (Skobowiat et al. 2011; 2013a). In a mouse model UVB was able to stimulate circulating levels of CRH, urocortin, β-END, ACTH and corticosterone (CORT), and to increase the expression of skin urocortin, β-END and CORT genes and proteins. UVB induced mechanisms of cutaneous HPA axis regulate the body homeostasis and require intact pituitary function for the systemic effect. (Skobowiat and Slominski 2015.)

2.2.2 p53, pro-opiomelanocortin and α-melanocyte stimulating hormone

UVR as an external stressor triggers human skin cells to produce CRH and related peptides urocortin 1 and 2 (Slominski et al. 2007). CRH binds to CRH receptor type 1 (CRH-R1) and induces the release of POMC-derived β-END, ACTH and α-MSH peptides, which for example have respective, anti-inflammatory, melanogenic, and protective functions in the skin (Skobowiat et al. 2011).

p53 is a transcription factor and tumor-suppressor protein with an essential role in response to UVR induced DNA damage in skin cells. In keratinocytes p53 induces transcriptional activation of POMC following UVR. p53 up-regulates POMC gene at mRNA and protein levels. Disruption in p53 function leads to abnormal cell survival responses and has a crucial role in the development of skin cancer. (Cui et al. 2007.)

UVR-induced POMC expression and POMC-derived α-MSH and ACTH have an essential role in melanogenesis and subsequent cutaneous pigmentation (Paus et al. 1999; Miller and Tsao 2010; D'orazio et al. 2013). In addition, α-MSH induced

pigmentation protects against UVR by enhancing repair of DNA photoproducts (Miller and Tsao 2010).

2.2.3 β-endorphin

The endogenous opioid peptide β-END is derived from its precursor β-lipotropin and exerts influence through opioid receptors in the skin (Slominski et al. 2012). Both cutaneous β-END and β-lipotropin are degraded from POMC and induced by UVR (Paus et al. 1999; Cui et al. 2007; Skobowiat et al. 2011; Slominski et al. 2012). β-END is known for generating analgesic and euphoria related feelings in humans. Tanners often report feelings of relaxation and positive mood after UVR exposure and these psychological effects may be explained by the production of cutaneous β-END induced by UVR (Slominski et al. 2012). It has been suggested that UVR addiction may be result from stimulation of β-END expression in the skin and plasma (Nolan et al. 2009; Skobowiat and Slominski 2015). Both exogenous and endogenous opioids function in the neuroendocrinological pathways of the skin and at systemic levels (Slominski et al. 2012). β-END binds specifically with μ-type opioid receptors, leading to morphine-like effects (Fell et al. 2014). However, β-END may also interact with other mechanisms which are as yet unidentified (Nguyen et al. 2012).

According to *in vitro* and *in situ* studies β-END and μ-type opioid receptors were expressed in human epidermal melanocytes cultures (Kauser et al. 2003), and in *in vitro* studies β-END expression was increased in UVR-exposed keratinocyte and melanocyte cultures of skin samples (Skobowiat et al. 2011; Slominski et al. 2012). In an *in vivo* human study β-END was present in keratinocytes of the follicular matrix and in sweat duct cells, but was absent from epidermal keratinocytes (Wintzen et al. 2001a). In a mouse study β-END was detected in the skin 12–24 h after UVR exposure. Maximum expression of β-END was after 12 h greatest effective influence was observed for UVC and UVB (Skobowiat and Slominski 2015). The effect of UVR on circulating β-END levels is inconsistent. Some studies have been unable to verify that UVR exposures increase the circulating levels of β-END (Wintzen et al. 2001b; Gambichler et al. 2002a; Kaur et al. 2006a), whereas one small study has shown this (Fallazadeh and Namazi 2009).

2.2.4 Cortisol and interleukin-6

Human skin expresses the genes coding for the key enzymes required for cutaneous corticosteroid synthesis, which leads to cutaneous production of cortisol and corticosterone (CORT) (Slominski et al. 2012). Cortisol production has been demonstrated in both *in vitro* and *in vivo* studies in hair follicles and in normal human epidermal and dermal skin cells including keratinocytes, melanocytes and dermal fibroblasts (Slominski et al. 2012). Cortisol production of the skin cells is regulated by ACTH and cAMP pathway, Interleukin-1 (IL-1) as well as by tissue injury and UVR (Slominski et al. 2005; Vukelic et al. 2011; Skobowiat et al. 2013b).

The activation and deactivation of cortisone to cortisol in the skin is mediated by two enzymes: 11b-Hydroxysteroid dehydrogenase type 1 (11b-HSD1) and 11b-hydroxysteroid dehydrogenase type 2 (11b-HSD2). Cortisol production is dependent on UVR wavelength, and UVB and UVC have been shown to be able to produce cortisol in the skin. (Skobowiat et al. 2013b.) However, it is unclear how UVR regulates cutaneous corticosteroidogenesis (Slominski et al. 2014).

UVR-induced immunosuppression is determined by dose and wavelength. UVR has a capability to modulate growth factors and induce primary cytokines (e.g. Interleukin 1, -6 and -10) in the skin. (Murphy 2009.) UVR induced IL-6 synthesis and release have both local inflammatory and immunosuppressive reactions and systemic effects through entering the circulation (Urbanski et al. 1990; De Vos et al. 1994). Urbanski et al. (1990) showed elevated IL-6 concentrations in circulation after whole body UVR exposure and the increase was greatest when the subjects' clinical sunburn reactions were most intense.

2.3 Benefits and disadvantages of UV light on humans

2.3.1 Photocarcinogenesis

Ultraviolet radiation (from sunlight or tanning beds) is a complete carcinogen and risk factor of skin cancers, including squamous cell carcinoma, basal cell carcinoma and malignant melanoma (IARC 1992; van der Rhee 2016). The incidence of melanoma and nonmelanoma skin cancers is rising despite extensive campaigns intended to reduce public exposure to natural and artificial UVR (Murphy 2009; WHO 2012). UVR causes DNA damage in the form of photoproducts, oxidative

stress and inflammation in the skin, and both UVA and UVB radiation may cause local and systemic immunosuppression, which increases the risk of skin cancers (Murphy 2009; D'Orazio et al. 2013; Fajuyigbe and Young 2016; Hart and Norval 2018). UVB wavebands are biologically the most active part of the solar spectrum reaching the earth and inducing DNA photoproducts, of which the most important are cyclobutane pyrimidine dimers and thymine dimers (Murphy 2009; Sklar et al. 2013). Unrepaired photoproducts lead to mutations in the epidermis and result in the development of skin cancers (Lan et al. 2016). In addition to UVR, skin cancers are caused by non-modifiable (e.g. genetic) risk factors. Genetic risk factors include, for example, light coloured eyes and naturally fair skin tone, red or blonde hair, freckles, many common moles or dysplastic nevi and skin type that burns easily. (Gandini et al. 2005.)

2.3.2 Vitamin D

2.3.2.1 Vitamin D production

The sources of Vitamin D (VitD) for humans are sunlight, diet and dietary supplements. Sunlight or artificial sources of UVB radiation provide more than 90% of circulating VitD status in normal circumstances, and the cutaneous photosynthesis of VitD has its maximum spectral effectiveness at about 297 nm (Lehmann and Meurer 2010). When the skin is exposed to sunlight, UVB radiation wavelength 290–315 nm penetrates the skin and results in the formation of previtamin D3 from 7-dehydrocholesterol (Holick 1981 and 2007). In the skin previtamin D3 is converted into vitamin D3, the process, which is thermally induced, and takes several hours (Webb 2006, Holick 1981 and 2007). Previtamin D3 can also be photoisomerised further into one of two inert isomers (lumisterol and tachysterol) or back to 7-dehydrocholesterol (Webb 2006).

Cutaneous VitD is biologically inactive and is activated in the liver and in the kidneys. In circulation vitamin D3 is bound to vitamin D-binding protein and transported to the liver, where it is hydroxylated to 25-hydroxyvitamin D [calcidiol, 25(OH)D] by the 25-hydroxylase enzyme. 25-hydroxyvitamin D is used to determine an individual's VitD status and the normal circulating levels of 25(OH)D range from 25 nmol/L to 200 nmol/L (Holick 2007; Lehmann and Meurer 2010). After hydroxylation in the liver 25-hydroxyvitamin D is transported to the kidneys, where it is metabolized into its hormonally active form 1,25-dihydroxyvitamin D [calcitriol,

1,25(OH)D] by the enzyme 25-hydroxyvitamin D-1α-hydroxylase (Lehmann and Meurer 2010, Holick 2007). The normal circulating levels of 1,25(OH)D are between 75 pmol/L-200 pmol/L (Lehmann and Meurer 2010). Renal hydroxylation is regulated by circulating phosphorus and calcium levels and plasma parathyroid hormone (Holick 2007). In addition to its actions in the kidneys, 1,25-dihydroxyvitamin D also has biological effects in other VitD receptor-positive target tissues such as bones, intestines and the parathyroid gland (Lehmann and Meurer 2010).

The best indicator of VitD status is the 25(OH)D concentration measured in serum or plasma. VitD deficiency is generally recognized as a 25(OH)D level below 20 ng/mL and insufficiency defined as levels between 21–29 ng/mL (Holick 2007). The US Institute of Medicine (IOM) has concluded that serum 25(OH)D concentration of 50 nmol/L meets the requirements of at least 97.5% of the population and recommends a dietary daily intake of 15 μg VitD for people aged 1-70 years and of 20 μg for people older than 70 years (Ross et al. 2011).

2.3.2.2 Vitamin D deficiency and depression

Sunlight is a major source of circulating VitD for most humans. Vitamin D, especially its deficiency, is associated with some psychiatric conditions such as depression. The aetiopathogenesis of depression includes genetic, social, psychological and biochemical factors, where genetic vulnerability and stress play a key role. The pathophysiology of depression is linked to structural and functional brain abnormalities, including HPA axis hyperactivity and the inflammatory response system, low levels of brain neurotrophins and reduced activity in noradrenergic and serotonergic neurotransmission. (Palazidou 2012.) The role of VitD in the development and function of the brain may play a role in neuropsychiatric diseases, including depression (Eyles et al. 2005 and 2013). The 1,25-dihydroxyvitamin D receptor and 1α-hydroxylase enzyme responsible for the formation of active VitD, are both found in human brain, which suggests that VitD may have autocrine or paracrine properties in the brain (Eyles et al. 2005).

The link between VitD and depression has been the focus of numerous studies, with inconsistent results, including longitudinal and cross-sectional studies as well as randomised controlled trials (RCT). VitD insufficiency has been hypothesized to be linked to depression but the epidemiological evidence is insufficient (Milaneschi et. al 2014). Milaneschi et al. (2014) found significantly lower 25(OH)D levels in subjects with current depression than in healthy controls (1,102 currently depressive,

790 remitted depressive, 494 healthy controls). In addition, in individuals with current depression, 25(OH)D was inversely associated with symptom severity, suggesting a dose-response gradient and with risk of having a depressive disorder at two-year follow-up (Milaneschi et al. 2014). van den Berg et al. (2016) studied 367 depressed compared to 132 non-depressed elderly individuals in a two-year follow-up prospective cohort study. They found no association between serum VitD status in two-year depression status nor in the course of remission (van den Berg et al. 2016).

2.3.3 Tanning controversy

2.3.3.1 Melanin synthesis: purpose and process

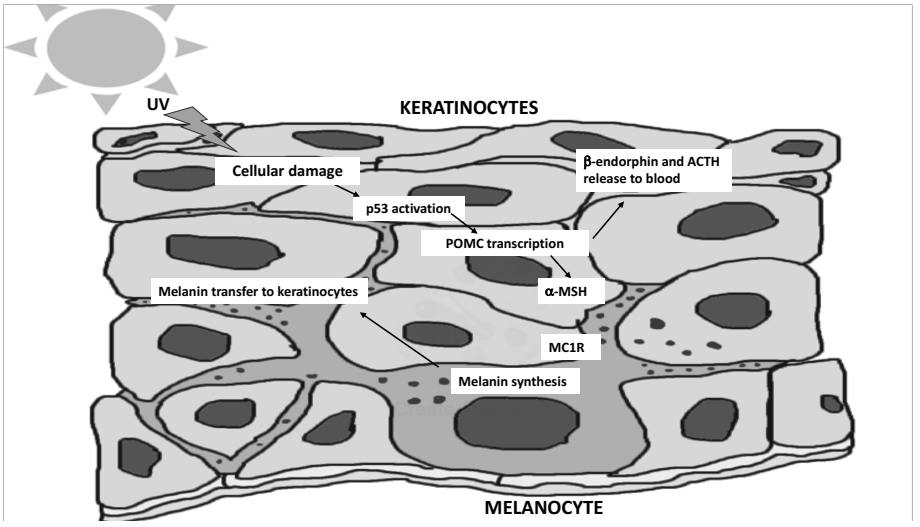
UV-induced DNA damage is a necessity for melanin production, i.e. tanning, which is a part of the self-defence and repair mechanisms against cell injuries caused by UVR. Melanin is formed in melanocytes in a process called melanogenesis. Melanin exists in two main chemical forms: 1) eumelanin (dark pigment) and 2) pheomelanin (light-coloured pigment). Eumelanin is much more potent for blocking UVR, thus fair-skinned people are sensitive to UVR and their risk for skin cancer is high. Dark-skinned and fair-skinned individuals have similar pheomelanin levels and the amount of epidermal eumelanin determines an individual's skin colour, UV sensitivity and cancer risk. (D'Orazio et al. 2013.) Eumelanin is more efficient in photoprotection, while UVR-exposed pheomelanin may induce free radicals and play a role in UV-induced cell injuries. Pheomelanin is found in people with red hair and skin phototypes I and II, in whom skin tumors are found more often. (Valverde et al. 1995; Miller and Tsao 2010.)

Epidermal exposure to UVB and UVA leads to the activation of p53, which up-regulates transcription of the POMC gene. POMC gene encodes production and secretion of several bioactive products including α-MSH, ACTH and endorphin. α-MSH binds to melanocortin 1 receptor (MC1-R) located on melanocytes, which leads to adenylyl cyclase activation and cyclic adenosine-monophosphate (cAMP) generation and melanin production. Melanocytes use the amino acid tyrosine to produce melanin, which is stored in melanosomes and further transported to keratinocytes. The accumulation of melanin in epidermal keratinocytes results in better protected skin against UVR insults. (D'Orazio et al. 2013, Sklar et al. 2013.) UVR induced melanogenesis may also involve other signalling pathways and direct

effects of UVR on melanocytes and there is some disagreement over the details of the process (D'Orazio et al. 2013). MC1-R genetic polymorphisms influence individual differences in pigmentation and different responses to UVR exposure. People with red hair and light skin have higher incidence of MC1-R mutations, which may result in UVR-induced decrease in eumelanogenesis and reduction in pigmentation. (Wolf et al. 2016.) (Figure 2)

Skin complexion is traditionally determined by the Fitzpatrick skin type system, which classifies individuals into six skin phototypes (I–VI) based on self-reported tanning and sunburning sensitivity (Fitzpatrick 1988). Skin pigmentation is divided into three types: immediate pigment darkening, persistent pigment darkening and delayed tanning. Immediate and persistent pigment darkening are consequences of the redistribution, oxidation and polymerization of pre-existing melanin. Delayed tanning is due to increased melanocyte activity and proliferation. Immediate pigment darkening occurs within minutes after UVA exposure and lasts for a maximum of two hours. (Sklar et al. 2013; Fajuyigbe and Young 2016.) With UVA exposures greater than 10 J/cm2 from tanning beds the immediate pigment darkening is more intense, leaving persistent pigment darkening that may last for 24 h. Delayed tanning is induced by UVB and typically becomes visible two or three days after exposure and lasts for a few weeks or months. (Sklar et al. 2013.) Delayed tanning is the eligible form of skin pigmentation.

Figure 2. Melanogenesis



Epidermal exposure to UVR results in cellular DNA damage and activation of protein p53, which up regulates transcription of the POMC gene. POMC encodes production of bioactive products (e.g. α-MSH, ACTH and β-endorphin). α-MSH binds to the melanocortin 1 receptor (MC1R) located on melanocytes leading to melanin production. Melanin is packaged in melanosomes and then transferred to keratinocytes.

2.3.3.2 Tanning behaviour: background

Tanning is fairly recent trend. For centuries pale skin was an indication of high social status while a tan signified low social status due to working outdoors as a manual laborer. For example, eighth-century Japanese women even went as far as to use lead and mercury-based whiteners on their skin. (Sarnoff 2013; Fitzpatrick 2014.) In 1855 Arnold Rikli, the "sun doctor", was the first to use light therapy to treat tuberculosis in Slovenia, and by the early 20th century, the therapeutic benefits of sunlight were recognized. In the 1920s, the French fashion designer Coco Chanel unintentionally got sunburn while travelling on the French Riviera. Thereafter tanned skin became a trend, and the sun was taken to represent pleasure and relaxation as well as health. The first sunscreen products became available in the 1920s. Later, in 1959, the first self-tanner, Man-Tan, was introduced, and the first UV tanning beds appeared in 1978 in the USA. (Sarnoff 2013.)

2.3.3.3 Tanning dependence

Despite awareness of the harmful effects of UVR, many sunbathers and indoor tanners continue to tan (Feldman et al. 2004; Nolan et al. 2009; O'Leary et al. 2014). The difficulty in ceasing tanning may be due to an addiction to UVR (Feldman et al. 2004; Nolan et al. 2009; Harrington et al. 2011; Ashrafioun and Bonar 2014), which shows signs of both dependence (physiological dependence) and addiction (psychological dependence) (Poorsattar and Hornung 2010). The release of endogenous opioids during UVR exposure may explain people's tanning behaviour (Feldman et al. 2004; Kaur et al. 2006b; Fell et al. 2014; Skobowiat and Slominski 2015). Other explanations may include cultural, health related and socioeconomic models (Warthan et al. 2005; Poorsattar and Hornung 2007).

Physiologic tanning dependence may cause tolerance and withdrawal symptoms due to adaptive changes and discontinuation (Feldman et al 2004; Kaur et al. 2006b). Kaur et al. (2006b) showed that blocking opioid receptors with systemic naltrexone

reduced preference for UV light in frequent tanners and induced withdrawal-like symptoms is some. These symptoms were not detected in any of the infrequent tanners given naltrexone (Kaur et al. 2006b). In another study the researchers observed that frequent tanners were capable of distinguishing a UV bed from a non-UV bed and showed an overwhelming preference (95%) to tan in the UV-emitting bed, suggesting that UVR was a reinforcing stimulus. In the same study the tanners reported more relaxed mood and even pain relief after UVR exposure. (Feldman et al. 2004.)

Recent experimental studies have shown that the biochemical mechanism behind tanning dependence may include the role of β-END expression in the skin induced by UVR (Feldman 2004; Nolan 2009; Fell et al. 2014; Skobowiat and Slominski 2015). Frequent low dose UVB exposures induced epidermal β-END synthesis and increased plasma β-END levels in mice (Fell et al. 2014). Also, naloxone, an opioid antagonist, was capable to elicit withdrawal signs in mice after chronic UVB exposure (Fell et al. 2014; Skobowiat and Slominski 2015).

2.3.3.4 Definition and measurement of tanning dependence

Although excessive indoor tanning was first described in the 2000s, it does not so far have a definite diagnosis code number in the ICD-10 nor in the DSM-5 (Heckman et al. 2008; APA 2013). It has been proposed that indoor tanning behaviour may be related to obsessive-compulsive disorder, seasonal affective disorder (SAD), anorexia, body dysmorphic disorder and depression in addition to psychic and physic addiction (Hillhouse et al. 2005; Warthan et al. 2005; Hillhouse et al. 2010; Petit et al. 2014; Heckman et al. 2016). Warthan et al. (2005) published a theoretical framework for the assessment of tanning-related substance-related disorder in 2005. The Diagnostic and Statistical Manual of Mental Disorders (DSM), the official guide to diagnosing mental disorders published by the American Psychiatric Association (APA 2013), has been used to evaluate possible UVR tanning dependence. The DSM-IV-TR (2000) definition for substance dependency includes three or more of the following symptoms in a 12-month period: tolerance, withdrawal, difficulty controlling use, negative consequences, significant time or emotional energy spent, putting off or neglecting other activities and desire to cut down (APA 2013).

The modified CAGE (m-CAGE for Cut down, Annoyed, Guilty, Eye-opener) is a clinically used tool to diagnose substance-related disorders and can be used to assess problematic tanning behaviour (Warthan et al. 2005; Harrington et al. 2011).

In earlier studies the m-CAGE showed problematic tanning behaviour in indoor tanning contexts in 12–33% of respondents, who mainly represented students or frequent indoor tanners (Warthan et al. 2005; Poorsattar and Hornung 2007; Heckman et al. 2008; Mosher and Danoff-Burg 2010; Harrington et al. 2011; Ashrafioun and Bonar 2014). The m-CAGE has been criticized for discrepancies in wording between studies, detecting subjects with no recent indoor-tanning history, and for giving too high percentages for tanning related problems (Schneider et al. 2015).

The self-administered measure SITAD (Structured Interview for Tanning Abuse and Dependence) was developed to detect both indoor and outdoor tanning dependence and abuse (Hillhouse et al. 2012). SITAD is based on opioid use items adapted from the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (First et al. 1995). In a piloting indoor tanning study 10.8% of participants met the criteria of indoor tanning abuse, and 5.4% appeared tanning dependent (Hillhouse et al. 2012).

Based on earlier questionnaire surveys including the use of DSM-IV, m-CAGE and SITAD, the UVR-related addiction prevalence ranges from 5.4% to 53% (Warthan et al. 2005; Poorsattar and Hornung 2007; Heckman et al. 2008; Mosher and Danoff-Burg 2010; Harrington et al. 2011; Hillhouse et al. 2012; Ashrafioun and Bonar 2014; Cartmel et al. 2017, Mays et al. 2017; Miller et al. 2018) (Table 1).

Recently a new instrument called the Behavioral Addiction Indoor Tanning Screener (BAITS), has been developed. This is a 7-item assessment designed to detect tanning behaviours parallel to behavioural addictions such as feelings of diminished control and strong urges to practice indoor tanning. BAITS has earlier been proven a valid and reliable tool for screening indoor tanning addiction. (Stapleton et al. 2016; Diehl et al. 2018.) The BAITS was initially validated in a study with a sample of college students, of whom nearly 9% were screened positive for symptoms of indoor tanning addiction and reported an average of 47 indoor tanning sessions in the past six months (Stapleton et al. 2016). In another study testing the validity and reliability of BAITS a total of 19.7% of current and 1.8% of former indoor tanning users screened symptomatic of a potential indoor tanning addiction (Diehl et al. 2018).

