

## INVESTIGATIVE REPORT

## Skin Pigmentation Kinetics after Exposure to Ultraviolet A

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**Multiple exposures to ultraviolet radiation (UVR) are the norm in nature and phototherapy