Table 1. Questionnaire surveys detecting the prevalence of UVR-related tanning dependency

Author (Year)	Participants n (male/female) aged yr Mean (SD)	Study design	Use of solarium n (%)	Dependency diagnosis tool	Dependent n (%)
Miller et al. (2018)	2572 (1415/1157) 16.06 (0.43)	High school students	Not given	m-CAGE	m-CAGE: 181 (7.02)
Cartmel et al. (2017)	499 (374/125) 38.5 (4.8)	White non-Hispanic participants	Not given	DSM-IV m-CAGE	DSM-IV and m-CAGE 88 (22.6)
Mays et al. (2017)	389 (0/389) 23.3 (3.0)	Non-Hispanic white young adult women	389 (100)	DSM-IV m-CAGE	DSM-IV and m-CAGE 122(24.4)
Ashrafioun et. al (2014)	530 (149/381) 19.5 (1.7)	University students	Not given	DSM-IV m-CAGE	DSM-IV: 165 (31) m-CAGE: 65 (12)
Hillhouse et. al (2012)	325, of which 296 finished (210/115) 21.8 (5.85)	College students	Not given	SITAD	Abuse: 32 (10.8) Dependent: 16 (5.4)
Harrington et. al (2011)	100 (36/64) 29.3 (9.41)	Beauty salon solarium	100 (100)	DSM-IV m-CAGE	DSM-IV: 41 (41) m-CAGE: 33 (33)
Mosher and Danoff-Burg (2010)	421 (133/284; 4 not reported) 92.9% of respondents aged 18-21	University students	237 (56)	DSM-IV m-CAGE	DSM-IV:* 90 (39) m-CAGE:* 70 (31)
Heckman et. al (2008)	400 (100/300) 21 (5.42)	University students/ community	152 (38)	DSM-IV m-CAGE	DSM-IV: 93 (23) m-CAGE: 44 (11)
Poorsattar et. al (2007)	375 (131/244)	University campus	56 (15)	m-CAGE	m-CAGE: 46 (12)
Warthan et. al (2005)	145 (47/98)	Beach-goers	Not given	DSM-IV m-CAGE	DSM-IV: 77 (53) m-CAGE: 32 (26)

* Data on 229 indoor tanners (8 of the 237 indoor tanners were omitted from subsequent analyses due to missing values on the mCAGE or mDSM-IV-TR measures)

DSM-IV, The Diagnostic and Statistical Manual of Mental Disorders (DSM), Fourth Edition; m-CAGE, m-CAGE for Cut down, Annoyed, Guilty, Eye-opener; SITAD, Structured Interview for Tanning Abuse and Dependence.

2.3.4 Exposure to light: behavioural influences

2.3.4.1 Seasonal affective disorder

Seasonal affective disorder (SAD) is a type of depression that recurs with a seasonal pattern and occurs most frequently in the winter months (Rosenthal et al. 1984a; Partonen and Lönnqvist 1998). According to the Diagnostic and Statistical Manual of Mental Disorders DSM-5 criteria, depression with a seasonal pattern is defined as a major depression that begins and ends during a specific season every year (with full remission for the remaining seasons), lasts for at least two years, and has more seasons of depression than without depression over a lifetime (APA 2013). Symptoms of SAD consist of depressed (sad) mood and low energy; individuals with SAD may feel fatigue, irritated and crave carbohydrates. They may moreover suffer from hypersomnia, weight gain, difficulties in concentrating and loss of social activity. (Melrose 2015.) Seasonal pattern disorders may vary in severity and some people may suffer from a milder form of SAD known as subsyndromal SAD (S-SAD) (Kasper et al. 1989).

2.3.4.2 Epidemiology and pathogenesis of SAD

The previous epidemiological studies on SAD, as assessed with the Seasonal Pattern Assessment Questionnaire (SPAQ), have shown a prevalence of 4–10% in general population (Miller 2005), while the estimated lifetime prevalence of affective disorders as based on a diagnostic interview is about 8% (Partonen and Lönnqvist 1998). The occurrence of SAD and S-SAD together is estimated to be 11–21% (Miller 2005). The prevalence of SAD varies across regions and populations between different studies and countries (Magnusson and Partonen 2005). Women experience SAD four times more often than men, and the age of onset is approximately between 18 and 30 years (Melrose 2015). An earlier overview including 20 studies on the epidemiology of SAD showed that SAD is more prevalent at higher Northern

latitudes and in certain ethnic groups (Magnusson 2000). However, some other studies have failed to show any connection between latitude and prevalence of SAD (Mersch et al. 1999; Levitt and Boyle 2002; Axelsson et al. 2004). Genetic, socio-cultural and psycho-social factors may also play a role (Mersch et al. 1999; Axelsson et al. 2004).

There are many theories of regarding the biochemical mechanisms explaining the pathogenesis of SAD, even though the mechanisms of the actions underlying SAD are not fully understood. These hypotheses include abnormal circadian phase shifting, abnormal serotonin neurotransmission and abnormal melatonin secretion (Miller 2005). Earlier studies of tryptophan (a serotonin precursor) depletion have shown that disturbed serotonergic activity may play a role in winter SAD (Neumeister et al. 1998a and 1998b). Patients with SAD were fed on beverages without tryptophan during the summer remission and they suffered of transient depressive relapse (Neumeister 1998a). In another study tryptophan depletion or catecholamine depletion after bright light therapy (BLT) triggered depression in patients with SAD (Neumeister 1998b). McMahon et al. (2014) found that individuals with SAD fail to downregulate their serotonin transporter levels appropriately in winter. Individuals with SAD have higher cerebral serotonin transporter binding in winter than do people without SAD (McMahon 2014). Darkness at night triggers melatonin synthesis in humans, leading some researchers to believe that abnormal melatonin synthesis may play a role in SAD (Miller 2005). Earlier studies have shown delayed nocturnal onset of melatonin secretion in SAD (Lewy et al. 1987; Rice et al. 1995; Wehr et al. 2001). Circadian rhythm responds to the rhythmic light-dark cycle that takes place daily and throughout the year (Melrose 2015). The circadian rhythm has been found to be timed differently in people with SAD differing more from 24 h and peaking at less regular times (Partonen and Lönnqvist 1998). Wehr et al. (2001) showed that for patients with SAD the duration of the night-time period of melatonin secretion was longer in winter than in summer, while for healthy volunteers no change in the period of melatonin secretion was found.

Also, the secretion of hormones other than melatonin and night-time body temperature may be disturbed in SAD. Avery et al. (1997) found that night-time cortisol minimum secretion was phase-delayed and was corrected with light treatment, but thyroid-stimulating hormone levels did not differ significantly before or after light treatment. The minimum body temperature during the night was delayed in people with SAD and was corrected after light therapy (Avery et al. 1997).

2.3.4.3 Treatment of SAD

The useful, noninvasive, rapid and most researched treatment for SAD is bright light therapy (BLT) given in the form of scheduled daily exposures to visible light. The effect is thought to be mediated through the eyes (Partonen et al. 1996; Partonen and Lönnqvist 1998; Privitera et al. 2010), possibly via serotonin induction (Gupta et al. 2013). It was first thought that BLT would normalize the phase-shift delay in those with SAD (Miller 2005). The other theories are that it lengthens the photoperiod in winter and suppresses the secretion of melatonin by the pineal gland (Miller 2005). BLT was earlier administered with an artificial light intensity of 2500 lx in the mornings for three hours, but nowadays the standard is most often bright light intensity of 10,000 lux for 30–45 minutes per session (Meesters and Gordjin 2016). The response to daily treatment with BLT is usually seen 1-2 weeks (Partonen and Lönnqvist 1998; Meesters and Gordjin 2016). However, the symptoms of SAD tend to return for most patients after treatment and it is possible to maintain the remission with BLT given five times a week (Partonen and Lönnqvist 1998). An overall positive response rate is up to 70% (Miller 2005). Typical adverse events are visual complaints such as eyestrain and increased risk of age-related macular degeneration, nausea, dizziness, difficulty sleeping and headaches. Relative contraindications are photosensitizing medications such as lithium, melatonin, phenothiazine antipsychotics and certain antibiotics, preexisting retinal diseases and recent eye surgery. (Melrose 2015; Meesters and Gordjin 2016.)

Since SAD is thought to be linked with a dysfunction in brain serotonin activity, the drug treatment usually involves second generation antidepressants, such as Selective Serotonin Reuptake Inhibitors (SSRIs) (Melrose 2015). Some promising results and positive effects have also been reported of the use of other antidepressants, such as agomelatine, moclobemide, citalopram and bupropion (Meesters and Gordjin 2016). Cognitive Behavioral Therapy (CBT) may provide a useful treatment for people with SAD (Melrose 2015; Meesters and Gordjin 2016).

2.3.4.4 Association of SAD and tanning dependence

Studies have shown associations between frequent indoor tanning and seasonal affective disorder (SAD) (Hillhouse et al. 2005; Hillhouse et al. 2010; Petit et al. 2014; Heckman et al. 2016). It has been proposed that individuals favour indoor tanning as a form of self-treatment on account of its mood-elevating properties (Petit et al. 2014; Heckman et al. 2016). SAD and symptoms of SAD were reported to be more

common among female college students who indoor tanned more frequently: 12 out of 27 (44%) frequent indoor tanners met the criteria for SAD compared with 14 out of 56 (25%) non-tanners (Hillhouse et al. 2005). Also, SAD was found to be three times more frequent in subjects with tanning dependence than among those who were not tanning dependent (Cartmel et al. 2017). A study with 139 female indoor tanners found that there was an association between presence of SAD and tanning to improve mood, tanning to relax and more problematic tanning (Culnan et al. 2015), and a study with 74 female frequent indoor tanners revealed higher than expected rates of SAD, body dysmorphic disorder and elevated stress levels (Blashill et al. 2016). However, a study of 306 female university students found no significant association between SAD and tanning dependence (Heckman et al. 2014).

Hillhouse et al. (2010) in a randomized controlled clinical trial studied the robustness of appearance-focused intervention to reduce indoor tanning and prevent skin cancer among female indoor tanners presenting with SAD (200 participants in the intervention group and 230 in control group). It has been suggested that individuals who report pathological motives for tanning or symptoms of SAD often report that tanning also improves their appearance. It was found that a tailored appearance-focused skin cancer prevention intervention was robust enough to reduce indoor tanning among subjects exhibiting SAD symptoms. (Hillhouse et al. 2010.)

2.3.4.5 Behavioural trait of morningness-eveningness

Chronotype is a measure of interindividual differences in daily activity patterns and sleep-wake cycles. People can be categorized into three chronotypes according to the timing of physiological functions and their preference in daily activities: morning types (advanced sleep period), neither types and evening types (delayed sleep period). (Horne and Östberg 1976; Duffy et al. 2001.) Morning types, also known as early larks, perform best physically and intellectually in the morning hours. Evening types, also known as night owls, perform physically and intellectually at their best in the late afternoon or the evening (Urbán et al. 2011). The physical and intellectual preference of neither types is later than in that of morning types but earlier than that of evening types (Adan et al. 2012). The proportion of chronotypes in population (morning types, neither types, evening types) varies in different studies depending on the data used. Broms et al. (2014) analysed the age-standardized chronotype of 567 men in Finland in a 23-year follow-up study (chronotype assessed in 1985 and 2008) and found that 9.5% of participants were evening type. Also, the chronotype

shifted towards more morning type with increasing age and on the individual level the clearly morning type preference tended to be more persistend with increasing age. One explanation for this finding was that the relative risk of dying was 1.3-fold higher in the evening types than in the morning types and survival for evening types, compared to morning types, started decreasing more rapidly after approximately 56 years of age. (Broms et al. 2014.)

Individual circadian preference is relatively stable during adulthood (Broms et al. 2014). However, there are changes in the tendency of sleep period across development and a shift in choronotype from morningness to eveningness occurs in adolescence (Adan et al. 2012). Analysing cross-sectional data from a range of age-groups, the midpoint of sleep becomes later from the age of 10 years until about 20 years, when the peak of eveningness is reached (Roenneberg et al. 2004). Randler et al. (2017b) showed that the morningness-eveningness preference changes in relation to gender and the difference between boys and girls arise during puberty and remain until 30 years. The peak of eveningness in girls is around the age of 16 and in boys 17 (Randler et al. 2017b). This shift in circadian preference is thought to be a part of pubertal development (Hagenauer et al. 2009) but also effected by social and environmental factors (Wittman et al. 2006).

Chronotype has a genetic basis (Barclay et al. 2010) and is modified by individual (e.g. age, sex (Adan et al. 2012), environmental (e.g., photoperiod, latitude (Borisenkov et al. 2010; Leocadio-Miguel et al. 2017; Randler et al. 2017a) and social factors (e.g. lifestyle, time schedules at school and work (Leonhard and Randler 2009)). Genetic variations in clock genes contribute to the chronotype (Ko and Takahashi 2006). People living at higher latitudes seem to have increased tendency toward late chronotype (Borisenkov et al. 2010; Leocadio-Miguel et al. 2017). There is evidence of an association between chronotype and non-communicable diseases and earlier studies have found that evening types are at greater risk of developing diabetes 2 and metabolic syndrome (Merikanto et al. 2013a; Yu et al. 2015). Also, evening chronotype has been found to be linked to other negative outcomes, such as poorer academic performance (Tonetti et al. 2015), sleep problems (Giannotti et al. 2002), health-related risky behaviours, such as smoking, alcohol consumption and physical inactivity (Urbán et al. 2011) and increased risk of injuries or accidents (Giannotti et al. 2002). Earlier studies have shown that eveningness is associated with unhealthier dietary habits, including lower VitD intakes (Sato-Mito et al. 2011; Kanerva et al. 2012).

Various self-report questionnaires have been developed to assess individual circadian preference and the most commonly used questionnaires have been

validated. The most frequently used measures are the Morningness-Eveningness Questionnaire (MEQ; Horne and Östberg 1976) and the Composite Scale of Morningness (CSM; Smith et al. 1989). The MEQ has 19 items and according to a composite score individuals are assigned to one of the five categories: definite evening type, moderate evening type, neither type, moderate morning type, and definite morning type (Horne and Östberg 1976). There is also a reduced an abridged version of the MEQ which has been validated and is reliable and quicker to complete (Adan et al. 1991). The CMS questionnaire is made up of 13 questions and places individuals into one of the three categories: evening type, intermediate type and morning type (Smith et al. 1989). The CMS includes items from the MEQ and from the diurnal type scale (Horne and Östberg 1976; Torsvall and Åkerstedt 1980). Another widely used instrument for assessing chronotype is the Munich ChronoType Questionnare (MCTQ; Roenneberg et al. 2003a). The MCTQ assesses an individual's chronotype according to the phase of entrainment as a clock time for the midpoint of sleep on days off, and the questions are about sleep and activity times (Roenneberg et al. 2003a).

2.3.4.6 Chronotype, the skin and hormones

The human circadian system regulates a wide variety of biological (e.g., hormone levels), psychological (e.g., mood) and behavioural (e.g., alertness) rhythms (Hidalgo et al. 2009). The mammalian circadian system is organized in a hierarchy of oscillators that operate at the cellular, tissue and systems levels. At the top of this hierarchy is the suprachiasmatic nucleus (SCN) in the hypothalamus that drives rhythms in activity and rest, feeding, body temperature and hormones. (Mohawk et al. 2012; Takahashi 2017.) The SCN co-ordinates independent peripheral oscillators resulting in a coherent rhythm at the organism level (Mohawk et al. 2012). The circadian oscillators consist of a network of transcription-translation autoregulatory feedback loops that involve several gene products and various output rhythms with a period close to 24 hours (Mohawk et al. 2012; Takahashi 2017).

The synchronization of the human circadian rhythm to the 24-hour rotation of the earth is an active process called entrainment and uses signals from the environment (time-givers or Zeitgebers) (Roenneberg et al. 2003b). Light is the most powerful time-giver for all circadian systems, but social cues also affect circadian rhythms (Roenneberg et al. 2007). A time-giver (e.g. light) induces phase shifting of circadian rhythms at the molecular level causing delays or advances in the phase of rhythms (Roenneberg 2003b).

As a peripheral tissue the skin has its own circadian rhythm which is under the influence of the suprachiasmatic nucleus (SCN) (Plikus et al. 2015; Dakup and Gaddameedhi 2017). Epidermal circadian rhythms have been widely studied, and, for example, Wu et al. (2018) identified 298 epidermal rhythmic genes in humans. Most cell types in the epidermis display circadian variations in metabolic actions such as proliferation, migration and differentiation, mitosis and vitamin synthesis (Sherrat et al. 2019). Also, the skin's circadian clock regulates UVR triggered cellular responses including sunburn apoptosis, inflammatory cytokine induction and erythema (Gaddameedhi et al. 2015). Earlier mouse and human studies have shown that UVB-induced skin carcinogenesis is associated with the skin's circadian fluctuations, which may affect protection against UVR (Gaddameedhi et al. 2011 and 2015; Nikkola et al. 2018). Brown et al. (2008) studied the length of the circadian period of cultured human fibroblasts measured by lentiviral bioluminescence assay and found that behavioural chronotypes were linked to different molecular circadian rhythms of fibroblasts: subjects with circadian preference towards morningness had a shorter period than subjects with circadian preference towards eveningness.

Many hormones have a role in the mammalian circadian system, and of these glucocorticoids and melatonin are the most widely studied. Cortisol has a diurnal pattern with highest levels after awakening, decreasing over the day and having lowest levels at bedtime (Dockray and Steptoe 2011). In addition to cortisol, levels of catecholamines (epinephrine and norepinephrine) are suppressed at night-time (Lange et al. 2010). Melatonin is synthesized in the pineal gland and secreted into the circulation at night, if it is dark enough, under the control of the SCN, which is synchronized with the external light-dark cycle (Pfeffer et al. 2018).

The association between chronotype and cortisol levels has remained inconsistent (Bailey and Heitkemper 2001; Kudielka et al. 2006; Dockray and Steptoe 2011). Earlier studies found an association between morningness-eveningness and cortisol-awakening-response (CAR): the morning-types showed higher salivary cortisol levels than evening-types immediately after awakening (Randler and Schaal 2010; Weidenauer et al. 2019). Dockray and Steptoe (2011) found no similar association. However, among healthy women evening-types had higher salivary cortisol levels than morning-types on leisure days throughout the day. Also, the seasonal variation in the photoperiod may influence the individual's cortisol status (Hansen et al. 2001; Adamsson et al. 2017). Adamsson et al. (2017) found no seasonal difference in salivary cortisol concentrations in a group of Swedish office workers. By contrast, a Danish study found highest urinary cortisol concentrations during the dark period of the year (Hansen et al. 2001).

The human circadian system regulates the immune system (Adams et al. 2013). Production of circulating proinflammatory cytokines, such as interleukin-6 (IL-6), peaks during night-time (Lange et al. 2010). The circadian secretion of IL-6 correlates with sleep/sleepiness and elevated IL-6 levels are associated with disorders of excessive daytime sleepiness such as narcolepsy and obstructive sleep apnea (Vgontzas et al. 2005). Mondin et al. (2016) in a cross-sectional study with 215 participants found an association between lower IL-6 levels and evening preference in participants with bipolar disorder.

2.3.4.7 Chronotype and mental health

Chronotype has been reported to be associated with mental health. Evening chronotype in particular has been increasingly recognized to correlate with increased risk of mood disorders, such as major depressive disorder (MDD), bipolar disorder and SAD (Au and Reece 2017; Taylor and Hasler 2018). In addition, eveningness has been connected to other mental health problems, for example alcohol dependence, anxiety, attention deficits and antisocial behaviours (Taylor and Hasler 2018).

Many studies have discovered the association between evening chronotype and depression and affective disorder (Drennan et al. 1991; Chelminski et al. 1999; Merikanto et al. 2013b; Jeong Jeong et al. 2015; Au and Reece 2017; Van den Berg et al. 2018; Vetter et al. 2018; Haraden et al. 2019). A recent review of 26 studies found a link between chronotype/eveningness/later circadian timing and mood as evening-type being a potential risk factor for depression, but the directionality remained unclear (Bauducco et al. 2020). Table 2 presents a summary of longitudinal studies published in 2018 and 2019 on young and adult participants investigating the association between chronotype and depression and depressive symptoms (Van den Berg et al. 2018; Vetter et al. 2018; Druiven et al. 2019; Haraden et al. 2019) (Table 2).

The mechanism underlying the association between evening chronotype and mental illness includes environmental and genetic factors. Environmental factors, such as early work start times and light exposure, cause sleep and circadian disturbances, e.g. sleep loss, which act on the neural and psychological mechanisms in the brain, for example neural processes related to reward and affective regulation. (Taylor and Hasler 2018.) Shared genetic factors may contribute to the link between chronotype and mental health. In a twin study, genetic contributions to chronotype were associated with depression and it was concluded that chronotype and

depression are both heritable and share common genetic factors. (Toomey et al. 2015.)

Table 2. Longitudinal studies on chronotype and depression and depressive symptoms

Author (Year)	Participants n (male/female)	Duration	Chronotype Measurement	Depression Disorder/Symptoms (Diagnosis)	Key findings
Haraden et al. (2019)	202 (89/113) aged 9-17.5 yr	4-yr follow-up	MESC	CDI (Symptoms)	Eveningness was associated with elevated symptoms of depression. Depression predicted eveningness 18 months later.
Vetter et al. (2018)	32 470 female participants aged 25-42 yr	4-yr follow-up	MEQ	Physician/clinician-diagnosed depression and/or antidepressant medication use	Significant linear trend of increasing depression risk across from early, intermediate and late chronotypes (highest risk)
Van den Berg et al. (2018)	742, of which 115 finished 1 yr follow-up (189/553) aged 18-56 yr	1-yr follow-up	MEQ	QIDS-SR (Symptoms)	Eveningness at baseline predicted more depressive symptoms after one year and relationship was mediated by poor sleep quality
Druiven et al. (2019)	505 (143/113 at 2 yr; 132/283 at 4 yr) * aged 18-65 yr	2-yr and 4-yr follow-up	MCTQ	CIDI	A later chronotype did not predict a persistent course of depressive disorder at 2 and 4 year follow-up

* 90 drop-outs during the study

MESC, Morningness/Eveningness Scale in Children; CDI, Children's Depression Inventory; MEQ, Morningness-Eveningness Questionnaire; QIDS-SR, Quick Inventory of Depressive Symptomatology – Self-Rated; MCTQ, Munich Chronotype Questionnaire; CIDI, Composite International Diagnostic Interview.

3 AIMS OF THE RESEARCH

The incidence of melanoma and non-melanoma skin cancers has risen despite campaigns intended to raise the population's awareness of the harmful effects of UVR. Frequent tanners are usually more knowledgeable about skin cancer risks, but continue to seek a tan, even to the extent that they can be regarded as being addicted to UV light. Excessive sun-seeking behaviour resembling UVR addiction has a biochemical background, which can be verified in clinical experimental studies. In addition, the prevalence of UVR-related addiction in population can be evaluated by using validated questionnaires. Even though many sunbathers express symptoms of seasonal affective disorder (SAD), no connection with tanning dependence has been demonstrated. Skin exposure to UVR induces a chain of neural and humoral reactions that may affect an individual's mood. Human circadian rhythmicity manifests in different biological and behavioural functions, from hormone secretion to well-being. Psychological symptoms, such as depressed mood, are associated with evening chronotype. Whether UVR can improve individual's mood independently and according to chronotype has been less well studied.

The specific aims of the present thesis were:

To study the expression of NB-UVB induced epidermal neuroendocrine mediators (NECM) (POMC, α-MSH, p53 and β-endorphin) in skin biopsies (I).

To assess the prevalence of UVR-related tanning addiction and the association of tanning dependence and symptomatic seasonality among Finnish sunbathers using validated questionnaires (II).

To study NB-UVB induced change in mood independently and according to individuals' chronotype that may be associated with the change in circulating vitamin D, β-endorphin, cortisol and Interleukin-6 status (III).

4 MATERIAL AND METHODS

4.1 Healthy subjects (I-III)

Altogether 13 healthy volunteers and 229 beachgoers participated in the three studies (I-III) of the present thesis (Table 3). In study I and III one volunteer withdrew from the study for personal reasons and, in addition, in study III one volunteer met the exclusion criterion. The Regional Ethics Committee of Tampere University Hospital District approved the study protocols.

The table 3 shows the narrow-band UVB (NB-UVB) exposures to which participants were submitted (I and III), and the frequency of tanning non-dependent and dependent/abusing participants and the seasonality of the participants (II).

Table 3. Demographic data on the healthy volunteers participating to the three studies (I-III)

Study	Healthy subjects n (male/female)	Mean age, years (range)	Skin Phototypes, II/III (n)	NB-UVB total exposures SED (n)	NB-UVB cumulative mean dose SED (J/cm-2) (n)
I	12 (1/11)	40 (23-62)	75% (9) / 25% (3)	1 (7) / 3 (5)	0.17 (7) / 0.51 (5)
III	11 (0/11)	42 (24-62)	72.7% (8) / 27.3% (3)	7 (5) / 8 (6)	1.19 (5) / 1.36 (6)

Study	Healthy subjects n (male/female)	Mean age, years (range)	Skin Phototypes, I-II/III/IV (n)	Non-Dependent % (n)	Dependent or Abused % (n)	Seasonality Normal / S-SAD / SAD % (n)
II	229 (36/193)	34 (18-59)	22.7% (52) /46.3% (106) /31.0% (71)	66.4% (152)	33.6% (77)	43.2% (99) / 26.2% (60) / 16.2% (37)

The first (I) and third (III) studies were carried out at the Department of Dermatology and Venereology, Tampere University Hospital, Tampere, Finland, at a latitude of 61°N, during winter months (October-November) in 2014. Altogether 13 healthy subjects volunteered for studies I and III. All subjects gave their informed consent to participate. The inclusion criteria were no phototherapy, solarium visits, sunny holidays or VitD supplementation during the preceding two months. The exclusion criteria were sun-

sensitive Fitzpatrick skin phototype I (Fitzpatrick 1988) and pregnancy. In addition, in study III a further exclusion criterion was use of antidepressants.
SED, Standard erythema dose.

4.2 Methods

4.2.1 Narrow-band UVB exposures

In studies I and III the initial NB-UVB dose for all the subjects was 0.17 J/cm2, corresponding to one standard erythema dose (SED). This is equivalent to the Commission Internationale de l'Éclairage (CIE) erythema-weighted irradiance of 10 mJ/cm2 CIE (CIE 1999). In study I seven volunteers received whole-body NB-UVB exposures of 0.17 J/cm2 (1 SED) on day one, whereas five other volunteers received a total cumulative dose of 0.51 J/cm2 (1 SED + 2 SED) on two consecutive days. In study III all volunteers received a total of four whole body NB-UVB exposures administered on consecutive days and the dose was increased gradually according to the skin phototype, up to 0.34 J/cm2 (2 SED) for five volunteers and 0.51 J/cm2 (3 SED) for the remaining six volunteers. The total cumulative dose ranged from 1.19 J/cm2 (7 SED) to 1.36 J/cm2 (8 SED). Room conditions were fixed for lighting and temperature (+23°C), and the NB-UVB exposures were administered in late afternoons. The volunteers' eyes were protected with opaque goggles to avoid any non-intentional effects through the eyes. The irradiations were provided using a Waldmann UV 7002 cabin equipped with 40 TL01 NB-UVB tubes (Schulze & Böhm, Brühl, Germany). No skin burns, erythema reactions or increases in pigmentation were observed 24 h after the last NB-UV-B exposure.

4.2.2 Skin biopsies and β-endorphin, POMC, p53 and α-MSH expression

In study I a pair of 4 mm punch biopsies was taken from each volunteer's buttocks skin. The first biopsy was taken immediately before the first NB-UVB exposure and the second biopsy 24 hours after the last NB-UVB exposure. The biopsies were taken under local anaesthetic using 1% lidocaine without epinephrine. The biopsies were put in 4% formalin and embedded in paraffin and sectioned at 4 μm thickness for further processing. To assess the change in expression of p53, β-END, POMC

and α-MSH the immunohistochemical staining was performed according to the instructions given by the manufacturers. The staining of p53, β-END, POMC and α-MSH was interpreted and graded by eye. For tumor suppressor protein p53 the grading varied from negligible to faintly positive, definitely positive and strongly positive while the grading for β-END, POMC and α-MSH was from no change to a detectable (positive) change. Positive control tissue from normal pituitary gland was used to confirm the quality and proper functioning of the reagents. The methods are described in detail in study I.

4.2.3 Measurement of serum 25(OH)D, β-endorphin, cortisol and Interleukin-6 concentrations

Blood samples to determine the serum 25(OH)D concentrations were taken before each NB-UVB exposure and on the fifth day of the study when no NB-UVB exposure was given. The samples were protected from light, centrifuged and the serum stored at –70°C. The serum 25(OH)D concentration was measured by chemiluminescent microparticle immunoassay using the Architect system (Abbott Laboratories, Abbott Park, IL, USA). Quality control was further assured by participation in the International External Quality Assessment Scheme (DEQAS) (Miettinen et al. 2014). Due to the short duration of the study, the participants' dietary intake of VitD was not determined, but they were asked to keep their diets constant.

Serum immunoreactive β-END was measured using an ELISA method developed at the University of Oulu, Finland. Samples and synthetic human β-END calibrators were incubated with synthetic β-END -containing C-terminal biotin and rabbit anti-human β-END-antiserum "BK22" (Vuolteenaho 1984) at a final dilution of 1/100000. After overnight incubation at +4°C, streptavidin-horseradish peroxidase conjugate (Pierce, Thermo Fisher Scientific Inc., Waltham, MA, USA) was added, followed by 3,3',5,5'-tetramethylbenzidine substrate (TMB One, KemEnTec Diagnostics A/S, Taastrup, Denmark). The reaction was stopped with 2-N sulphuric acid and the wells were scanned at A450 nm with a Varioskan Flash Plate Reader (Thermo Scientific, Thermo Fisher Scientific Inc., Waltham, MA, USA). Serum cortisol was measured using a Ref 52611 ELISA kit from IBL International GmbH, Hamburg, Germany and IL-6 was measured with a DY206 ELISA kit from R&D Systems, Inc., Minneapolis, MN, USA, according to the instructions provided by the manufacturers.

4.2.4 Questionnaires

4.2.4.1 Structured Interview for Tanning Abuse and Dependence

In study II the Structured Interview for Tanning Abuse and Dependence (SITAD) was used to evaluate participants' addiction to outdoor or indoor tanning (Hillhouse et al. 2012) (Appendix 1). After obtaining permission from Professor Joel Hillhouse to use SITAD, the English version of SITAD was translated into Finnish following the instructions of the World Health Organization (WHO 1948). One question concerning "recurrent tanning-related legal problems" was removed as irrelevant. The methods to fulfill the criteria of tanning dependence, tanning abuse and non-dependence are described in detail in study II.

4.2.4.2 Seasonal Pattern Assessment Questionnaire

In study II the seasonality of the volunteers was assessed by means of the Seasonal Pattern Assessment Questionnaire (SPAQ) (Rosenthal et al. 1984b; Kasper et al. 1989; Magnusson 1996). The SPAQ itself is not a diagnostic scale, but has been adapted for screening. The Global Seasonality Score (GSS) was used to determine the potential presence of SAD or S-SAD (Magnusson 1996). The GSS is used for assessing lifetime seasonal variations, including changes in the duration of sleep, social activities, mood, weight, appetite and energy level, each scored from 0 to 4 and maximum score being 24. In addition, it elicitis if these seasonal variations are regarded as a problem (B2, scored from 0 to 5) (Magnusson 1996) (Table 4). The participant was defined as having SAD (i.e. not a diagnosis of SAD but symptoms equivalent to SAD), when GSS ranged from 11 to 24 points and the severity of seasonal problems was graded as at least moderate (B2: 2-5). Having S-SAD (i.e. symptoms equivalent to S-SAD) was defined when GSS ranged from 11 to 24 points with no seasonal problems or only mild ones (B2: 0-1), or GSS ranging from 9 to 10 points with at least mild problems (B2: 1-5). Having normal seasonality was defined when GSS ranged from 0 to 8 points, or 9 to 10 points but without experiencing seasonal problems (Magnusson 1996) (Table 4) (Appendix 1).

Table 4. Seasonality score table.					
Seasonality	GSS		B2		
Normal	9-10	and	0	or	GSS 0-8
Subsyndromal SAD	11-24	and	0-1	or	GSS 9-10 and B2 1-5
SAD	11-24	and	2-5		

4.2.4.3 Visual Analogue Scale

In study III the Visual Analogue Scale (VAS) was used to assess the subjects' current state of mood, including a mean of the total of four separate mood dimensions: satisfaction, fatigue, wellbeing and irritation (Folstein and Luria 1973). Stress was excluded from the original five VAS dimensions due to irrelevant relation to the other four dimensions when calculating the average VAS of the mood state. All these items were marked on a scale from 0 to 100 mm line, whose ends were anchored as best ever (values closer to zero) versus worst ever. The instruction was administered as follows: "At the moment, how are your feelings of satisfaction / fatigue / wellbeing / irritation /stress? Please indicate by making a mark on the line. The VAS was carried out daily before each NB-UVB exposure and 24 hours after the last exposure.

4.2.4.4 Morningness- Eveningness Questionnaire

A Morningness-Eveningness Questionnaire (MEQ-6, v.1.2 FI, National Institute for Health and Welfare, Helsinki, Finland) based on the original Horne & Östberg Morningness-Eveningness (MEQ-19) questionnaire translated to Finnish was used to define the chronotype in study III (Horne and Östberg 1976). The modified instrument comprises six questions. All the answers were scored and summed for a total Morningness-Eveningness score of 5 to 27 points. The participants were classified into morning or evening chronotypes ("Larks" vs. "Owls") based on the last question in MEQ-6, where the definitely morning and rather morning than evening chronotypes were classified as "Larks", and definitely evening and rather evening than morning chronotypes were classified as "Owls" (Appendix 1).

4.2.5 Statistical analyses

In study II, for statistical analyses individuals with tanning dependence or tanning abuse were combined to form a single group in the analyses, as were individuals with SAD or S-SAD. Mean values and their standard deviations (SD) were calculated for continuous variables and frequencies were calculated for categorical variables. Statistical significances for the hypothesis of linearity across categories of tanning dependence were evaluated using the Cochran–Armitage test for trend and analysis of variance (ANOVA) with an appropriate contrast. The logistic regression adjusted for confounding factors tested the relationship of tanning dependence and seasonality. Simulations were used to calculate post hoc power for the relationship of tanning dependence and seasonality using the actual data from the study. All the analyses were performed using Stata Statistical Software, Release 15.1 (StataCorp LP, College Station, TX, USA). The significance level for the study was set at 5% ($P<0.05$).

In study III, the results were expressed as means with standard error of mean (SEM) and 95% confidence intervals (CIs). Sample size being small and some variables skewed, resampling-based (Bootstrap or Monte-Carlo) methods were used to achieve an acceptable significance level (p-value) and 95% CIs. The Fisher-Pitman permutation test for paired replicates (exact p-value) was used to assess the mean changes (within subjects) in mood and physiological biomarkers between baseline and day 5 values. Changes in baseline and day 5 values between chronotypes were compared using bootstrap-type independent-sample t-tests. Statistical significance for the hypothesis of linearity across the 5-day periods of biomarkers was tested using bootstrap-type repeated-measures analysis of variance (rANOVA). The method of Cohen was used to calculate the effect size (d); an effect size of 0.20 is considered small, 0.50 moderate and 0.80 large; CIs for the effect sizes were obtained by bias-corrected bootstrapping. Correlation coefficients were calculated using Spearman's method. The normality of the variables was tested using the Kolmogorov-Smirnov test. No adjustment was made for multiple testing. Statistical analyses were carried out using Stata statistical software, release 14.1 (StataCorp, College Station, TX, USA), and IBM Statistical Package for the Social Sciences (SPSS) Statistics, version 25 (International Business Machines Corporation, Armonk, NY, USA). The significance level for the study was set at 5% ($P<0.05$).

5 RESULTS

5.1 Effect of narrow-band UVB exposures on cutaneous β-endorphin, POMC, p53 and α-MSH expression (I)

The sub-erythemal NB-UVB doses induced a faint to moderate nuclear staining of p53 and the faint expression of cytoplasmic β-END, while NB-UVB-induced changes in the staining of POMC or α-MSH were only detected in single volunteers. The biopsies taken 24 hours after the last NB-UVB exposure showed an increase in nuclear p53 staining, which was definite (++) in 3 samples and detectable in all other samples when compared to basal levels. A faint (+) positive epidermal (mostly cytoplasmic) β-END staining was found in the majority (11/12) of biopsies. There was no difference in β-END expression in the response to two different NB-UVB doses. The pituitary control sections showed very strong β-END (+++), POMC (+++) and α-MSH (+++) expression. The responses of p53, β-END, POMC and α-MSH expression to NB-UVB exposures in study I are presented in Table 5.

Table 5. Expression of skin p53, β-END, POMC and α-MSH after NB-UVB exposure

Volunteer	Age	Skin photype	Cumulative UV-dose (SED)	α-MSH	POMC	p53	β-END
1	23	II	1			+	+
2	24	II	1			+	+
3	31	III	1			+	+
4	43	II	1		+	+	
5	53	II	1			+	+
6	62	III	1			+	+
7	35	II	1	+		+	+
8	42	II	3			+	+
9	33	II	3			++	+
10	50	II	3			++	+

11	49	II	3			++	+
12	36	III	3			+(+)	+(+)

5.2 Prevalence of tanning dependence and abuse and connection with seasonal affective disorder among sunbathers (II)

Altogether 229 replies out of 393 questionnaires were received giving a response rate of 58.5%. According to SITAD, data from 152 (66.4%) respondents met the criteria of non-dependent, 18 (7.9%) tanning dependence and 59 (25.8%) tanning abuser. According to SPAQ, 99 (43.2%) respondents displayed normal seasonality, 60 (26.2%) S-SAD and 37 (16.2%) SAD. Of the respondents with tanning dependence or abuse 36 (56.2%) had SAD-like (S-SAD or SAD) symptoms, whereas of non-dependent respondents 61 (46.2%) had SAD-like symptoms. There was no connection between tanning dependence/abuse and seasonality ($p=0.54$) among respondents.

5.3 Association of tanning dependence and abuse and characteristics of sunbathers (II)

Of the sunbathers 50.2% (n=115) sunbathed regularly whenever it was possible regardless of their tanning dependence/abuse status. Of the tanning dependent/abused sunbathers 76.6% (n=59) engaged in regular sunbathing more frequently than did non-dependent sunbathers (36.8% (n=56) ($p<0.001$). As many as 93.9% (n=215) of sunbathers had sometimes tanned with intention and those with tanning-dependence or abuse did so significantly ($p=0.036$). Of tanning dependent/abusing sunbathers 59.7% (n=46) had ever used solarium vs. 40.1% (n=61) of non-dependent sunbathers, showing a linear significant difference ($p=0.004$). The proportion of photosensitive respondents decreased significantly as degree of tanning dependence increased ($p=0.034$).

5.4 Effect of narrow-band UVB exposures on mood in general and according to chronotype (III)

In study III five volunteers reported morningness (Larks) and six eveningness (Owls). The mean VAS of mood state included only four dimensions: satisfaction, fatigue, wellbeing and irritation. The baseline mean of the four VAS dimensions of mood in 11 subjects was 28.6 ± 15.9 mm and improved to 21.2 ± 12.6 mm during the five days (Note! the values closer to zero depict better state of mood). Average improvement of VAS was -7.4 mm and was significant (p=0.038).

At the individual level, perceived mood improved in eight participants (73%), five of whom were Owls, and the mean VAS score was higher (i.e. the mood was worse) in the Owls than in the Larks on each day. On the other hand, the mean VAS score decreased more in the Owls (from 34±19.2 to 22.3±15.4 mm) than in the Larks (from 22.1±8.29 to 20.0±9.97 mm), i.e. the mood state improved more in the Owls from day 1 to day 5 (Figure 3 and Table 6 and 7), the effect being significant for the change on the VAS-wellbeing dimension (p=0.021).

Figure 3. Mean VAS (mm) of mood of 11 subjects according to the chronotype on days 1-5.

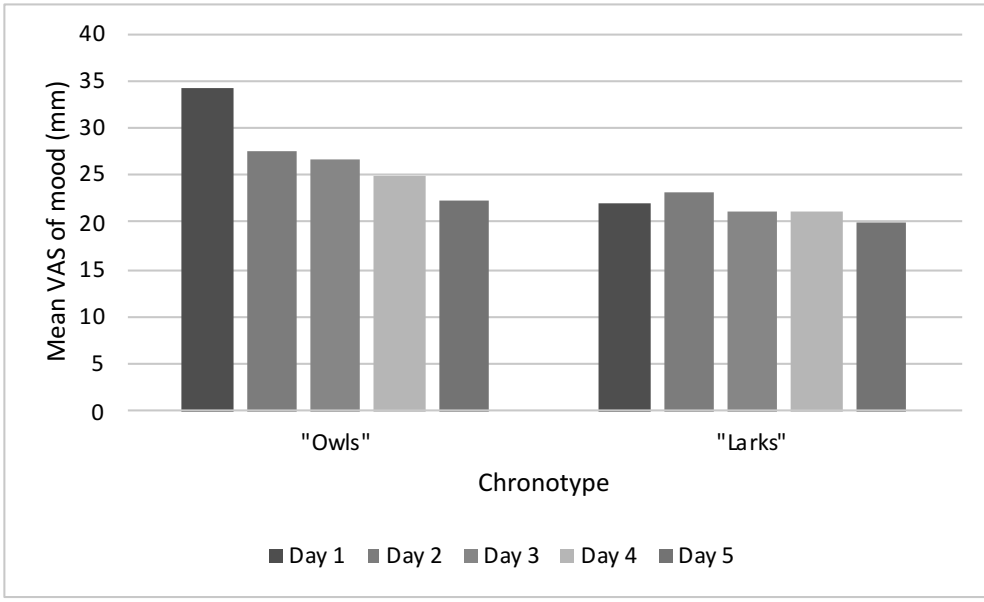


Table 6. Measurements of the subjects' mean VAS of mood (mm) in the group of "Larks" on days 1-5		
SAMPLE	MEAN (mm)	SD±
DAY 1	22.1	8.29
DAY 2	23.1	12.8
DAY 3	21.2	11.7
DAY 4	21.1	11.8
DAY 5	20.0	9.97

SD: Standard Deviation

Table 7. Measurements of the subjects' mean VAS of mood (mm) in the group of "Owls" on days 1-5		
SAMPLE	MEAN (mm)	SD±
DAY 1	34.1	19.2
DAY 2	27.5	11.8
DAY 3	26.7	13.2
DAY 4	24.9	16.9
DAY 5	22.3	15.4

SD: Standard Deviation

5.5 Effect of narrow-band UVB exposures on participants' serum 25(OH)D, β-endorphin, cortisol and Interleukin-6 concentrations (III)

The four NB-UVB exposures significantly improved 25(OH)D3 concentrations of all the 11 volunteers from the initial mean values of 75.5 ± 28.0 nmol/L to 83.7 ± 27.0 nmol/L over the five days, the average increase being 8.3nmol/L ($p<0.001$). Mean circulating immunoreactive β-END increased from 203±129 fmol/mL to 242±174 fmol/mL, but the increase was insignificant ($p=0.41$). Circulating cortisol also increased from 391±81 nmol/L to 470±139 nmol/L, not reaching significance ($p=0.16$). Unlike the other mediators, mean IL-6 concentration seemed to decrease from 1.12±0.66 pg/mL to 0.76±0.19 pg/mL, the decrease being significant ($p=0.025$).

Table 8 depicts the mean±sd daily concentrations of the circulating markers of 25(OH)D3, β-END, Cortisol and IL-6 grouped by chronotype. The mean

25(OH)D3 levels were lower in the Owls throughout, as were the mean IL-6 levels. Mean β-END was higher in the Owls than in the Larks at the end of the study, but there was overall fluctuation in both groups. Mean cortisol levels were higher in the Owls on each day (Table 1). NB-UVB induced circulating mediators did not show any correlation with the perceived changes in the current mood state. The impact of UVR irradiations on circulating biomarkers is described in detail in Study III.

Table 8. Mean serum vitamin D (25(OH)D3), β-END, cortisol and IL-6 levels of participants, grouped by chronotype (Larks/Owls)

	Baseline	Day 2	Day 3	Day 4	Day 5
"Larks"					
25(OH)D3 (nmol/L)	81.4±29.9	81.4±31.5	84.2±30.8	85.0±29.0	88.8±29.2
β-END (fmol/mL)	203±121	278±188	110±93.4	225±163	230±205
Cortisol (nmol/L)	364±75.9	367±86.0	332±104	421±181	421±175
IL-6 (pg/mL)	1.46±0.71	1.56±1.25	1.20±0.54	0.83±0.22	0.82±0.22
"Owls"					
25(OH)D3 (nmol/L)	70.5±28.2	72.2±27.4	74.0±28.1	76.3±25.8	79.5±27.0
β-END (fmol/mL)	203±148	179±63.0	266±167	250±187	253±162
Cortisol (nmol/L)	413±84.4	507±146	522±105	464±93.5	467±118
IL-6 (pg/mL)	0.83±0.51	0.67±0.24	0.80±0.31	0.63±0.23	0.72±0.17

Absolute values are given as means±SD (Standard Deviation)

6 DISCUSSION

6.1 Narrow-band UVB treatment increases β-endorphin and p53 expression, but not POMC nor α-MSH expression in epidermal keratinocytes of human skin *in vivo* (I)

In our study (I) we confirm, to the best of our knowledge for the first time, that NB-UVB exposures induced increases in β-END expression *in vivo* in human epidermal keratinocytes of healthy adult volunteers. The incidence of melanoma and non-melanoma skin cancers rises despite extensive campaigns intended to reduce public exposure to natural and artificial UVR (IARC 2012). The vast majority of sunbathers and tanning booth users are very well aware of the cancer risks, but continue to seek tan, which could be a consequence of tanning dependence with a physiological, opioid-related background and mediated through UVR exposure of the skin (Wintzen et al. 2001a; Feldman et al. 2004; Fell et al. 2014; Skobowiat and Slominski 2015). Recent animal studies have shown induction of β-END formation in response to cutaneous UVB exposure and assessed the relationship between β-END and UVR-related addiction (Slominski et al. 2012; Fell et al. 2014; Skobowiat and Slominski 2015). In an earlier *in vitro* study with human skin culture and co-cultured keratinocytes and melanocytes, UVR induced β-END production was shown to be UVB dose dependent and highest at 24 hours after UVR exposure (Skobowiat et al. 2011). In the same study, the most significant effects on production of cutaneous HPA axis elements was observed after exposure to the more energetic wavelengths UVC and UVB, although β-END expression was also stimulated by UVA (Skobowiat et al. 2011). Earlier *in vitro* studies have shown UVR-induced β-END formation to be at its highest at 6 to 24 hours after UVR exposure (Slominski and Wortsman 2000; Cui et al. 2007). In an animal study with mice, a dose of 4 SED of BB-UVB activated β-END production with the maximal response observed at 12 hours after the UVB exposure (Skobowiat and Slominski 2015). Therefore, both the wavelength of UVR and the dosing of UVB as well as the timeframe of sampling may all be crucial to the outcome of the expression of β-END in the skin. In our study, the timing (24 hours after the last UVB exposure) and the UVB dose (1 SED or 3 SED) appeared to be favourable, resulting in a faint positive cytoplasmic β-

END staining of epidermal keratinocytes in 11 out of the 12 samples compared to the non-irradiated skin. Future studies should involve more sensitive and specific staining methods to show UVR-induced β-END expression in the skin and also study other possible dermal neuroendocrine mediators (NECM) than β-END proposed to be associated with tanning dependence.

The effect of UVR on circulating β-END levels is inconsistent, and only few studies have been published on humans *in vivo*. Some studies failed to show UVR to have impact on the circulating levels of β-END (Wintzen et al. 2001b; Gambichler et al. 2002a; Kaur et al. 2006a). One small study demonstrated this induction to exist (Fallazadeh and Namazi 2009). In a study of skin biopsies taken immediately after 9 tanning lamp exposures, β-END was detected in human keratinocytes of the follicular matrix and in the cells of sweat ducts, but not in epidermal keratinocytes (Wintzen et al. 2001a). Accordingly, the researchers suggested that cutaneous β-END formation could be due to up-take from the systemic circulation, or from the cutaneous nerves instead being formed on site in keratinocytes (Wintzen et al 2001a). These results differ from our findings. Possible explanations for these different outcomes may be the timeframes of sampling and the difference in the UVR exposure source used. Skobowiat et al. (2011) were able to show in their earlier *in vitro* study β-END formation in co-cultured keratinocytes and melanocytes after UVR exposure, and further studies on animals have also shown this (Fell et al. 2014; Skobowiat and Slominski 2015), therefore it seems clear that skin is not just a target of β-END up-take from the circulation, but is an active co-player and capable of producing β-END independently upon UVB exposure. More sophisticated methods, such as liquid chromatography-mass spectrometry could be used in future to investigate the formation of β-END on site *in vivo* in humans.

Recent animal studies have shown that UVB is a stronger inducer of β-END production than UVA (Fell at al. 2014; Skobowiat and Slominski 2015). Artificial UVR devices used to treat inflammatory skin diseases mostly emit UVB, while solarium lamps mainly emit UVA. The spectra of different UVR sources can be compared using previous measurement of lamp spectra (Ylianttila et al. 2005) and the solar spectrum calculated using FASTRT UV stimulating tool (Engelsen and Kylling 2005). Based on previous measurements of spectral comparison, with 1 SED erythema dose, the unweighted UVB is practically the same for the NB-UVB, solarium and solar spectra, and thus the sun and solarium spectra have sufficient amounts of UVB radiation to induce dermal β-END expression with UV doses of 1 SED or more. Therefore, the UVB radiation delivered from sunlight and solarium

lamps may be enough to have an important role of the UVR-related addictive behaviour.

It has been well-known for years that p53 expression is related to UVR exposures (Li et al. 1998; Sablina et al. 2005; Gilchrest 2011). To detect p53 expression in human keratinocytes the timeframe of sampling may be crucial. Cui et al. (2007) showed earlier that keratinocytes expressed p53 already 1 hour after UVB irradiation, the maximum expression being 3 hours after irradiation. In our study we detected definite positive p53 staining in all samples taken 24 hours after the last UVB exposure, but the expression might have been even stronger if we had taken the samples earlier. The finding that even very small doses of UVB without causing sunburn induced p53 expression in all biopsies, suggests that small UVR doses can also be harmful.

We were unable to show an increase in POMC or α-MSH expression in the samples (with the exception of single samples) after NB-UVB exposure. Their metabolism is fast (Diano 2011; Slominski et al. 2012), thus it is entirely possible that they were degraded during the timeframe of our study. However, taking many biopsies at different time-points in a human *in vivo* study causes much discomfort and permanent scars to the volunteers. Thus, many timepoints for biopsy sampling in one study are usually not an option. In our present study, we chose to take the biopsies 24 hours from after irradiation because the MED is defined then and the erythema after UVB is also maximal at 24 hours from exposure. In our future studies, it will be possible to choose a different timepoint such as three hours after UVR to take a biopsy. Also, various technical limitations may have prevented detection of α-MSH, which is a very small peptide. It may thus have failed to be retained in the tissues during the fixation procedure. The positive control for the α-MSH staining showed that the IHC staining was working. POMC is a large molecule, but it is likely to be processed quite rapidly to its derivative peptides, and the rapid degradation of POMC may prevent its identification. Accordingly, the expression and detection of a target product may be a question of adjusted timing rather than that these were not present in the skin. In our future studies, different timing for collection of samples must be considered.

6.2 Sunbathing dependence is frequent among sunbathers but show no connection with seasonal affective disorder (II)

The motivations for tanning behaviour may vary. Before study II, we hypothesized that people with tanning dependence would be more often characterized by SAD-like symptoms and that purposeful tanning could be an attempt to improve mood. In the study we found tanning dependence and symptomatic seasonality to be frequent among the sunbathers and beachgoers, but the phenomena showed no connection. Actually, according to the SITAD questionnaire, 33.6% of the respondents were classified as tanning-dependent or abusing, and according to the SPAQ, 42.4% of the respondents displayed seasonality of SAD or S-SAD. Thus, our finding differs from that of Hillhouse et al. (2005), who earlier found a positive connection between excessive indoor tanning and SAD among college students in Tennessee, USA. Cartmel et al. (2017) also found SAD, alcohol dependence and "exercise addiction" to be significant predictors for tanning dependence. However, the motivation in these could have been appearance-related rather than physiological dependence (Hillhouse et al 2005; Cartmel et al. 2017). The difference from our study could be attributable to a different geographic location influencing abundance of sunlight, but also to the source of tanning. There is a significant seasonal variation in daylight and sunshine in Finland, which does not easily facilitate the development of tanning dependence, whereas frequent use of solarium and solarium-related tanning dependence were possible throughout the year for the college students, therefore, between these two studies the target groups differed from each other. This could in part explain why our results did not show a significant connection with UVR-related tanning dependence and symptomatic seasonality.

Although SAD and S-SAD are frequent in the Northern Hemisphere, earlier studies have failed to show a significant association of latitude with the prevalence of SAD (Mersch et al. 1999; Levitt and Boyle 2002; Axelsson et al. 2004). Grimaldi et al. (2009) detected in a Finnish national population-based survey the frequency of SAD to be 2.6%, which is less than in our sample of sunbathers, but to diagnose someone as having a definite SAD diagnosis, the individual needs to be interviewed and examined. One of the explanations for the higher prevalence of SAD in our study is that we did not evaluate the other possible coexisting psychological conditions of the respondents which may have contributed to symptoms similar to those of SAD.

In the present study, the majority of all respondents (93.4%) had sometimes sunbathed with the specific intention of tanning their skin. Many individuals seek to

tan despite being aware of the link between UVR and skin cancer. Earlier studies on indoor tanning have shown that appearance enhancement has been the most commonly reported reason given for indoor tanning (Asvat et al. 2010; Ingledew et al. 2010). There are unlicensed and largely untested self-administered products of synthetic analogues of α-MSH called "Melanotan-II" sold in the internet to stimulate melanogenesis and promote sunless skin pigmentation. The use of Melanotan-II was detected among young people attending fitness centres (Hjuler and Lorenzen 2014). However, Melanotan-II is more potent and stable than endogenous α-MSH and may even hyper-stimulate pigment synthesis resulting in atypical nevi and potentially develop melanoma (Langan et al. 2010). The possible association of α-MSH and tanning dependence is not known.

In addition to appearance related motivations, the other reasons proposed for indoor tanning are relaxation, enhanced mood, stress relief and improved energy (Feldman et al. 2004; Kourosh et al. 2010; Stapleton et al. 2010). It has been suggested that tanners may use indoor tanning to enhance their mood and as a form of self-medication (Hillhouse et al. 2005 and 2010). Therefore, future campaigns addressing the role of appearance motivations and mood-related intentions to sunbathe will be needed to reduce harmful sunbathing behaviour and the incidence of sunbathing-related skin cancers.

When studying UVR-related addiction among sunbathers and indoor tanners, it is important to consider the cultural differences in tanning behaviour. Many studies on UVR-related tanning addiction have been conducted in the USA, where indoor tanning is more frequent and less controlled by the authorities than in Finland, and the climate is more favourable to sunbathing. In a study using SITAD among college students in Tennessee, USA, the prevalence of tanning dependence was 5.4% and that of abuse was 10.8% and 53.7% of respondents engaged in indoor tanning (Hillhouse et al. 2012). Although in our study the total number of tanning-dependent subjects and tanning abusers was higher than in an earlier study (Hillhouse et al. 2012), the prevalence of indoor tanning was lower (46.7% of respondents had experience of ever using a tanning booth indoors). There is a shortage of studies assessing sunbathing-related tanning addiction compared to indoor tanning dependence. However, sunlight could be even more addictive than indoor tanning, since sunlight contains an abundance of UVB, which is a stronger inducer of β-END production than UVA (Fell at al. 2014; Skobowiat and Slominski 2015). There is an urgent need to perform larger studies in sunny holiday resorts targetting the prevalence of sunlight related addiction.

6.3 Narrow-band UVB treatment improves mood of healthy subjects independently and differently depending on chronotype (III)

Before embarking on study III, we hypothesized that NB-UVB exposures would elicit a change in the mood states of healthy volunteers independently and differently depending on chronotype, and that such a change might be associated with skin-borne circulating mediators. Our study showed that four exposures of sub-erythematosus NB-UVB induced significant improvement in mean current mood state over the five days. A mood-enhancing effect of UVR has been reported earlier (Gambichler et al. 2002b; Taylor et al. 2009; Veleva et al. 2018). A review of seven studies found that UVR was capable of improving mood in six of the studies, supporting a positive effect of UVR on mood (Veleva et al. 2018). A double-blind study with fibromyalgia patients showed an increased positive affect between mood (positive affect, well-being, relaxation and reduced pain levels) and UVR (Taylor et al. 2009). Volunteers exposed to whole-body UVA weekly over a three-week period felt more balanced, less nervous, more strengthened and more satisfied with their own appearance than the control group (Gambichler et al. 2002b).

One possible explanation for the positive effect of UVR exposure on mood via the skin is through the VitD pathway. Our study showed that the baseline 25(OH)D levels correlated with the baseline mood state. However, improvement in 25(OH)D in circulation did not correlate with improvement in mood state. We also detected a highly significant improvement in 25(OH)D concentration as a consequence of four daily consecutive NB-UVB exposures. An earlier study showed a significant positive correlation between active circulating VitD metabolite 1,25(OH)2D levels and affective state/wellbeing after three full-body BB UV-B exposures (Biersack et al. 2016). Edström et al. (2010) reported that UVR improved in parallel with both VitD status and mood state. However, a meta-analysis found no significant reduction in depression after VitD supplementation (Gowda et al. 2015). The association between UVR, VitD and mood is still poorly understood, and the tentative positive connection between UVB-induced VitD synthesis and current mood state does not exclude the possibility of other UVR-inducible mediators acting on mood. More research is still needed to clarify this.

The mood-enhancing property of UVR through the skin may involve a cutaneous analogue of the hypothalamic-pituitary-adrenal (HPA) axis and the local immune system (Skobowiat et al. 2011; Slominski et al. 2012 and 2013). Skin is a potential source of numerous UVR-inducible neuro-active compounds (Slominski and

Wortsman 2000; Slominski et al. 2012; Denda et al. 2013). In humans, β-END is connected with pleasure and euphoric feelings (Skobowiat et al. 2011; Slominski et al 2012; Fell et al. 2014), and UVR-induced production of β-END in the skin could influence wellbeing and mood. In the present study, the circulating β-END levels increased during UVR exposures, albeit insignificantly. Our finding can neither exclude nor confirm the possibility that UVR-induced β-END formed in the skin affects mood in humans. The effects of UVR exposures on circulating β-END levels have remained inconsistent in the few published studies on humans (Wintzen et al. 2001b; Kaur et al. 2006a). In addition, the local serotonergic/melatonergic system of the skin may also affect mood (Slomiski and Wortsman 2007). A study with UVA radiation reported higher serum serotonin levels in individuals exposed to UVA than in non-exposed controls. The authors explained this finding to occur possibly by a cutaneous pathway, since all participants wore opaque goggles designed to block out UVA radiation and accordingly also excluding the tentative retinal mediation of serotonergic effects. (Gambichler et al. 2002b.)

We found four NB-UVB exposures to induce a significant decrease in serum IL-6 levels. Urbanski et al. (1990) showed a peak in IL-6 levels at 12 hours after BB-UVB exposure, but the doses used were aggressively high. We used low physiologic and non-erythematous UVR dosing and drew the blood samples 24 hours after UVR exposures. IL-6 is thought to be important in depression, since IL-6 levels were found to be higher in depressed patients than in healthy controls, although the results have been inconsistent across studies (Yang et al. 2007). In the present study, NB-UVB seemed to induce an increase in cortisol levels, although this did not reach a significant extent. Earlier, both UV-B and UV-C wavelengths have been shown to elevate cortisol levels in the skin (Fitzgerald et al. 2006). Regarding the connection between circulating cortisol and mood, it is known that corticosteroid treatment can induce psychiatric symptoms, most commonly hypomania and mania, but also anxiety, whereas long-term use of corticosteroids appears to increase the risk for depression (Kenna et al. 2011). The NB-UVB-induced increase in circulating cortisol could be associated with improved mood state, but we were not able to verify any such correlation.

We discovered that the volunteers having the chronotype of eveningness had a worse current mood state throughout the study, and they tended to show more improvement in their mood upon UVR exposure than did the volunteers with the chronotype of morningness. Some earlier studies have shown the association of chronotype with mood disorders with the evening-bound orientation being prone to depression (Hidalgo et al. 2009; Merikanto et al. 2013b; Jeong Jeong et al. 2015; Au

and Reece 2017). Since the participants in our study were healthy volunteers who did not suffer from depression and as we did not interview or specifically screen them for possible depressive symptoms, no direct comparison can be made with present and past research. Future studies need to include volunteers with an ongoing depressive episode that has been diagnosed using a structured clinical interview.

Chronotype and circadian preferences may be associated with mood in different ways through the preference for daily activities and the subsequent timing of physiological functions. Evening-types achieve feeling best mainly during the evening hours and prefer later bedtimes and wake-up times (Urbán et al. 2011; Adan et al. 2012). In extreme cases, one is awake late into the night and wakes up at midday, which results in a sleep-wake pattern being in desynchronization with the environment and the majority of society (Bauducco et al. 2020). Both light exposures and social activities influence mood (Stephenson et al. 2012; Bauducco et al. 2020). Because evening-types tend to become active later in the afternoon, they may expose themselves to a significant amount of sunlight. An earlier study has shown that UVB-induced skin carcinogenesis is associated with the circadian fluctuations, with humans more prone to sunburn erythema after UVB exposure in the evening than morning (Nikkola et al. 2018). As a result, those with preference to eveningness may get sunburn and tanned more easily than those with preference to morningness because of the lack of protection provided by their internal clock against sunlight in the evening hours. This should be taken into account when planning interventions to stop the harmful tanning behaviour as well as when investigating the motivation behind excessive sun-seeking behaviour.

6.4 Limitations of the study

The limitations of studies I and III were that we had no control group or sham treatment, and that the total number of volunteers remained limited (12 volunteers in study I and 11 volunteers in study III). In study I, a different schedule of sampling could have resulted in a stronger expression of neuropeptides (β-END, p53, POMC, α-MSH) in the skin biopsies. Also, the skin biopsies were taken at only two timepoints. However, taking more biopsies was not possible due to ethical limitations, since invasive biopsies always cause a definite discomfort. However, when our study was launched, there was no solid existing evidence of the optimal timepoint for sampling to show peak levels of expression. In study III evaluating the influence of UVR on mood, all the volunteers were healthy, but the outcomes could

have been more pronounced in the depressed individuals. In addition, study III included only female volunteers (since no men volunteered).

In study II, investigating the prevalence of tanning dependence among sunbathers and the association of tanning dependence with SAD, a confounding factor relevant to our results was that summer 2015 was very rainy and chilly for sunbathing, which may have, for instance, influenced the development of addictive behaviour. The study was implemented only in summertime, which may contributed to the outcome, since the symptoms of seasonality might have been higher in winter and the level of sunbathing dependence lower. The results in study II are thus context-dependent and confined to beachgoers in Southern Finland.

7 CONCLUSIONS AND FUTURE PROSPECTS

The conclusions and future prospects of the present studies investigating the effects of NB-UVB exposures on the cutaneous formation of β-END, p53, POMC and α-MSH and on circulating VitD, β-END, cortisol and IL-6 levels, as well as the effect of NB-UVB exposures on mood individually and according to chronotype and the prevalence of UVR-related tanning dependence and SAD among Finnish sunbathers, are presented as follows:

Narrow-band UV-B is capable of inducing human epidermal β-END formation in keratinocytes in vivo and offers an explanation for the development of UVR-related tanning addiction (I)

Our study was the first clinical study to show that human epidermal keratinocytes are capable of inducing β-END expression *in vivo* at 24 hours after the last NB-UVB exposure. This finding increases the understanding of the importance of the skin being hormonally interactive organ and, further, the understanding of the biochemical background of addiction-related sun-seeking behaviour. In our study, we aimed to study NB-UVB-induced neuropeptide expression in skin biopsies, and the immunohistochemical staining was performed with polyclonal antibodies. In the future, different methods, such as fluorescent immunohistochemical system, could yield more consistent data. Other options in future studies are microarray analyses (microRNA and mRNA) of skin biopsies to analyse responses in gene expression related to neuropeptide production after UVR exposure. In the future, specific and sensitive analysis methods based on an analytical chemistry technique known as liquid chromatography-mass spectrometry could also be used to analyse the concentrations of neuropeptides in plasma and saliva after UVR exposure.

Earlier studies investigating the effects of UVR on neuroendocrine modulators of the skin have been mainly based on animal and cell culture studies (Slominski and Wortsman 2000; Cui et al. 2007; Skobowiat et al. 2011; Slominski et al. 2012; Fell et al. 2014; Skobowiat and Slominski 2015). Future studies need to focus on humans, and, when possible, to be conducted as placebo-controlled clinical experimental studies with sufficient sample size and statistical power, but these demand more extensive funding.

In addition to β-END, an endogenous peptide with opioid activity, other proteins including neurohormones, neurotransmitters and neuropeptides formed in the skin may play a role in developing tanning addiction. For example, enkephalins are endogenous opioid peptides produced in the human body and may represent a part of additional UVB-induced skin responses that can activate opioid receptors on the cutaneous sensory nerves (Slominski et al 2011). Also, the sense of well-being after UVA exposure may be due to increases in the circulating serotonin and decreases in melatonin levels produced via a cutaneous pathway having its starting point in the skin (Gambichler et al. 2002b). It is also of note that solarium rays contain sufficient UVB to deliver UVB effects even if they are considered as UVA sources.

Tanning dependence may be associated with the human neuroimmune mechanisms where UVR-damaged skin cells release alarmins which trigger epidermal inflammation in the epidermis and activate pathways leading to the development of addiction (Iacopetta et al.2018). Future human studies investigating the biochemical background of sun-seeking behaviour are warranted to include several other proteins and chemical signalling molecules which are associated with developing tanning addiction.

In conclusion, our findings support that human keratinocytes express β-END, which may be involved in the development of UVR-related addiction, and which may explain why sun-screening campaigns tend to fail. In the future, new human *in vivo* studies using diverse sampling schedules, more sensitive and specific staining methods, different analytical methods and different UVR sources are warranted to confirm our findings.

UVR-related tanning dependence is not associated with seasonality and multiple factors are likely to play a role in developing tanning dependence (II)

In the present study we found no connection between tanning dependence and symptomatic seasonality among sunbathers, differing from some earlier studies (Hillhouse et al. 2005; Hillhouse et al. 2010; Petit et al. 2014; Heckman et al. 2016). This finding may relate to the context of summertime sunbathing in our Northern four-season country, differing from cohorts using tanning booths. Harmful sun-seeking habits probably have multiple determinants, and when planning interventions to prevent UVR-related skin cancers, multiple factors explaining the background of tanning behaviour must be considered. Various factors including physiological, cultural, health-related and socioeconomic ones may cause individuals to develop tanning dependence (Feldman et al. 2004; Warthan et al. 2005; Poorsattar

and Hornung 2007; Robinson et al. 2012). In addition, a possible mechanism for tanning dependence is the opiate-like effects triggered by UVR exposure (Kaur et al. 2006b; Fell et al. 2014). Fell et al. (2014) showed in mice that opioid receptor blockade (naloxone) reversed systemic analgesia caused by UVR and induced withdrawal symptoms after chronic UVR exposure, and in another study on frequent tanners the opioid blockade induced withdrawal-like symptoms (Kaur et al. 2006b). Future placebo-controlled (sham vs. UVR exposure) studies should investigate whether naloxone as an opioid-receptor antagonist could significantly reduce the subjective reward of UVR exposure, and if its effect will be greater in UVR-addicted than in non-addicted persons.

Ultraviolet radiation may induce addiction through the central nervous system (CNS), and a genetic basis may be involved in this induction when tanning dependence develops. Exposure to UVR was shown to produce increases in neural dopamine response among frequent tanners when using UVR tanning bed, but not when using a sham tanning bed without UVR (Aubert et al. 2016). Another study found that frequent indoor tanners exhibited increases in blood flow in reward-based brain regions when using UVR tanning bed versus sham tanning bed (Harrington et al. 2012). In addition, recent studies have shown a genetic basis for indoor tanning addiction by demonstrating associations of tanning behaviour with the genes of addiction reward pathways (Flores et al. 2013; Mays et al. 2020). In the future, an interesting study could be to investigate the mechanisms of the action of β-END as well as neurotransmitters in the brain, which may play a role in sun-seeking behaviour in humans. Also, genetic influences warrant further investigation to identify personalized measures and methods to detect genetic differences in tanning behaviour.

We found that tanning dependence and SAD-like symptoms were frequent among sunbathers, suggesting that sunbathing may attract people presenting with SAD and possibly help to overcome symptoms on account of the mood-enhancing properties of UVR, as suggested earlier (Petit et al. 2014; Heckman et al. 2016). In our study smoking or drinking habits were not significantly associated with tanning dependence or abuse, unlike in earlier studies, in which tanning dependence has been associated with substance use including alcohol, tobacco, marijuana and other drugs (Warthan et al. 2005; Mosher and Danoff-Burg 2010; Ashrafioun and Bonar 2014). In the future new interventions to stop tanning dependence and develop treatment approaches require addressing the underlying co-occurring psychological and behavioural factors. Also, supplementary interviews and experimental controlled

studies are needed to target the impacts of sunlight on mood and behaviour more profoundly.

In our sample using the SITAD questionnaire among beachgoers the frequency of tanning dependence was 8% and that of tanning abuse was 26%, which is less than in a study by Warthan et al. (2005) using the mCAGE measure and the mDSM-IV-TR measure among 145 sunbathers on Galveston Island, Texas (26% met the mCAGE and 53% the mDSM-IV-TR criteria for tanning addiction). We chose the SITAD measure to detect tanning abuse and dependence since we found it best suited for the study at the time we collected the data. SITAD was developed to detect tanning abuse and dependence accurately and is suitable for the assessment of both outdoor sunbathing and indoor tanning (Hillhouse et al. 2012). However, as yet there is no consensus as to which questionnaire is the most valid method for screening tanning dependence, so future research is needed to continue developing and validating clinically useful self-report measures to assess tanning dependence. In addition, when assessing the prevalence of tanning dependence, feasible instruments with psychometric properties for screening and personal interviews are needed.

NB-UVB exposures improve mood but mechanisms need further studying (III)

In the present study, four sub-erythematous exposures of UVR had a significant impact on perceived mood, serum 25(OH)D and IL-6 levels. The improvement in mood was greater in participants with their preference toward eveningness rather than morningness. We also observed increases in serum cortisol and β-END levels, which, however, remained insignificant. Our findings support the hypothesis that UVR-exposed human skin could interact with the body and brain but showing the exact mechanisms of action in humans remains a challenge. When examining the effects and mechanisms of NB-UVB exposures on mood, further studies with larger sample sizes for double-blind randomized clinical trials need to have a focus on cohorts of individuals with depressive disorders assessed using a structured clinical interview for diagnosis. Also, it will be important to determine the therapeutic range and duration of UVR exposure that is efficient to improve mood, and this will require repeated measurements and follow-up.

In our study the NB-UVB exposures were administered in the afternoon. The hour of the day of UVR exposure could be of importance given that the circadian clock genes play a role in regulating the sensitivity of the skin to UVR damage, as erythemal response, sunburn-induced apoptosis and induction of inflammatory cytokines have been shown to have circadian fluctuations (Gaddameedhi et al. 2015).

Evening-types tend to feel most alert later in the afternoon and may expose themselves to a significant amount of sunlight in the afternoons. In our present study, the impact of chronotype gained little attention. Whether the interactions of UVR exposure with circadian clock proteins affect individual skin cancer risk needs confirmation in future human studies.

An earlier study with patients suffering from SAD found no association between chronotype and the success of bright light therapy (Knapen et al. 2016). Bright light therapy administered in the form of scheduled daily exposures to visible light is a useful treatment for SAD, in which the effect is thought to be mediated through the eyes (Partonen et al. 1996; Partonen and Lönnqvist 1998; Privitera et al. 2010). In our study we used NB-UVB, which penetrates the skin and may influence mood through pathways other than bright light therapy. Future studies are needed to investigate the neural, endocrine and immune impacts of UVR via the skin that regulate mood, if any, and further, whether it would be worthwhile to try NB-UVB phototherapy for patients suffering from depressive symptoms.

We expected that some of the measured changes in the circulating mediators (VitD, β-END, cortisol, IL-6) would correlate with improvement in mood states, but there was no correlation between the subsequent UVR-induced increase or decrease in the circulating mediators and improvement in mood. The findings of the beneficial effects of UVR on mood demands further research with larger samples to better understand the importance and interactions of the skin with other tissues as a sensory organ also capable of influencing mood.

To sum up, minimal NB-UVB exposures are effective in increasing the expression of β-END and nuclear p53 expression in human skin *in vivo*. Our findings indicate that β-END formed in keratinocytes could be involved in the development of addiction to sunlight and partially explain why sun-screening campaigns tend to fail. Even small doses of NB-UVB induced p53 staining in all samples verifying its crucial role in tumor suppression and, moreover, its protective nature in the development of skin cancers. Sunbathing-related tanning dependence and symptomatic seasonality are both frequent among Finnish sunbathers and may promote sun-seeking risk behaviour, but still, they appear to be separate phenomena. Sub-erythematosus exposures to NB-UVB improve mood, especially among those with evening preference for their daily activities and induce a significant change in the circulating VitD and IL-6 levels. The mood-enhancing effect of UVR may be the reason for seeking a tan, but the exact mechanisms remain unclear. However,

sunbathing used as a relaxant or stimulator of mood should be considered in future skin cancer prevention programmes.

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APPENDIX 1: QUESTIONNAIRES

MEQ

Olettaen sopivat ympäristön olosuhteet, kuinka helppoa sinulle on aamuisin vuoteesta nouseminen?

- ☐ ei lainkaan helppoa
- ☐ ei kovin helppoa
- ☐ melko helppoa
- ☐ hyvin helppoa

Kuinka virkeäksi tunnet itsesi aamuisin ensimmäisen ½ tunnin aikana?

- ☐ hyvin väsyneeksi
- ☐ melko väsyneeksi
- ☐ melko levänneeksi
- ☐ hyvin levänneeksi

Oletetaan, että olet päättänyt ruveta harrastamaan jotakin urheilulajia. Ystäväsi suosittelee sinulle harjoitusohjelmaksi 2 kertaa viikossa tunti kerrallaan. Paras aika hänelle on aamuisin kello 7.00–8.00. Pitäen mielessäsi vain oman 'parhaalta tuntuu' -rytmisi, kuinka luulisit suoriutuvasi?

- ☐ olisin hyvässä vireessä
- ☐ olisin kohtuullisessa vireessä
- ☐ tuntuisi melko vaikealta
- ☐ tuntuisi hyvin vaikealta

Oletetaan, että sinun täytyy osallistua 2 tunnin rasittavaan fyysiseen työhön. Voit täysin vapaasti suunnitella aikataulusi. Ottaen huomioon vain oma 'parhaalta tuntuu' -rytmisi, minkä vaihtoehdon valitsisit?

- ☐ kello 8.00-10.00
- ☐ kello 11.00-13.00
- ☐ kello 15.00-17.00
- ☐ kello 19.00-21.00

Oletetaan, että voit valita työaikasi. Oletetaan, että työpäivä on 5 tunnin mittainen, työ on mielenkiintoista ja palkkaa maksetaan tulosten mukaan. Mitkä viisi PERÄKKÄISTÄ tuntia valitsisit? Rastita valitsemasi viisi PERÄKKÄISTÄ kellonaikaa.

☐ *1-2*	☐ *2-3*	☐ *3-4*	☐ *4-5*	☐ *5-6*	☐ *6-7*	☐ *7-8*	☐ *8-9*
☐ *9-10*	☐ *10-11*	☐ *11-12*	☐ *12-13*	☐ *13-14*	☐ *14-15*	☐ *15-16*	☐ *16-17*
☐ *17-18*	☐ *18-19*	☐ *19-20*	☐ *20-21*	☐ *21-22*	☐ *22-23*	☐ *23-24*	☐ *24-01*

On niin sanottuja "aamuihmisiä" (aamunvirkku, illantorkku) ja "iltaihmisiä" (illanvirkku, aamuntorkku). Kumpaan ryhmään sinä kuulut? Valitse sopivin seuraavista vaihtoehdoista.

- ☐ Ehdottomasti "aamuihmisiin"
- ☐ Enemmän "aamu-" kuin "iltaihmisiin"
- ☐ Enemmän "ilta-" kuin "aamuihmisiin"
- ☐ Ehdottomasti "iltaihmisiin"

MEQ-19, original (E) 1976, J.A. Horne & O. Östberg, Int J Chronobiol 1976;4:97-110.
MEQ-6, v.1.2 (FI) 2014, Timo Partonen, THL, Helsinki.

The the Structured Interview for tanning Abuse and Dependence, SITAD© Questionnaire – Finnish Version (Auringonotto- ja solariuminkäyttökysely ”SITAD”)

1. Otatko aurinkoa/käytätkö solariumia mielestäsi runsaasti? K☐ E☐
   a) Pitävätkö muut auringonottoajat/solariumin käyttäjät auringonottoasi/solariumin käyttöäsi runsaana? K☐ E☐
2. Oletko koskaan ottanut aurinkoa/käynyt solariumissa vähentääksesi stressiä, voidaksesi paremmin tai kohentaaksesi mielialaasi? K☐ E☐
3. Oletko koskaan ajatellut, että auringonotto/solariumissa käynti olisi sinulle ongelma? K☐ E☐
   a) Oletko koskaan kokenut, että auringonotto/solariumin käyttö ohjaa tai kontrolloi elämääsi? K☐ E☐
   b) Onko auringonottosi/solariumin käyttösi koskaan tuntunut hallitsemattomalta? K☐ E☐
   c) Ovatko ystäväsi tai perheesi koskaan ehdottaneet, että sinun pitäisi vähentää auringonottoa/solariumin käyttöä tai lopettaa se? K☐ E☐
      i) Jos kyllä, jatkoitko auringonottoa/solariumin käyttöä siitä huolimatta? K☐ E☐

4. Koetko, että auringonotto/solariumin käyttö on ajoittain tärkeämpää kuin muut asiat elämässäsi, kuten ystävät, perhe, koulunkäynti tai työnteko? K☐ E☐
   a) Oletko koskaan jättänyt menemättä töihin tai kouluun auringonoton/solariumin vuoksi? K☐ E☐
   b) Olisitko valmis olemaan poissa töistä tai koulusta auringonoton/solariumin vuoksi? K☐ E☐
   c) Onko työntekosi tai koulunkäyntisi kärsinyt joskus auringonoton/solariumin vuoksi? K☐ E☐
      i) Jos ei: Oletko laiminlyönyt perhettäsi tai kotitöitäsi auringonoton/solariumin vuoksi? K☐ E☐

5. Voisitko kuvitella, että jatkaisit auringonottoa/solariumin käyttöä, vaikka sinulla olisi diagnosoitu ihosyöpä? K☐ E☐
   a) Onko sinulla joskus diagnosoitu ei-pahanlaatuinen ihosairaus, minkä vuoksi sinun pitäisi välttää auringonvalolle altistumista? K☐ E☐
      i) Jos kyllä: Oletko joka tapauksessa jatkanut auringonottoa/solariumin käyttöä? K☐ E☐
   b) Pidätkö nykyistä auringonottoa/solariumin käyttöäsi epäterveellisenä? K☐ E☐
   c) Onko sinulle koskaan diagnosoitu ihosyöpää? K☐ E☐
      i) Jos kyllä: Oletko jatkanut auringonottoa/solariumin käyttöä diagnoosista huolimatta? K☐ E☐

6. Onko auringonotto/solariumin käyttösi aiheuttanut sinulle ongelmia toisten ihmisten kuten perheenjäsenten, ystävien, puolison tai opiskelu- tai työtovereiden kanssa? K☐ E☐
   a) Luopuisitko mielummin läheisestä ihmissuhteesta kuin autingonotosta/solariumin käytöstä? K☐ E☐
7. Huomasitko aloittaessasi auringonoton/solariumissa käyntejä, että otit/käytit sitä useammin kuin olit alunperin suunnitellut? K☐ E☐
   a) Jos ei: Olitko auringossa/solariumissa yhdellä kertaa kauemmin kuin olit ajatellut? K☐ E☐
   b) Oletko koskaan ottanut aurinkoa/käynyt solariumissa, vaikka olisit ajatellut että auringonotton/solariumin käytön lisääminen tekee sinusta enemmänkin vähemmän viehättävän? K☐ E☐
   c) Oletko koskaan ottanut aurinkoa/käynyt solariumissa niin usein, että olet ajatellut sen vahingoittavan ulkonäköäsi? K☐ E☐
8. Oletko koskaan yrittänyt vähentää tai lopettaa auringonottoa/solariumin käyttöä onnistumatta siinä? K☐ E☐

a) Oletko koskaan ajatellut, että auringonoton/solariumin käytön lopettaminen olisi vaikeaa, vaikka haluaisit lopettaa? K☐ E☐

9. Käytitkö erityisen paljon aikaa auringonottoon/solariumin käyttöön tai jonkin sellaisen asian tekemiseen mikä mahdollisti auringonoton/solariumin käytön? K☐ E☐

10. Oletko luopunut osittain tai kokonaan yhteisestä ajasta ystävien tai perheen kanssa tai muiden tärkeiden asioiden kuten työn, urheilun, harrastusten tai mielenkiinon kohteiden parissa vietetystä ajasta auringonoton/solariumin käytön vuoksi? K☐ E☐
    a) Onko auringonotto/solariumin käyttö sinulle tärkeämpää kuin suhteesi:
        i) puolisoosi? K☐ E☐
        ii) ystäviisi? K☐ E☐
        iii) perheeseesi? K☐ E☐

11. Onko auringonottosi/solariumin käyttösi aiheuttanut sinulle psyykkisiä ongelmia, kuten masentuneisuutta, jännittyneissyyttä tai univaikeuksia? K☐ E☐
    a) Onko auringonottosi/solariumin käyttösi aiheuttanut sinulle fyysisiä ongelmia tai pahentanut jo olemassa olleita fyysisiä ongelmia? K☐ E☐

12. Oletko huomannut, että tarvitset nykyään paljon enemmän aurinkoa/solariumissa käyntejä saadaksesi sellaisen tunteen kuin halusit (esim. hyvänolon tunteen, vähemmän stressaantuneen tai rentoutuneen olotilan) kuin aloittaessasi auringonoton/solariumissa käyntejä? K☐ E☐
    a) Jos ei: Oletko huomannut, että nykyään samalla määrällä auringonottoa/solariumin käyttöä on vähemmän vaikutusta kuin ennen? K☐ E☐

13. Onko sinulla koskaan ollut vieroitusoireita eli huonovointisuutta vähentäessä tai lopettaessasi kokonaan auringonoton/solariumin käytön? K☐ E☐
    a) Onko sinulla fyysisiä oireita, jos et pysty ottamaan aurinkoa/käyttämään solariumia normaalin aikataulusi mukaan tai joudut jättämään jonkin käyttökerran väliin? K☐ E☐
    b) Jos kyllä: Mitä seuraavista oireista sinulla oli?
        i) alakuloisuutta tai masennusta? K☐ E☐
        ii) huonovointisuutta tai oksentelua? K☐ E☐
        iii) kipua tai arkuutta? K☐ E☐
        iv) nenänvuotoa tai silmien vuotoa? K☐ E☐
        v) hikoilua? K☐ E☐
        vi) ripulia? K☐ E☐
        vii) lisääntynyttä haukottelua? K☐ E☐
        viii) kuumetta? K☐ E☐
        ix) univaikeuksia? K☐ E☐
        x) ärtyneisyyttä? K☐ E☐
        xi) jännittyneisyyttä tai levottomuutta? K☐ E☐
    c) Jos vieroitusoireita: Jos et ole ottanut aurinkoa/käyttänyt solariumia hetkeen, niin otatko aurinkoa/käytätkö solariumia ehkäistäksesi ennalta sitä, ettei sinulle tule huono olo? K☐ E☐
    d) Oletko koskaan ottanut aurinkoa/käyttänyt solariumia, kun olet voinut huonosti, jotta voisit paremmin? K☐ E☐

A. Missä määrin seuraavat seikat voinnissasi vaihtelevat <u>vuodenaikojen mukaan</u>?

	ei muutosta	lievää vaihtelua	vaihtelee jonkin verran	selvää vaihtelua	erittäin huomattavaa vaihtelua
1. Unen pituus	O	O	O	O	O
2. Sosiaalinen aktiivisuus	O	O	O	O	O
3. Mieliala	O	O	O	O	O
4. Paino	O	O	O	O	O
5. Ruokahalu	O	O	O	O	O
6. Toimintatarmo	O	O	O	O	O

B. Jos koet mitään kohdassa A. mainituista vuodenaikojen mukaan tapahtuvista muutoksista, tunnetko että <u>nämä muutokset ovat</u> sinulle <u>ongelma</u>?

O Ei
O Kyllä

<u>Jos vastasit kyllä</u>, onko tämä ongelma

lievä	kohtalainen	huomattava	vakava	lamaannuttava
O	O	O	O	O

PUBLICATIONS

PUBLICATION I

Narrow-band Ultraviolet B Radiation Induces the Expression of β-endorphin in Human Skin *in vivo*

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Narrow-band ultraviolet B radiation induces the expression of β-endorphin in human skin *in vivo*

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ABSTRACT

Background: Ultraviolet radiation (UVR) from the sun and solaria has addictive properties that may develop into dependence. In mice, UVR addiction was connected to β-endorphin (β-END) formed in the skin after UVR exposure. In humans, the formation of β-END in skin keratinocytes has not been confirmed *in vivo*.
Objective: To determine with immunohistochemistry if sub-erythematous narrow-band UV-B (NB-UV-B) exposures stimulate p53 mediated expression of pro-opiomelanocortin (POMC), β-END and α-melanocyte stimulating hormone (α-MSH) in human skin keratinocytes *in vivo*.
Methods: Within 12 healthy volunteers, 7 received a single 1 standard erythema dose (SED) of NB-UV-B on their whole body, and 5 volunteers received a cumulative dose of 3 SED delivered on two subsequent days i.e., 1 + 2 SED. Skin biopsies were taken immediately before the first exposure and at 24 h from the last UV-B exposure to assess p53, β-END, POMC, and α-MSH expression.
Results: Nuclear p53 expression increased in all samples taken at 24 h after NB-UV-B exposure. UV-B irradiation also increased epidermal β-END expression in 11 out of 12 samples taken at 24 h after UV-B exposure. The brownish staining was localized in the cytoplasm of keratinocytes and around the nuclei, being more pronounced in the basal cell layers. POMC and α-MSH staining showed no obvious meaningful increase since only one section of each showed any change compared with basal levels.
Conclusions: Our study is the first to show that UV-B exposures increase β-END expression in epidermal keratinocytes of human skin *in vivo*, which could be the link to proposed UVR addiction.

1. Introduction

Ultraviolet radiation (UVR) is a potent skin carcinogen, which induces DNA mutations in epidermal cells of the skin and simultaneous immunosuppression [1,2]. Sunbathing and the use of tanning booths increase the risk for skin cancers. Tanners admit the cancer risks, but continue to sunbathe and tan [3–5]. This could be due to a form of behavioral addiction linked to UVR exposure [3,4,6]. Based on questionnaire surveys, addiction towards ultraviolet radiation (UVR) or solaria is common, with a prevalence ranging from 5% to 55%, and varies according to the target group and questionnaire [6,7,8]. Withdrawal-like symptoms were produced in 50% of frequent tanners when given the opioid antagonist naltrexone [9]. Chronic exposure to opioids results in tolerance, requiring an increase in the dose, and physical dependence. In an experimental sham study, frequent solarium users seemed to sense with their skin the true solarium from a sham device [3]. Skin pathology is proposed to influence human emotional states, releasing a variety of chemical mediators that act on the brain [1,10].

UVR exposure activates p53 in the skin and directly controls induction of prohormone pro-opiomelanocortin (POMC) and melanocortins (MSH) by up-regulating the POMC gene at both mRNA and protein levels [11]. POMC and MSH, in specific α-melanocyte stimulating hormone (α-MSH), as well as adrenocorticotropins (ACTH) have a profound role in regulating melanogenesis and subsequent tanning [1,12,13]. α-MSH binds to the melanocortin 1 receptor (MC1R) on the surface of melanocytes to activate adenyl cyclase and generate cyclic adenosine-monophosphate (cAMP) [1]. α-MSH may also have enforcing effects [5] or even increase the risk of melanoma [14].

Exogenous opioids have addictive properties delivered through opioid receptors. In humans, β-endorphin (β-END) is linked with feelings of euphoria. β-END and its immediate precursor β-lipotropin are

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endogenous opioid peptides degraded from POMC in the skin upon UVR exposure [11,12,15,16]. β-END interacts specifically with the OP_3-opioid receptor, formerly named the μ-type opioid receptor, evoking morphine like effects [17,18]; however, other mechanisms may be involved [19]. β-END plays a role in melanin synthesis and tanning, expressing potent dendritogenic effects in epidermal melanocytes [20]. β-END has also been implicated in the pathogenesis of various dermatoses, including psoriasis and atopic dermatitis, possibly related to β-END functioning as a differentiation factor.

In vitro studies have confirmed increased expression of β-END in incubated human skin samples and keratinocyte and melanocyte cultures when exposed to UVR [15,16]. In mice, β-END formed in the skin mediates UVR-related addiction [18]. Skobowiat and Slomiski [21] showed that mice with UVB-induced mechanisms originating in the skin regulate body homeostasis through the Hypothalamic–Pituitary-Adrenal axis (HPA); however, an experiment showing systemic impact in an intact functioning pituitary is required. Stimulation of β-END expression in the skin and plasma could lead to UVR addiction [4,21]. In mice, the epidermal expression of β-END was most pronounced 12–24 h after UVR exposure, and the more energetic wavelengths of UV-C and UV-B appeared to be more effective [21]. Differences in regulation of the POMC system between different species cannot be excluded [20]. In a human *in vivo* study, β-END was detected only in the keratinocytes of the follicular matrix and the cells of the sweat ducts, but not in epidermal keratinocytes [22]. A preliminary study showed an increase in plasma β-END [23], while other studies have not been able to confirm this [9,17,24,25].

In this *in vivo* study, we used immunohistochemistry (IHC) to verify whether narrow-band whole-body UV-B exposures have an impact on p53 expression and subsequently form neuroendocrine mediators: β-END, POMC, and α-MSH in the skin of human volunteers *in vivo*.

2. Materials and Methods

2.1. Volunteers and Study Protocol

The Regional Ethics Committee of Tampere University Hospital District approved the study protocol. All volunteers gave their informed consent for participation. The study was implemented in late autumn from October to November 2014 at the Phototherapy Unit of Tampere University Hospital, Tampere, Finland.

Healthy volunteers with skin phototypes II-III could participate [26]. Exclusion criteria included a history of photosensitivity, usage of drugs, extensive scarring of the skin or having any tanning bed or sunlight exposures in the preceding 2 months. At onset, one already randomized female volunteer withdrew from the study due to an illness. Accordingly, 12 volunteers, 11 females and one male, completed the study. The mean age was 40 (23–62) years, nine presenting skin phototype II and three belonging to phototype III [26]. According to a computerized randomization list, 7 volunteers received one single 1 SED narrow-band UV-B (NB-UV-B) exposure on their whole body, while 5 received a higher dose of 3 SED, which was administered in two sessions (1 SED and 2 SED) on subsequent days. No skin burns, erythema reactions or increases in pigmentation/tanning was detected at 24 h after the last UV-B exposure.

Room conditions were fixed for lighting, temperature (+23 °C), and exposures, and samplings were administered in late afternoons. This was regarded as a neutral time of day with regard to the diurnal secretion of neuroendocrine mediators and the circadian clock [27]. Before the biopsy, volunteers were asked to rest for 20 min. During the irradiations, eyes were covered with opaque goggles to avoid any non-intentional effects through the eyes.

2.2. Ultraviolet Radiation Source

A Waldmann UV 7002 phototherapy cabin equipped with 42 TL01 NB-UV-B tubes (Schulze & Böhm, Brühl, Germany) was used for irradiations. The irradiance of the cabin was measured by the author (LY) from STUK (the Nuclear Safety Authority, Finland) using an Ocean Optics S2000 spectroradiometer. The readings indicated that no correction coefficient was needed to calculate the doses. After correction for systematic errors and stray light, it was estimated that the uncertainty (2σ) of the measurement of the Ocean Optics S2000 was approximately 14% [28]. The measurements were traceable to the National Institute of Standards and Technology, USA. One SED is defined as an erythemal effective radiant exposure of 10 mJ cm-2 CIE [29], and it is equivalent to an unweighted physical dose of 170 mJ cm-2 from the NB-UV-B lamps.

2.3. Skin Biopsies and Immunohistochemistry

The change in expression of p53, β-END, POMC and α-MSH was assessed by taking a pair of 4 mm punch biopsies from the volunteers' buttocks skin: the first sample immediately before the first NB-UV-B exposure and the second at 24 h after the last UV-B dose. We used 1% lidocaine without epinephrine. The biopsies were put in 4% formalin, embedded in paraffin and sectioned at 4 μm thickness for further processing. Immunohistochemical (IHC) staining was performed according to protocol of manufacturer with a monoclonal anti-p53 antibody (Leica Novocastra clone D0-7 number NCL-p53-D07; Leica Biosystems Newcastle Ltd., Balliol Business Park West, Benton Lane, Newcastle Upon Tyne, NE12 8EW, United Kingdom) using a Ventana BenchMark XT immunostainer (Ventana Medical Systems, Tucson, Arizona), and with the polyclonal antibodies anti-ACTH 7-23 (Bioss Antibodies bs-0004R; Bioss Inc. 500 West Cummings Park, Suite 6500 Woburn, MA 01801), anti-MSH (Bioss Antibodies bs-1848R; Bioss Inc. 500 West Cummings Park, Suite 6500 Woburn, MA 01801), and beta-endorphin (Biosite LS-C23035-250; LifeSpan Biosciences, Inc. Seattle, WA, USA). The Ventana Ultraview DAB Detection Kit and Ventana CC1-buffer pH 8.5 was used as a pretreatment. To confirm the quality and proper function of the reagents, we used a positive control from tissue of a normal pituitary gland. An experienced dermatopathologist, second author (RH-O), and the first author (AJ) jointly interpreted the results. Staining the tumor suppressor protein p53 was graded using 4 grades: negligible, faintly positive (+), definitely positive (++), and strongly positive (+++). Changes in the expression of β-END, POMC and α-MSH were graded by eye from no change to a detectable change (positive), defining also the site of increase (nuclear or cytoplasmic). The settings of the microscope and camera were kept constant. No corrective measures were taken to adjust the color balance to confirm the comparability of the samples. A 200X magnification of the sections was used for the evaluation.

3. Results

The basal level p53 staining showed some solitary positively stained nuclei (Fig. 1a), whereas the staining of β-END (Fig. 1c, e), POMC (Fig. 2a) and α-MSH (Fig. 2d) was nearly negligible. The biopsies taken at 24 h after UV-B exposure showed an increase in nuclear p53 staining, which was definite (++) in 3 samples from sun-sensitive phototype II volunteers, and weaker, but detectable in all other samples, when compared with the basal level (Fig. 1a, b). We also found a faint (+) positive β-END staining in the epidermis of the majority (11/12) of sections. This was principally cytoplasmic, and seemed more pronounced in the basal layers (Fig. 1d, f). Some β-END staining was observed also in the peri-nuclear area. The response from two different sub-erythematous doses of UV-B showed no difference. The pituitary tissue control section expressed strong β-END (+++) staining as was expected (Fig. 1g).

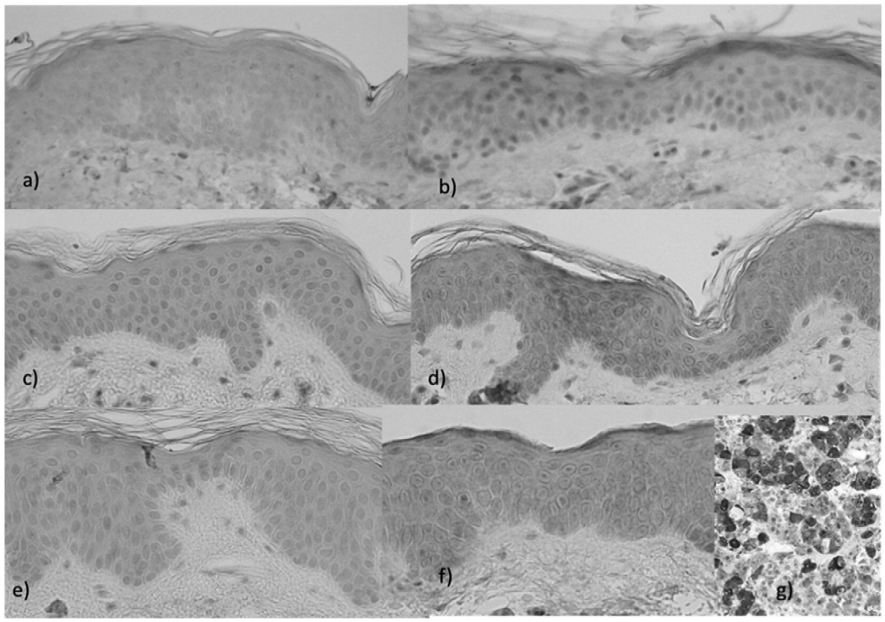

Fig. 1. The top panel shows epidermal nuclear p53 staining that increased from the basal level with a few nuclei (a) to definitely positive expression at 24 h after UV-B exposure (b). β-END expression increased in the cytoplasm and around the nuclei at 24 after UV-B exposure (d, f) compared with the basal level (c, e); however, it remained faint compared with the control pituitary tissue (g). Magnification of 200X.

Fig. 2a and d show the POMC and α-MSH expressions at basal levels before UVR exposure and the response at 24 h after UV-B exposure (Fig. 2b, e). We were unable to show any changes from the basal level for either of the two neuropeptides, excluding a minor change in only one section of each POMC and α-MSH. The pituitary control sections showed very strong POMC (+++) and α-MSH (+++) expression (Fig. 2c, f).

4. Discussion

In our study we showed mild increases in β-END expression *in vivo* in human epidermal keratinocytes 24 h after a sub-erythematous narrow-band UV-B exposure, which was more visible in basal layers. To our knowledge, this is the first clinical study to show expression changes in humans. These results are important regarding the increased

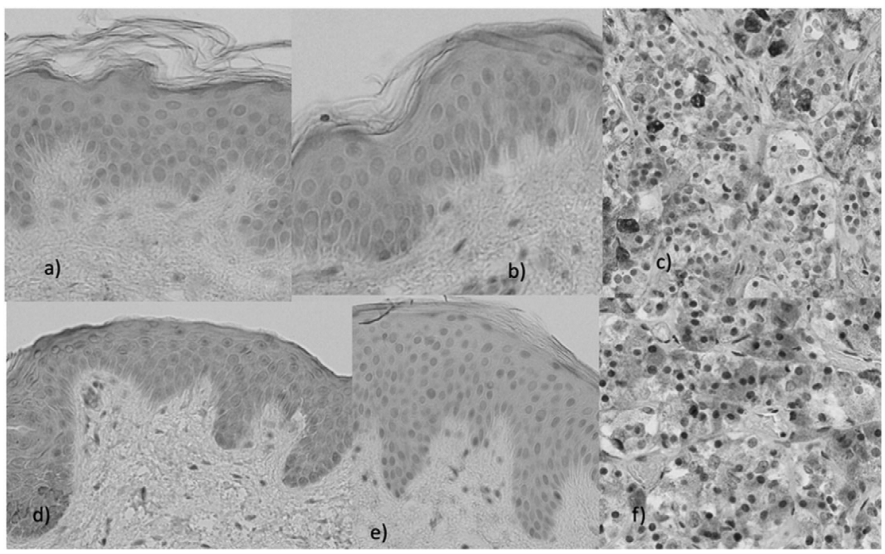

Fig. 2. No definite changes in POMC or α-MSH staining were detected in the epidermis following UV-B exposure. The upper panel shows POMC expression before (a) and at 24 h post UV-B in the skin (b), and the positive pituitary control POMC staining (c). The lower panel shows α-MSH expression before (d) and at 24 post UV-B exposure (e), and staining of the positive α-MSH control pituitary tissue (f). Magnification of 200X.

interest in the proposed UVR related addiction [3,4]. Two earlier clinical *in vivo* studies have increased our understanding of skin as an endocrine organ influencing mood [3,9]. Feldman et al. [3] showed in a randomized study that volunteers frequently using a solarium were capable of recognizing the UV-A solarium from a sham [3]. In another study, opioid blockade with naltrexone reduced UV preference in frequent tanners, however, they exhibited withdrawal-like symptoms [9].

These reinforcing effects of UVR were suggested to be, at least partially, due to dermal endogenic opioid formation.

Wintzen et al. [22] performed a clinical human *in vivo* study detecting β-END immunoreactive material only in part of the samples being localized in keratinocytes from the follicular matrix and ductal cells of the sweat glands, but not in epidermal keratinocytes. Therefore, they concluded that β-END in the skin originates from systemic circulation or cutaneous nerves [22]. The protocol differed from ours with two points, which may have influenced the different outcomes. They took the biopsies immediately after the UVR exposure, whereas we took samples 24 h after it. In addition, Wintzen et al. [22] used Cleo Natural® tanning lamps, Philips, emitting both UV-B and UV-A with close resemblance to the sunlight spectrum, whereas we used narrow-band UV-B TL01 lamps (Fig. 3). In addition, the number of exposures also differed.

Using human skin organ culture and co-cultured keratinocytes/melanocytes, Skobowiat et al. 2011 [15] showed that stimulation of the POMC genes was dependent on UVR wavelengths and doses and that significant production of proteins occurred only after exposure to the more energetic wavelengths UV-C and UV-B, although β-END was also stimulated by UVA. Both epidermal keratinocytes and melanocytes have been connected with β-END expression [30]. Therefore, the wavelengths and dosing of UV-B may have an influence on the results for humans. In a culture model, POMC gene activation was detected 10 h after UVR, whereas increases of POMC peptides were significant 10–24 h afterwards [31]. In human and mouse keratinocytes, expression of POMC proteins was higher at 6 h after UVR compared with 24 h [11]. In mice, broad-band UV-B increased the expression of urocortin, β-END and corticosterone genes and proteins to the maximum 12 h after UV-B exposure [21]. In our study, the expression of β-END could have been stronger if we took biopsies earlier, at 12 h post UV-B exposure. Skobowiat et al. 2011 [15] showed that using human skin organ culture and co-cultured keratinocytes/melanocytes was significant, and UV-B dose dependent increases in β-END immunoreactive signals were found within the cytoplasm of keratinocytes. This was distributed throughout the epidermis and consistent with our findings. In summary, our studies confirm that human skin is a potential source for β-END production *in vivo*.

Basal formation of α-MSH is low [32], and its metabolism is fast due to degradation [33]. We were unable to show any convincing changes in POMC or α-MSH protein expression. This could be due to fast degradation or to some undefined technical limitation in our systems. α-MSH is a very small peptide, and we may have failed to retain it in the tissue during sample processing. Conversely, POMC is a larger molecule, but is also rapidly degraded.

UV-B irradiation of human foreskin *ex vivo* showed that virtually every keratinocyte expressed p53 after 1 h of UVR, with maximum expression after 3 h. α-MSH was maximally expressed after 3–6 h of UVR throughout the epidermis [11]. In another study using normal human breast skin *ex vivo*, p53 was maximally expressed at 12–24 h after UV-B exposure [34]. The p53 expression in our samples could have been even stronger if we took earlier biopsies.

To understand the different outcomes from the studies by us and Wintzen et al. [22], we compared the spectra of different UVR sources using our previous measurements [35] (Fig. 3, Table 1). The solar spectrum (solar zenith angle 40°, total ozone column 350 DU) was calculated using the FASTRT UV simulating tool [36]. The unweighted (physical) UV doses for UV-B and UV-C (250–320 nm) and total UV (250–400 nm) were calculated for the same 1 SED erythema dose. With 1 SED erythema dose, the unweighted UV-B and UV-C doses are practically the same for the narrow-band UV-B, solarium and the solar spectra. The amount of UV-A received is considerably higher with solarium lamps and the sun compared with the narrow-band UV-B lamps. Based on the spectral comparison, the sun and solarium spectra emit sufficient amounts of UV-B radiation to induce an increase in β-END expression e.g., with total UV dose of 1 SED or more. Animal studies have shown that UV-B is a definite inducer of β-END and stronger than UV-A [18,21]. Skobowiat et al. [15] showed significant production of CRH, POMC, and ACTH proteins after UV-B and UV-C exposure, however, β-END was also stimulated by UV-A, proposing that this is due to overlapping spectral or alternative effects [15]. UVR effects on cortisol activity were also waveband dependent in human skin *ex vivo* [37]. In conclusion, solarium and sunlight induced UVR dependence could be due to UV-B involved in the spectrum of tanning devices and the sunlight because extended tanning sessions deliver significant amounts of UV-B as shown in Table 1.

In the skin, the roles of various neuropeptides seem to be overlapping. POMC and its degradation products ACTH and α-MSH promote tanning, however, β-END has this capacity too [20]. *In vitro* α-MSH induced melanin synthesis and reduced UVR induced damage in DNA [38]. Effects of α-MSH and its synthetic derivatives include enforcement of tanning, nausea, somnolence and penile erections [39]. Neuropeptides comprise a complex interaction system capable of modulating cell growth and immune responses, and many of these functions are verified in hair follicles [12]. Currently, the best documentation for local cutaneous production of endogenous peptide with opioid activity has been provided for β-END; however, the roles of several other proteins remain to be elucidated [40], and future studies should include the enkephalins, another family of endogenous opioid peptides binding to opioid receptors in the skin [40].

A limitation of our study was that biopsies could only be taken twice due to ethical constraints.

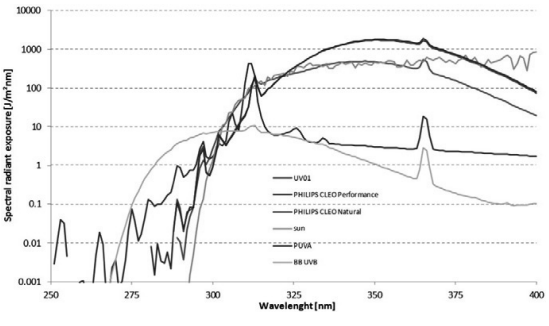

Fig. 3. Spectral radiant exposure for different medical and solarium lamps and the sun with the same 1 SED erythemal dose.

Table 1
Unweighted (physical) UV doses for different medical and solarium lamps and the sun with the same 1 SED erythemal dose.

Lamp Type	Erythemal Dose	Unweighted (Physical) Dose	
		250–320 nm	250–400 nm
NB UVB (TL01)	1 SED	140 mJ/cm^2	170 mJ/cm^2
BB UVB (UV21)	1 SED	24 mJ/cm^2	36 mJ/cm^2
PUVA (Waldmann PUVA)	1 SED	110 mJ/cm^2	7400 mJ/cm^2
Philips Cleo Performance®	1 SED	110 mJ/cm^2	7000 mJ/cm^2
Philips Cleo Natural®	1 SED	160 mJ/cm^2	2300 mJ/cm^2
sun (350 DU, 40°zenith angle)	1 SED	160 mJ/cm^2	3800 mJ/cm^2

5. Conclusions

Our findings indicated that narrow-band UV-B was capable of inducing β-END formation in keratinocytes in the epidermis of humans *in vivo* and offered an explanation for the development of UVR addiction in humans. Further studies are warranted to use different time-schedules of sampling - realizing that the optimal timing for one peptide may not be optimal for another - as well as other, more sensitive methods for expression detection.

Conflicts of interest

All authors declare no conflicts of interest.

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Tanning dependence and seasonal affective disorder are frequent among sunbathers but are not associated

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Tanning dependence and seasonal affective disorder are frequent among sunbathers but are not associated

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ABSTRACT

Ultraviolet radiation (UVR) is a known risk factor for skin cancers. Those who are tanning dependent seek out UVR exposure. Many tanners have expressed symptoms of seasonal affective disorder (SAD), but conclusive evidence of a connection with tanning dependence is lacking. We evaluated the frequency of tanning dependence or abuse and symptoms of SAD among Finnish sunbathers and analysed whether phenomena are associated which could indicate a common biological mechanism. Sunbathing related tanning dependence/abuse among Finnish sunbathers were assessed using the Structured Interview for Tanning Abuse and Dependence measure (SITAD), and symptoms of SAD were assessed with the Seasonal Pattern Assessment Questionnaire (SPAQ). Of 229 sunbathers, 8% (n = 18) were classified as tanning-dependent, and 26% (n = 59) were classified as tanning abusers. Additionally, 16% (n = 37) met the criteria for SAD, and 26% (n = 60) met the criteria for subsyndromal seasonal affective disorder (S-SAD), but there was no significant association between tanning dependence or abuse and SAD or S-SAD. Sunbathing dependence or abuse and SAD/S-SAD were frequent among sunbathers, and they may promote sun-seeking risk behaviour. However, within this sample, tanning dependence and SAD/S-SAD were not associated.

1. Introduction

Ultraviolet radiation (UVR) exposure has been causally linked to the development of non-melanoma skin cancers and malignant melanomas (Gandini et al., 2011; Tierney et al., 2015). Many sunbathers and indoor tanners are aware of the harmful effects of UVR but continue to seek a tan (Feldman et al., 2004; Nolan et al., 2009; O'Leary et al., 2014). Some of the difficulty in ceasing tanning has been attributed to UVR addiction (Ashrafioun and Bonar, 2014; Feldman et al., 2004; Harrington et al., 2011; Nolan et al., 2009), which has been unofficially regarded as a subtype of behavioural addiction.

A theoretical framework for the assessment of tanning-related substance-related disorder proposes that exposure to sunlight accelerates the synthesis of endogenous endorphins in the skin, which may reinforce tanning behaviour and, at least in some circumstances, explain the addictive nature of sunbathing (Warthan et al., 2005). In an earlier study blocking opioid receptors with systemic naltrexone reduced the UVR preference and induced withdrawal-like symptoms in some frequent tanners (Kaur et al., 2006). In mice, frequent low-dose UV-B exposures induced epidermal β-endorphin synthesis, increased plasma β-endorphin levels and raised the pain threshold (Fell et al., 2014). Moreover, naloxone was capable of eliciting withdrawal signs in mice after chronic UV-B exposure, and this was related to formation of β-endorphin in the skin (Fell et al., 2014; Skobowiat and Slominski, 2015). Artificial narrow-band UV-B exposures also increased β-endorphin expression in human skin in vivo (Jussila et al., 2016), and earlier human studies detected increased levels of endorphins after UVR, although subsequent studies in men have not confirmed this finding (Belon, 1985; Gambichler et al., 2002; Levins et al., 1983; Warthan et al., 2005; Wintzen et al., 2001).

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The modified Cut down, Annoyed, Guilty, Eye-opener (m-CAGE) measure detected problematic indoor tanning behaviour in 11% to 33% of respondents, mostly representing university/college students or frequent indoor tanners (Ashrafioun and Bonar, 2014; Harrington et al., 2011; Heckman et al., 2008; Mosher and Danoff-Burg, 2010; Poorsattar and Hornung, 2007; Warthan et al., 2005). Using the criteria of the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR), for substance dependence but being modified for tanning dependence, tanning dependence was found in 23–53% of participants (American Psychiatric Association, 2000; Ashrafioun and Bonar, 2014; Harrington et al., 2011; Heckman et al., 2008; Mosher and Danoff-Burg, 2010; Warthan et al., 2005). Using the self-administered Structured Interview for Tanning Abuse and Dependence (SITAD), 5.4% of the participants met the criteria for tanning dependence and 10.8% for tanning abuse. The respondents were randomly selected college students in East Tennessee State University (First et al., 1995; Hillhouse et al., 2012). SITAD was based on the criteria for substance dependence but modified to detect tanning dependence and abuse behaviour, whereas the modified m-CAGE and DSM-IV-TR were intended to identify substance dependence (Warthan et al., 2005).

Seasonal affective disorder (SAD) is a condition of regularly occurring depression during autumn or winter, with remission in the following spring or summer (Rosenthal et al., 1984). Subsyndromal seasonal affective disorder (S-SAD) shows similar but milder symptoms (Partonen and Lönnqvist, 1998). SAD/S-SAD is common in northern latitudes, where the amount of sunlight is scarce in wintertime (Magnusson, 2000). Excessive indoor tanning and SAD have shown a positive relationship (Heckman et al., 2016; Hillhouse et al., 2010, 2005; Petit et al., 2014), which may suggest that individuals may indoor tan as a form of self-treatment on account of its mood-enhancing properties (Heckman et al., 2016; Petit et al., 2014). SAD was reported earlier to be three times more frequent among subjects with tanning dependence than among subjects with no tanning dependence (Cartmel et al., 2017). However, among female university students SAD was not significantly associated with tanning dependence (Heckman et al., 2014). In a study with women indoor tanners, the presence of SAD was associated with more problematic tanning and tanning to improve mood and relax (Culnan et al., 2015). In another study, frequent indoor tanners had higher than expected rates of SAD, body dysmorphic disorder and elevated stress (Blashill et al., 2016). To the best of our knowledge, there are no studies on the association between tanning dependence and seasonal symptoms in northern latitudes, where SAD/S-SAD is a common finding. Our study is also the first study to use SITAD in an outdoor sunbathing context to assess sunbathing related tanning dependence/abuse.

In wintertime, people tend to travel to sunny resorts to get tanned, to improve their mood and to energise. Our aim was to assess the presence of sunbathing related tanning dependence among Finnish sunbathers using the SITAD measure with sunbathing related wording (Hillhouse et al., 2012), and to explore whether tanning dependence/abuse shows a positive relationship with SAD/S-SAD. We hypothesised that tanning dependence is frequent among sunbathers, and that some beach and parkgoers may sunbathe for the mood-enhancing effects in summertime.

2. Methods

The research plan was approved by the Ethics Committee of Pirkanmaa Hospital District (#R15001). The sunbathers were informed about the study and agreed to participate by responding anonymously either using an internet link or posting their answers in a prepaid envelope.

2.1. Participants and recruitment

Finnish-speaking adults encountered on beaches or in parks in July and August 2015 were eligible for inclusion in the study. We approached people on beaches and in parks in two Finnish cities, Tampere and Pori (61°N), and asked them to participate in the survey. The responses to the questionnaires were voluntary, no rewards were offered. No distinction was made between genders or between families, couples, groups or single people in terms of recruitment. A total of 393 questionnaires, 199 paper questionnaires and 194 internet links to questionnaires, were supplied.

2.2. Measures

The demographic and background data gathered included Fitzpatrick's skin phototyping (Fitzpatrick, 1988). The most sensitive skin phototype, type I, always burns and never tans; type II often burns and tans poorly; type III sometimes burns and tans easily; type IV never burns and tans rapidly; types V and VI refer to brown and black skin (Fitzpatrick, 1988). Since the skin phototype I does not tan, we proposed the number of respondents presenting the phototype I to remain low among sunbathers. The questions also delineated whether sunbathing was intended for the specific purpose of tanning, whether the individual sunbathed seldom/occasionally or frequently (defined whenever possible in purpose to tan), and the frequency of sunscreen use (never, occasionally, frequently, always). Smoking (yes/no and, if yes the number of cigarettes smoked per day and how soon the first cigarette is smoked after awakening) and alcohol consumption (never, once a month, 2–4 times a month, 4 times a week or more often and, if alcohol is consumed, the number of portions per day and how often six portions or more are consumed a day). These habits were asked elicited as addictive behaviours may coincide.

We used the SITAD measure to differentiate tanning-dependent individuals and abusers from non-dependent individuals. SITAD is based on opioid use items adapted from the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Axis I Disorders (SCID) (First et al., 1995). Depending on choice of wording, it can be used to detect either indoor or outdoor tanning dependence or both (Hillhouse et al., 2012). In the present study wording was adjusted to consider in specific sunbathing related tanning dependence. The SITAD questions differentiate tanning dependent, tanning abusers and non-dependent respondents (Hillhouse et al., 2012). For tanning dependence, the respondent needed to fulfil at least three of the following criteria: 1) loss of control over tanning; 2) unsuccessful efforts to cut down or control tanning; 3) a great deal of time spent on tanning, preparations for it, or recovery from it; 4) social problems due to tanning; 5) physical or psychological problems due to tanning; 6) tolerance of the effects of tanning; or 7) withdrawal symptoms attributable to tanning (Hillhouse et al., 2012). A respondent was defined as a tanning abuser if the criteria for dependence were not met but at least one of the following was present: 1) recurrent tanning accompanied by failure to fulfil role expectations at work or school, 2) physically hazardous recurrent tanning, or 3) continued tanning in the presence of recurrent social or interpersonal problems. There were altogether 13 main items with one to four sub-questions after removing one item, as “legal problems” was deemed inappropriate for the sunbathing context (Hillhouse et al., 2012). The measure was translated following the WHO guidelines (World Health Organization, 1948, http://www.who.int/substance_abuse/research_tools/translation/en/#). Completion of the SITAD measure takes about 5 min.

The presence of seasonal variations in mood and behaviour was evaluated using the Seasonal Pattern Assessment Questionnaire (SPAQ), which yields a numerical Global Seasonality Score (GSS) (Magnusson, 1996). The sunbathers were classified by means of the SPAQ into those fulfilling the criteria for SAD and S-SAD and for normal seasonal variations in mood and behaviour (normal seasonality).

Briefly, the seasons may give rise to changes in six items tied to well-being and behaviour (i.e., mood, appetite, weight, sleep duration, energy level, and social activity), which may vary from no change (0) to mild (1), moderate (2), definite (3) or severe (4). The sum of these item scores is the GSS, ranging 0–24 (Kasper et al., 1989; Magnusson, 1996; Rosenthal et al., 1987). Respondents reported whether these seasonal variations were a problem and, if they were, its severity (mild to disabling). Accordingly, a respondent was defined as having SAD when the GSS ranged from 11–24 points and the severity of seasonal problems was graded as at least moderate (Kasper et al., 1989). S-SAD was defined as a GSS score of 11–24 with no or only mild seasonal problems, or as a GSS score of 9–10 with at least mild problems. Normal seasonality was defined as a GSS score of 0–8, or 9–10 but with no seasonal problems.

2.3. Statistical methods

Mean values and their standard deviations (SD) were calculated for continuous variables and frequencies were calculated for categorical variables. Statistical significances for the hypothesis of linearity across categories of tanning dependence were evaluated by using the Cochran–Armitage test for trend and analysis of variance with an appropriate contrast. The relationship of seasonality and tanning dependence was tested with logistic regression adjusted for confounding factors. Simulations were used to calculate post hoc power for the relationship of seasonality and tanning dependence by using the actual data from this study. All analyses were performed using Stata Statistical Software, Release 15.1 (StataCorp LP, College Station, TX, USA).

3. Results

The response rate was 59% ($n = 229$), and 70% ($n = 161$) of responses arrived via the internet. There was a female predominance 84% (193/229) among responses. According to the tanning dependence measure SITAD targeting sunbathing related tanning dependence, 34% ($n = 77$) of sunbathers were screened as positive, with 8% ($n = 18$) fulfilling the criteria for tanning dependent and 26% ($n = 59$) for tanning abusers (Table 1).

Altogether 42% ($n = 97$) of the sunbathers were classified to display either SAD (16%, $n = 37$) or S-SAD (26%, $n = 60$). A total of 28% ($n = 63$) of sunbathers reported seasonal variations in mood and behaviour to be a definite problem, with 6% ($n = 14$) having severe or disabling problems. SAD/S-SAD and tanning dependence or abuse showed no association ($p = 0.54$) and the share of normal seasonality, S-SAD or SAD was equal independent of the tanning dependence classification. The history of ever having used a solarium and having sunbathed frequently to tan was both significantly associated with tanning dependence, whereas the proportion of photosensitive respondents decreased significantly as degree of tanning dependence increased, as seen in Table 1.

Altogether 50% ($n = 115$) of respondents sunbathed frequently whenever possible (Table 1), but there was a highly significant linear difference between non-dependent (37%), tanning abusers (75%), and tanning dependent respondents (83%) ($p < 0.001$). Of all respondents 94% ($n = 215$) had sometimes sunbathed with the specific intention of tanning their skin, but those who were tanning-dependent or abusers did so significantly ($p = 0.036$) more frequently. Table 1 shows that 67% of tanning-dependent and of 58% of abusers had tanned their skin indoors, compared to 40% of non-dependent beach and parkgoers, showing a linear significant difference ($p = 0.004$). Only 1.3% ($n = 3$) were frequent indoor tanners, and 20.1% ($n = 46$) had used solarium for a specific occasion, and 17.5% ($n = 40$) were random users. The frequency of using sunscreen, smoking or drinking habits were not significantly associated with tanning dependence/abuse (Table 1).

No statistical relationship between seasonality and sunbathing related tanning dependence was detected (p for linearity 0.35, post hoc power 74%). To evaluate if essential co-variates had an impact on the relationship between seasonality and tanning dependence we conducted an analysis 1) with the original crude data, 2) with the age and gender-adjusted data, and 3) with the co-variates smoking, drunkenness and sun sensitive skin (phototypes I-II) data in addition to age and gender (Table 2.). The models showed that co-variates had no impact on the outcome, nor were any significant associations between seasonality and tanning dependence detected.

Table 1
Characteristics of sunbathers.

	Non-Dependent $n = 152$	Abusers $n = 59$	Dependent $n = 18$	p-value*
Age, mean (SD)	33 (11)	33 (9)	37 (12)	0.30
Females, n (%)	124 (82)	53 (90)	16 (89)	0.16
Skin phototype, n (%)				0.034
I	1 (1)	2 (3)	0 (0)	
II	40 (26)	7 (12)	2 (11)	
III	67 (44)	33 (56)	6 (33)	
IV	44 (29)	17 (29)	10 (56)	
GSS mean (SD)	9.5 (4.7)	10.8 (5.0)	10.5 (4.2)	0.13
Seasonality, n (%)				0.54
Normal	91 (60)	28 (47)	13 (72)	
S-SAD	40 (26)	18 (31)	2 (11)	
SAD	21 (14)	13 (22)	3 (17)	
Ever used solarium, n (%)	61 (40)	34 (58)	12 (67)	0.004
Ever sunbathed to tan, n (%)	139 (91)	58 (98)	18 (100)	0.036
Sunbathes frequently to tan, n (%)	56 (37)	44 (75)	15 (83)	<0.001
Sunscreen use, n (%)				0.52
Never	21 (14)	6 (10)	1 (6)	
Occasionally	54 (36)	28 (47)	11 (61)	
Frequently	77 (51)	25 (42)	6 (33)	
Smoking, n (%)	38 (25)	13 (22)	6 (33)	0.74
Excessive use of alcohol, often, n (%)	23 (15)	10 (17)	2 (11)	0.88

Abbreviations: GSS, Global Seasonality Score; S-SAD, Subsyndromal Seasonal Affective Disorder; SAD, Seasonal Affective Disorder.
* p for linearity.

4. Discussion

In this study, we assessed sunbathing related tanning dependence among beach and parkgoers using SITAD, showing that 34% of the Finnish beach and parkgoers were tanning dependent or abusers (Hillhouse et al., 2012). This highlights that tanning addiction must be considered an important factor which may lead to excessive sunbathing and thus increase the risk of getting contracting skin cancer. SAD/S-SAD symptoms were detected in 42% of beach and parkgoers. Respective figures were published earlier in a population-based health examination study reporting S-SAD in 38.9% and SAD in 2.6% of the

Table 2
Relationship between seasonality and tanning dependence (dependent/abusers vs. others).

	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Seasonality			
Normal	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
S-SAD	0.32 (0.07–1.45)	0.31 (0.07–1.41)	0.34 (0.07-1.58)
SAD	0.81 (0.22–3.00)	0.79 (0.21–2.96)	0.75 (0.20–2.85)
p for linearity	$p = 0.35$	$p = 0.33$	$p = 0.34$

Model 1 was crude. Model 2 was adjusted for age and gender. Model 3 was adjusted for age, gender, smoking, excessive use of alcohol and skin phototype I–II.

Finnish adults (Grimaldi et al., 2009).

Warthan et al. assessed motivations for beachgoing among 145 sunbathers on Galveston Island, Texas. Using the mCAGE measure and the mDSM-IV-TR measure, 26% of sunbathers met the mCAGE and 53% the mDSM-IV-TR diagnostic criteria for tanning addiction (Warthan et al., 2005). Due to the use of different measures, these results are not directly comparable with ours, although they undeniably indicate the magnitude of the tanning dependence issue. The mCAGE has been criticised for the use of detecting tanning dependence. The criticism has arisen due to the different wording between studies, for screening subjects who had not tanned themselves indoors recently as problematic tanners and for giving too high percentages for tanning related problems (Schneider et al., 2015).

Hillhouse et al., who developed the SITAD measure, performed a pilot study showing that 16% of 296 college students met the criteria for indoor tanning dependence/abuse (Hillhouse et al., 2012). Interestingly, in our sample targeting sunbathing related tanning dependence, the frequency of tanning dependent or abusers was double. This gap could be related to the beach and park context, as well as the different cultures and northern geographic locations in our study with minimal sunlight for several months a year, which may be conducive to sun-seeking behaviour.

In the present study, up to 16% scored on the SPAQ to meet the criteria for SAD and thereby exceeding the figure of 2.6% detected by Grimaldi et al. in a national population-based survey (Grimaldi et al., 2009). Earlier, Hillhouse et al. reported that 81% of frequent indoor tanners exhibited either SAD or S-SAD, supporting the idea that tanning beds are used for their mood-enhancing effects (Hillhouse et al., 2005). To conclude, both sunbathing and indoor tanning seem to attract people presenting with more severe seasonality and may serve as an attempt to cure symptoms, as suggested earlier (Heckman et al., 2016; Petit et al., 2014). Other explanations are possible, since Grimaldi et al. reported 85% of a national cohort to follow some seasonal pattern in their mood and behaviour (Grimaldi et al., 2009). The discrepancy in seasonality figures between Hillhouse's study and ours could also be due to the season in which the studies were conducted and to other potential differences involving seasonal availability, proximity to home, engaging alone or with friends (Hillhouse et al., 2005).

Contradicting our hypothesis, in the present study SAD/S-SAD was not associated with sunbathing related tanning dependence. Another recent study found that meeting the criteria for SAD was associated with tanning dependence (Cartmel et al., 2017). Of specific types of psychiatric disorders, tanning dependence was associated with “being more likely to meet criteria for SAD” (Heckman et al., 2014). Nevertheless, consistent with our findings, Heckman et al., using the Positive Affect and Negative Affect Scale (PANAS), reported a lack of association between mood states, including both negative (upset, scared, irritable, nervous, jittery, afraid) and positive (feeling interested) mood states and tanning addiction and speculated that this finding might indicate that tanning-dependent individuals have developed tolerance of the mood-enhancing effects of tanning but continue to tan as a hallmark of dependence (Heckman et al., 2016; Watson et al., 1988).

In the northern regions, when daylight is scarce in wintertime, SAD symptoms are a frequent finding (Magnusson, 2000). Bright light therapy through the eyes may effectively cure SAD (Partonen and Lönnqvist, 1998), but it is not known if UVR exposures through the skin have a definite impact on mood. Whether exposures of the skin using visible light or UVR induce neuroendocrine changes needs further study (Fell et al., 2014; Felton et al., 2017; Jussila et al., 2016; Skobowiat and Slominski, 2015; Skobowiat et al., 2011). A recent study using brain imaging techniques with single photon emission computerised tomography (SPECT) showed that addicted tanners express stronger neural rewarding responses to UVR than do non-addicted tanners, supporting the existence of a cutaneous-neural connection (Aubert et al., 2016). UV induced endocannabinoids have also been linked with sunbathing and mood (Felton et al., 2017). Some studies propose that genetic involvement may play a role in tanning addiction (Cartmel et al., 2017, 2014; Khouja et al., 2018). Further research is therefore still needed as the mechanisms remain far from clear.

Excessive tanning of the skin is a frequent concern of health care authorities due to the ever-increasing prevalence of skin cancers, including melanomas (Gandini et al., 2011; Tierney et al., 2015). Our results show that one-third of beach and parkgoers met the criteria for sunbathing related tanning dependence/abuse, justifying the serious concern about the skin cancer risk in tanning-dependent individuals. New interventions are therefore urgently needed to halt addictions like harmful tanning behaviour. Hillhouse et al. presented an appearance-focused intervention booklet that might be useful, independent of SAD status, in preventing tanning dependence (Hillhouse et al., 2010).

The research group who published SITAD has now moved on to using another shorter instrument, the Behavioural Addiction Indoor Tanning Screener, BAITS (Diehl et al., 2018; Stapleton et al., 2016). BAITS was not available when we conducted our study; it is also designed for indoor tanning users and there are no published studies using BAITS in the sunbathing context.

There were some limitations in our study. First, the summer when data was collected was very rainy and chilly, allowing sunbathing on only very few days, which may have influenced the sample content and its size. The survey was implemented only in summertime, which may have impacted on the presence of SAD/S-SAD. The results are context-dependent and confined to Finnish beach and parkgoers.

5. Conclusions

Sunbathing related tanning dependence/abuse and symptomatic seasonality (SAD/S-SAD) were both frequent among Finnish sunbathers but not associated with each other. Further targeted experimental and longitudinal clinical studies are necessary to elucidate and understand these phenomena to improve efficacy in the prevention of skin cancers.

Disclosure of interest

The authors report no conflict of interest.

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Supplementary materials

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PUBLICATION III

Narrow-band ultraviolet B (NB UV-B) exposures improve mood in healthy individuals differently depending on chronotype

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Narrow-band ultraviolet B (NB UV-B) exposures improve mood in healthy individuals differently depending on chronotype

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Abbreviations: ultraviolet radiation (UVR), narrow-band UV-B (NB UV-B), Visual Analogue Scales (VAS), β-endorphin (β-END), Interleukin-6 (IL-6)

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Abstract

The evening chronotype is associated with psychological symptoms such as depressed mood, while skin exposure to ultraviolet radiation (UVR) may affect mood and behavior through neural and humoral routes. This pilot study aimed to investigate the impact of whole-body narrow-band (NB) UV-B exposure on current mood state and circulating 25-hydroxyvitamin D3 ($25(OH)D_3$), interleukin-6 (IL-6), cortisol and β-endorphin (β-END) levels in healthy participants. Here, eleven healthy women received full-body NB UV-B exposures on four afternoons, and the chronotype was assessed with a shortened version of Horne and Östberg's Morningness-Eveningness Questionnaire (MEQ). Perceived mood was evaluated using the Visual Analogue Scale (VAS), and serum $25(OH)D_3$, IL-6, cortisol and β-END concentrations were monitored daily. Decreasing VAS values showed mood to improve significantly over the five days after the four suberythematous NB UV-B exposures ($p=0.038$), and the more the circadian preference was inclined towards eveningness, the greater the improvement in the mood dimension of wellbeing ($p=0.021$). Baseline mood state was correlated with baseline $25(OH)D_3$ ($r = -0.54$, 95% CI: -0.86 to -0.09) and with baseline cortisol ($r = -0.57$, 95% CI: -0.87 to -0.04). During the NB UV-B exposures, $25(OH)D_3$ increased significantly, as expected, and IL-6 declined significantly by -0.35 (95% CI: -0.69 to -0.07) pg/mL from the initial values of 1.12±0.66 pg/mL ($p=0.025$). In conclusion, in our pilot study, NB UV-B exposure improved mood, especially among those with evening preference for their daily activities, as well as circulating $25(OH)D_3$ levels, whereas circulating IL-6 levels decreased.

Keywords: Chronotype; mood; Vitamin D; β-endorphin; cortisol; interleukin-6; UV-B

Introduction

Chronotype is a measure of individual differences in daily activity patterns and sleep-wake cycles whereby humans can be classified into three chronotypes based on the timing of physiological functions and their preference in daily activities: morning types (advanced sleep period), neither types, and evening types (delayed sleep period) (Duffy et al. 2001; Horne and Östberg 1976). The chronotype has a genetic basis and is influenced by environmental factors such as latitude and photoperiod (Randler and Rahafar 2017; Shawa et al. 2018). Higher latitudes are characterized by greater seasonal variations in photoperiod, and people living at high latitudes seem to have a tendency towards a late time of day preference (Borisenkov et al. 2010; Leocadio-Miguel et al. 2017). Previous studies suggest that the preference for eveningness may be associated with depressive symptoms and mood disorders (Au and Reece 2017; Hidalgo et al. 2009; Jeong Jeong et al. 2015; Merikanto et al. 2013).

Circadian rhythms are present in near all tissues throughout the human body, and the circadian system is responsible for regulating the approximate 24-hour rhythms in biological (e.g., hormone levels), psychological (e.g., alertness and mood) and behavioral (e.g., eating, exercising and sleeping) variables (Adan et al. 2012; Dibner 2019; Lemmer 2009). The individual morningness-to-eveningness preferences arise from the differences in the endogenous circadian clock and the daily schedule for activities as well as the interplay between these two systems (Barclay et al. 2014; Dijk and von Schantz 2005; Kerkhof 1985). Circadian rhythms are generated and maintained by the intrinsic clocks to track time and correspond to the temporal organization of the environment. Light exposure is the most potent time cue for synchronizing internal circadian rhythms with the transitions of the external light-dark cycle (Münch and Bromundt 2012; Roenneberg et al. 2007; Zeitzer et al. 2005).

Human skin is repeatedly exposed to solar ultraviolet radiation (UVR), a confirmed environmental carcinogen that induces mutations and simultaneous immunosuppression (Hart and Norvall 2018; Murphy 2009; World Health Organization 2012). The ultraviolet B (UV-B) wavebands (315-280 nm) are biologically the most active part of the solar spectrum reaching the Earth, and UV-B-induced DNA damage leads to the release of various pro-inflammatory mediators from keratinocytes, including interleukin-6 (IL-6) (Hart and Norvall 2018; Murphy 2009; Urbanski et al. 1990; World Health Organization 2012). In addition, UV-B radiation exerts strong biological actions on regulatory pathways related to immune balance, both vitamin-D-related and vitamin-D-independent (Skobowiat and Slominski 2015).

UV-B radiation induces the cutaneous photosynthesis of vitamin D, which leads to increased levels of its circulating form, i.e., 25-hydroxyvitamin D3 ($25(OH)D_3$). Vitamin D insufficiency (levels of 50–75 nmol/L) and deficiency (levels of <50 nmol/L) are common at high latitudes in winter unless vitamin D substitution is used (Holick 2007; Holick and Chen 2008). Low $25(OH)D_3$ status has been linked to poorer mental health and may explain the low vitality, energy and mood observed in winter (Berk et al. 2007; Harris and Dawson-Hughes 1993; Humble 2010; Jääskeläinen et al. 2015).

IL-6 and cortisol are first-line immune modulators that can be synthesized in the skin under stress conditions, including UVR exposure, and delivered in the circulation (Kirnbauer et al. 1991; Skobowiat et al. 2011; Skobowiat and Slominski 2015; Urbanski et al. 1990). Elevated IL-6 levels have been detected in patients presenting with major depressive disorder (Hodes et al. 2016; Liu et al. 2012), while cortisol has been associated with the immune system and mood (Shattuck and Muehlenbein 2015). Patients who did not respond and were resistant to antidepressant medications had increased circulating TNF-α and IL-6 levels and impaired

cutaneous glucocorticoid receptor function (Fitzgerald et al. 2006). A large proportion of depressed patients showed excessive cortisol production (Carpenter and Gruen 1982).

Beta-endorphin (β-END) is formed in the epidermal skin from proopiomelanocortin (POMC) upon UVR exposure (Fell et al. 2014; Skobowiat and Slominski 2015). Human studies of UVR-induced β-END are rare, however, and the results are conflicting. In mice, UV-B-induced β-END led to the development of UVR-addictive behavior, and it has been suggested that it may underlie tanning-dependent behavior in humans (Skobowiat and Slominski 2015).

This pilot study investigated the impact of whole-body narrow-band (NB) UV-B exposure on the current mood state, in general and according to chronotype, in healthy participants and the associations with the circulating $25(OH)D_3$, IL-6, cortisol and β-END levels. We hypothesized that a) NB UV-B exposure improves the current mood state and that b) this improvement occurs through the skin-borne circulating mediators and is reflected in changes in their circulating levels. Further, we hypothesized that these changes are greater in individuals with an eveningness preference for daily activities because a) they tend to have a higher ratio of phase advancing to phase delaying of circadian rhythms by light exposure (Emens et al. 2009) and b) they appear to benefit from the phase advance accompanied by improvements in self-reported depression and stress (Facer-Childs et al. 2019).

Materials and Methods

The study was implemented at the Department of Dermatology and Venereology, Tampere University Hospital, Tampere, Finland, 61°N, in October to November, when the availability of biologically effective skin-burning UV-B solar ultraviolet radiation was negligible. The protocol followed the principles of the Declaration of Helsinki and its amendments and was approved by the Regional Ethics Committee of Tampere University Hospital District (#

R14012). The volunteers were recruited through advertisements and provided informed consent. No compensation was given.

Volunteers

Twelve healthy women agreed to participate. The inclusion criteria were as follows: a) they had no phototherapy, solarium visits or sunny holidays during the preceding two months, and b) they had taken no vitamin D supplements during the preceding two months. The volunteers had to regard themselves as "healthy" and have not had any psychiatric interventions. Depression was not specifically screened. The exclusion criteria were a) sun-sensitive Fitzpatrick skin phototype I, b) the use of antidepressants or any psychotropic drugs, c) the use of medication with definite photosensitizing potential (specifically, amiodarone, dacarbazine, doxycycline, phenothiazine, furosemide, diclofenac, NSAIDs, ketoprofen, and piroxicam) and d) pregnancy. We also asked whether the volunteer was using any medication, and if so, we asked them to name it. Demographic data were collected with respect to age, gender, skin phototype (Fitzpatrick 1988) and body-mass index (BMI).

Narrow-band UV-B exposures

The volunteers received four full-body NB UV-B exposures in the afternoons of four consecutive days. Their eyes were covered with opaque goggles during the irradiations. The irradiance of the Waldmann UV 7002 phototherapy cabin (Herbert Waldmann GmbH & Co. KG, Villingen-Schwenningen, Germany), which was equipped with 42 TL01 tubes (Schulze & Böhm GmbH, Brühl, Germany), was measured beforehand using an Ocean Optics S2000 spectroradiometer (Ocean Optics, Inc., Largo, FL, USA). After correcting for systematic errors and stray light, it was estimated that the uncertainty (2σ) of the measurement was approximately 14%. These assessments were traceable to the National Institute of Standards and Technology (Gaithersburg, MD, USA). The initial NB UV-B physical dose was 0.17

J/cm^2, corresponding to one standard erythema dose (1 SED), which is equivalent to an erythemal effective radiant exposure of 10 mJ/cm^2 (Commission Internationale de l'Éclairage 1999). In the course of the four days, the dose was increased from 0.17 J/cm^2 to 0.34 J/cm^2 (2 SED) for 5 volunteers and to 0.51 J/cm^2 (3 SED) for 6 volunteers. The cumulative dose of the four irradiations thus ranged from 1.19 J/cm^2 (7 SED) to 1.36 J/cm^2 (8 SED).

Evaluation of mood state

To determine whether the four NB UV-B exposures affected the participants' mood state, they were asked to rate their mood on each day immediately before the scheduled NB UV-B exposure and on day five 24 hours after the last exposure, employing the Visual Analogue Scale (VAS), where values closer to zero depict better feelings (Folstein and Luria 1973). The eventual mood VAS used in the statistical analyses was the mean of four dimensions of mood, 1) VAS-satisfaction, 2) VAS-tiredness, 3) VAS-wellbeing and 4) VAS-irritation, each scored separately (in millimeters) on a 100-mm line with its ends anchored to the concepts of "best ever" versus "worst ever". The questions above the VAS scales asked "How do you rate your feelings of satisfaction/tiredness/wellbeing/irritation at the moment?"

Morningness-Eveningness Questionnaire (MEQ-6)

A Morningness-Eveningness Questionnaire (MEQ-6, v.1.2 FI, National Institute for Health and Welfare, Helsinki, Finland) based on the original Horne & Östberg Morningness-Eveningness (MEQ-19) questionnaire translated to Finnish was used to define the chronotype (Horne and Östberg 1976). The modified instrument comprises six questions. All the answers were scored and summed for the total Morningness-Eveningness score of 5 to 27 points. In our analyses, the participants were classified into morning or evening chronotypes ("Larks" vs. "Owls") based on the last question in MEQ-6, where the definitely morning and rather

morning than evening chronotypes were classified as “Larks”, and definitely evening and rather evening than morning chronotypes were classified as “Owls”.

Time schedule for blood sampling

Blood samples were taken prior to each NB UV-B exposure to measure circulating 25(OH)D_3, IL-6, cortisol and β-END levels. All samples were drawn at the same hour of the afternoon on five consecutive days. The first sample was taken on day 0 before the first exposure, and the last sample was taken 24 hours after the final NB UV-B exposure. The blood samples were protected from the light and centrifuged, whereupon the sera were separated out and stored at -70°C until used for the analyses.

Laboratory analyses

25(OH)D_3 was determined by a Chemiluminescent Microparticle Immunoassay using the Architect system (Abbott Laboratories, Abbott Park, IL, USA). Quality control was further ensured by participation in the International External Quality Assessment Scheme (DEQAS) (Miettinen et al. 2014). Due to the short duration of the trial, no nutritional analysis of the availability of vitamin D in the diet was performed, but the volunteers were asked to keep their diet constant during the experiment.

Serum immunoreactive β-END was measured using an ELISA method developed at the University of Oulu, Finland. Samples and synthetic human beta-endorphin calibrators were incubated with synthetic beta-endorphin-containing C-terminal biotin and the rabbit anti-human beta-endorphin antiserum “BK22” (Vuolteenaho 1984) at a final dilution of 1/100000. After overnight incubation at +4°C, streptavidin-horseradish peroxidase conjugate (Pierce, Thermo Fisher Scientific Inc., Waltham, MA, USA) was added, followed by 3,3',5,5'-tetramethylbenzidine substrate (TMB One, KemEnTec Diagnostics A/S, Taastrup, Denmark). The reaction was stopped with 2-N sulfuric acid, and the wells were scanned at $A_{450\ nm}$ with a

Varioskan Flash Plate Reader (Thermo Scientific, Thermo Fisher Scientific Inc., Waltham, MA, USA). Serum cortisol was measured using a Ref 52611 ELISA kit from IBL International GmbH, Hamburg, Germany, and IL-6 was measured with a DY206 ELISA kit from R&D Systems, Inc., Minneapolis, MN, USA, according to the instructions provided by the manufacturers.

Statistical analyses

We report the results as the means with standard error of mean (SEM) and 95% confidence intervals (CIs). Due to the sample size and some skewed distributions, resampling-based (Bootstrap or Monte-Carlo) methods were used in the analyses to define the significance level (p-value) and 95% CIs. Mean changes (within subjects) in mood and physiological biomarkers between day 5 and baseline values were assessed using the Fisher-Pitman permutation test for paired replicates (exact p-value). Changes on day 5 and baseline values between chronotypes were compared using bootstrap-type independent-sample t-tests (5000 replications). Statistical significance for the hypothesis of linearity across the 5-day periods of biomarkers was tested using bootstrap-type repeated-measures analysis of variance (rANOVA). Effect size (d) was calculated using the method of Cohen, where an effect size of 0.20 is considered small, 0.50 moderate, and 0.80 large; CIs for the effect sizes were obtained by bias-corrected bootstrapping (5000 replications). We used linear regression models to evaluate the contributions of the total Morningness-Eveningness score and the skin phototype as independent predictors of the dependent outcome of the change in VAS-wellbeing. We calculated the correlation coefficients using Spearman's method. The normality of the variables was tested using the Kolmogorov-Smirnov test. No adjustment was made for multiple testing, but this information can be obtained by multiplying the actual p-value by the number of comparisons made. Statistical analyses were carried out using the Stata statistical software, release 14.1 (StataCorp, College Station, TX, USA), and the IBM Statistical

Package for the Social Sciences (SPSS) Statistics, version 25 (International Business Machines Corporation, Armonk, NY, USA).

Results

Chronotype and other background characteristics

Eleven of 12 women completed the experiment, since one withdrew for personal reasons. Five volunteers had a preference towards morningness (Larks) and six towards eveningness (Owls). The mean age was 41.6 ±11.2 years. Eight volunteers (72.7%) displayed skin phototype II (often burns and tans poorly), and three (27.3%) displayed skin phototype III (sometimes burns and tans easily) (Fitzpatrick 1988). The mean BMI was 25.1± 5.1 kg/m^2.

Impact of NB UV-B exposures on mood

The mean VAS score (VAS-mean) of the eleven participants on the four mood dimensions (satisfaction, tiredness, wellbeing and irritation) was 28.6±15.9 mm before the NB UV-B exposures, which improved between day 5 and baseline significantly by a mean of 7.4 (95% CI: -14.5 to -0.3) mm by the end of the study (p=0.038, Figure 1). The declining VAS-mean depicted improved mood in eight of eleven participants (73%), five of whom were Owls. The VAS-mean was higher (i.e., the mood was worse) in the Owls than in the Larks on each day. On the other hand, the VAS-mean decreased from baseline to day 5 in the Owls, 11.8 (95% CI: -21.4 to -2.2) mm, and the Larks, 2.1 (95% CI: -11.6 to 7.5) mm, i.e., the mood state improvement was larger in the Owls, but the difference between the groups was not statistically significant (p=0.19) (Figure 1 and Table 1).

There was a difference in the change in the "VAS-wellbeing" mood dimension from baseline to day 5 between the Larks [1.0 (95% CI: -5.6 to 7.6)] and Owls [-16.8 (95% CI: -25.8 to -5.8)] amounting to -17.8 (95% CI: -31.92 to -3.75) mm on average (p=0.019). Furthermore, in the regression analysis, the total Morningness-Eveningness score alone significantly

explained the change in VAS-wellbeing (R^2 = 0.47; B = -2.10, 95% CI: -3.80 to -0.40; p=0.021), whereas the skin phototype alone did not. However, the model (with R^2 = 0.72), in which we entered both the total Morningness-Eveningness score (B = -2.54, 95% CI: -3.92 to -1.15; p=0.003) and the skin phototype (B = -15.34, 95% CI: -28.59 to -2.10; p=0.028), explained significantly the change in VAS-wellbeing, and showed greater improvement among the Owls and among those having skin phototype II. Adding age to this model (R^2 = 0.85) yielded similar results and pointed to the same two predictors as being significant.

Impact of UVR irradiations on circulating biomarkers

The baseline 25(OH)D_3 values ranged from 43 to 128 nmol/L, with two volunteers apparently being vitamin-D-deficient (below 50 nmol/L) and three displaying vitamin D insufficiency (levels 50-75 nmol/L) (Holick 2007; Holick and Chen 2008). The four NB UV-B whole-body exposures effectively improved the 25(OH)D_3 levels of all 11 volunteers by 8.3 (95% CI: 5.6 to 11.8) nmol/L from the initial values of 75.5 ± 28.0 nmol/L ($p<0.001$; effect size 1.50 (95% CI: 0.69 to 2.30)) (Figure 2a), and increases seemed detectable as early as after the first NB UV-B exposure. The increase was greatest in the volunteers with the lowest baseline values. None of the women were vitamin-D-deficient any longer by day five, but the five with the lowest baseline 25(OH)D_3 levels remained vitamin-D-insufficient (Holick 2007; Holick and Chen 2008).

The initial IL-6 values ranged from 0.34 to 2.21 pg/mL, showing a 7-fold interindividual variation, but the mean value seemed to decrease significantly by -0.35 (95% CI: -0.69 to -0.07) pg/mL from the initial level of 1.12±0.66 pg/mL (p=0.025; effect size 0.54 (95% CI: 0.09 to 1.31)) (Figure 2b).

Baseline cortisol values varied from 243 to 526 nmol/L, and among volunteers, the mean level had increased by 79 (95% CI:-18 to 177) nmol/L from the initial level of 391±81

nmol/L following the NB UV-B irradiations (Figure 2c), although this difference was not statistically significant (p=0.16).

The baseline β-END values showed a notable 45-fold interindividual variation, ranging from 18 to 362 fmol/mL giving an initial mean of 203±129 fmol/mL. This increased by 39 (95% CI: -50 to 129) fmol/mL following the NB UV-B exposures, but the difference was not statistically significant (p=0.41). β-END increased in six volunteers and decreased in five volunteers over the five days of the experiment (Figure 2d).

The mean serum 25(OH)D_3, β-END, cortisol and IL-6 levels on each day are shown by chronotype (Larks/Owls) in Table 1. The mean 25(OH)D_3 levels were lower in the Owls throughout, as were the mean IL-6 levels. Mean β-END seemed higher in the Owls than in the Larks at the end of the study, but there was considerable overall fluctuation in both groups. Mean cortisol levels seemed to be higher in the Owls on each day (Table 1).

Correlations between perceived mood and circulating biomarkers

Although the volunteers' baseline 25(OH)D_3 levels correlated with the baseline mood state (VAS mm) (r = -0.54, 95% CI: -0.86 to -0.09, p=0.085), the improvement in 25(OH)D_3 did not correlate with the improvement in mood state on day five (r= -0.14, 95% CI: -0.68 to 0.50, p=0.66).

The baseline IL-6 (pg/mL) values and perceived mood state (VAS mm) were not correlated (r = 0.06, 95% CI: -0.56 to 0.64, p=0.85), nor were the NB UV-B-induced increase in IL-6 and the improvement in mood (r = -0.44, 95% CI: -0.82 to 0.22, p=0.18), but the individual volunteers with higher baseline IL-6 clearly displayed a better mood state.

The volunteers' cortisol levels at baseline correlated with their mood state (r = -0.57, 95% CI: -0.87 to -0.04, p=0.065), but there was no correlation between the subsequent UVR-induced

increase in cortisol and the improvement in the mood VAS result (r= -0.03, 95% CI: -0.62 to 0.58, p=0.94).

Initial β-END values and mood VAS were not correlated (r = 0.43, 95% CI: -0.23 to 0.82, p=0.18), nor was there any correlation between the increase detected in β-END and the improvement in mood VAS (r= -0.15, 95% CI: -0.69 to 0.49, p=0.65).

Discussion

Our findings suggest a relationship between NB UV-B exposure and the mood state of the volunteers, referring to the fact that the improvement in mood was greater in those with their preference towards eveningness rather than morningness, the effect being significant for the change in the VAS-wellbeing dimension. NB UV-B significantly improved the balance of circulating vitamin D, and circulating IL-6 levels seemed to decrease. These findings suggest that further studies are required on the interactions between UVR, skin and mood.

There is evidence of an association between chronotype and mood disorders, as those with a circadian preference favoring eveningness appear to be prone to depression (Au and Reece 2017; Hidalgo et al. 2009; Jeong Jeong et al. 2015; Merikanto et al. 2013). In previous studies, mood assessments were used to evaluate depression or depressive symptoms over a longer period of time, e.g., during the past 12 months. In the present study, the volunteers who reported that they were evening chronotypes had a worse current mood state on average than the others throughout the five-day trial. The evening chronotypes also tended to show more improvement in their current mood state than the morning chronotypes following UVR exposure. However, because the participants in the current study were healthy, and as depression was not specifically screened and interviewed by us, any comparison between the current study and past research should be made with caution. To extend and deepen the scope of such generalizations, further studies need to be focused on volunteers with a current

depressive episode, as assessed using a structured clinical interview for diagnosis, to elucidate the effects and mechanisms of action of NB UV-B exposures on mood. Future studies are required to show the possible causal relationship between depressive symptoms and evening chronotype and, further, whether it could be worthwhile trying NB UV-B phototherapy for people suffering from depressive symptoms or other mood disorders.

The skin is capable of reacting to psychiatric and psychosocial conditions and is a potential source of numerous UVR-inducible neuroactive compounds (Denda et al. 2013; Slominski et al. 2012; Slominski and Wortsman 2000). We have shown earlier that NB UV-B exposure increases the expression of β-END in the human skin *in vivo* (Jussila et al. 2016), and mouse experiments have shown UV-B exposure to be capable of inducing β-END expression in the skin even to the point of developing addictive behavior towards UV radiation (Fell et al. 2014; Skobowiat and Slominski 2015). In the present study, the circulating β-END levels were increased during NB UV-B exposures, although not significantly, as there was up to 45-fold interindividual variation in these levels.

The present study demonstrated that the skin phototype together with the chronotype contributed significantly to the improvement in mood as assessed by means of scores on the subscale dimension of wellbeing on the VAS scales. The improvement was greater in the participants with skin phototype II than in those with skin phototype III. Phototype II burns more easily and tans less well than phototype III (Fitzpatrick 1988). Fair-skinned people are known to be more sensitive to the effects of UVR, which could explain the better mood-enhancing property of the UV-B exposures in our study. It has been reported in an earlier review that UVR was found to be capable of improving mood in six out of seven existing studies (Veleva et al. 2018). An explanation here might be that NB UV-B exposure influences mood and behavior through, e.g., thermoregulation, which in depressed individuals is often disturbed. The tentative warming of the body during UV-B exposure

might interfere with mood, as is shown for those with major depressive disorder, whereas warming with infrared light produces therapeutic benefits, which could occur through the deactivation of brown adipose tissue (Janssen et al. 2016).

We found that vitamin D status was lower in those volunteers who reported that their daytime peak was mainly in the evening rather than in the morning. One earlier study has shown that evening types have unhealthier dietary habits and that they ingest less vitamin D than morning types (Kanerva et al. 2012). To test the hypothesis that NB UV-B-induced increases in circulating vitamin D levels are associated with improved mood, much further study is necessary.

Four NB UV-B exposures with low nonerythematous physiological dosing also induced a significant decrease in IL-6 levels, and we also noted that the volunteers with evening preference displayed lower IL-6 levels throughout the study than those with morning preference. In an earlier study, Mondin et al. (2016) discovered that healthy individuals with a reversed day/night schedule had decreased IL-6 levels. In contrast to our findings, Urbanski et al. (1990) showed an increase in IL-6 levels after broadband (BB) UV-B exposure, with a peak value at 12 hours, but the BB UV-B doses used were aggressively high. However, further studies are necessary to verify any connection between UV-B exposure-induced IL-6 levels and mood.

We detected that UV-B seemed to induce an increase in cortisol levels, although not to a significant extent in our pilot study, and the average cortisol levels were slightly higher in the evening types than in the morning types on each day. Both UV-B and UV-C wavelengths have been shown previously to elevate cortisol levels in the skin (Fitzgerald et al. 2006). In the case of our study, the taking of the blood samples in the afternoon instead of the morning may be reflected in a decreased response, and this should be taken into account in further studies.-We did expect that the circulating mediators of the NB UV-B exposures we assessed

would correlate with the perceived changes in the current mood state, but they did not show any correlation with mood.

The limitations of our pilot study included the small number of volunteers, only female volunteers (since no men volunteered), and the lack of a control group. The groups of each chronotype were small, and some findings showed trends rather than reaching significance. Moreover, all the volunteers were healthy.

Conclusions

Our results indicate that four suberythematous exposures to NB UV-B can induce a change in perceived mood and 25(OH)D_3 as well as IL-6 levels. Mood improved more in evening chronotypes than morning chronotypes. Our findings support the hypothesis that UVR-exposed human skin transmits cues via reciprocal molecular interactions that affect mood regulation in the brain and contribute to behavior and the timing of daily activities. However, the demonstration of the exact mechanisms remains a major challenge.

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Disclosure of interest

The authors report no conflicts of interest.

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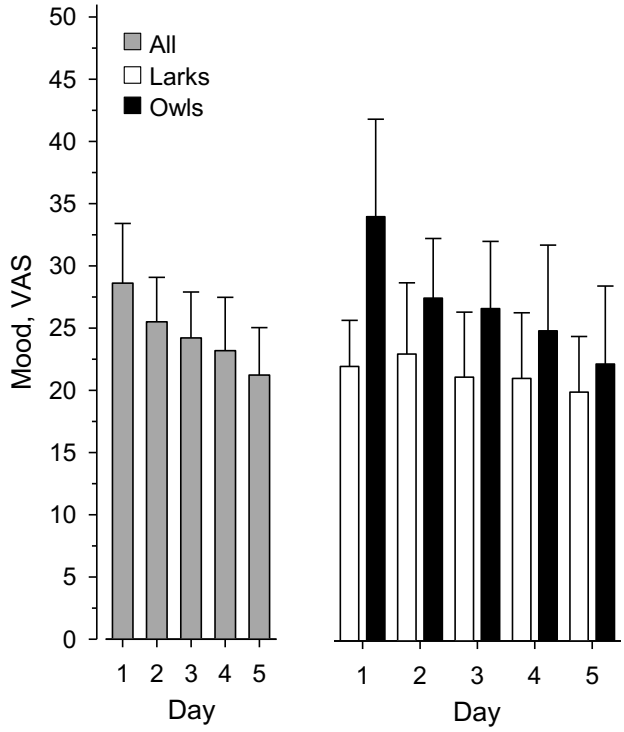

Figure 1. Perceived mood state immediately before NB UV-B irradiation on each day of the experiment and 24 hours after the last exposure. The bars depict the mean and SEM of the perceived mood of 11 participants on each day, taken all together (on the left, with gray bars), and by chronotype (Larks/Owls) (on the right, with white and black bars). The perceived mood state was evaluated as the mean of four dimensions of mood: 1) satisfaction, 2) tiredness, 3) wellbeing and 4) irritation. Each dimension was assessed on a 100-mm VAS scale, where a smaller value depicted a better mood.

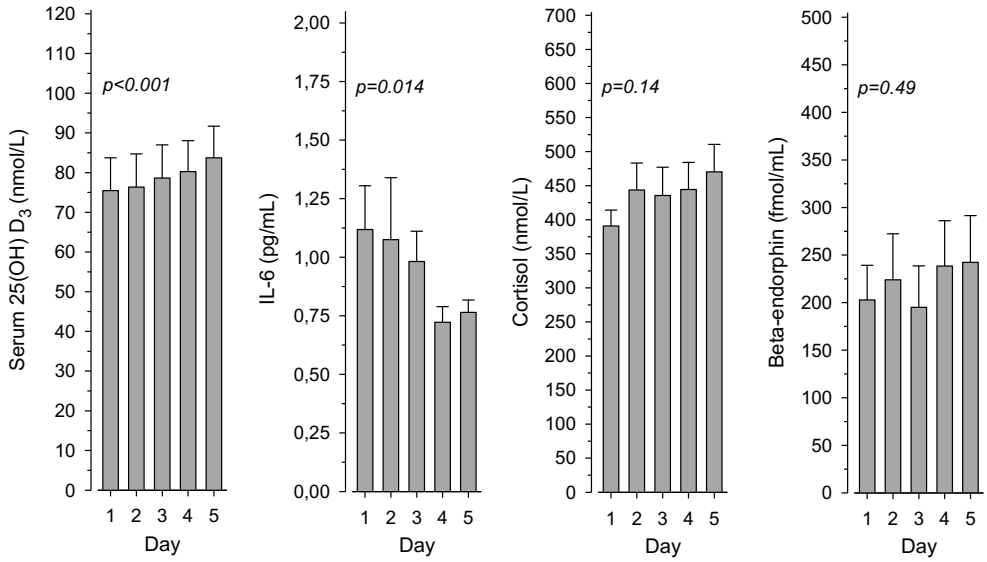

Figure 2a-d. The bars (from left to right) depict the means and SEM of a) serum 25(OH)D_3 (nmol/L), b) IL-6 (pg/mL), c) cortisol (nmol/L), and d) β-END (fmol/mL) on each day of the experiment. All blood samples were drawn immediately before the NB UV-B exposures or 24 hours after the last exposure. p-values show linearity across the 5-day periods.

Table 1. Mean serum vitamin D ($25(OH)D_3$), β-END, cortisol and IL-6 levels and mood VAS of the participants, grouped by chronotype (Larks/Owls).

	Baseline	**Day 2**	**Day 3**	**Day 4**	**Day 5**
"Larks"					
$25(OH)D_3$ (nmol/L)	81.4±13.4	81.4±14.1	84.2±13.8	85.0±13.0	88.8±13.1
β-END (fmol/mL)	203±54.0	278±84.0	110±42.0	225±73.0	230±92.0
Cortisol (nmol/L)	364±34.0	367±38.0	332±47.0	421±81.0	421±81.0
IL-6 (pg/mL)	1.46±0.32	1.56±0.56	1.20±0.24	0.83±0.10	0.82±0.10
VAS (mm)	22.1±3.71	23.1±5.73	21.2±5.22	21.1±5.27	20.0±4.46
"Owls"					
$25(OH)D_3$ (nmol/L)	70.5±11.5	72.2±11.2	74.0±11.5	76.3±10.5	79.5±11.0
β-END (fmol/mL)	203±60.0	179±63.0	266±68.0	250±76.0	253±66.0
Cortisol (nmol/L)	413±34.0	507±59.0	522±43.0	464±38.0	467±48.0
IL-6 (pg/mL)	0.83±0.21	0.67±0.10	0.80±0.12	0.63±0.09	0.72±0.07
VAS (mm)	34.1±7.85	27.5±4.80	26.7±5.40	24.9±6.89	22.3±6.27

Absolute values are given as mean±SEM.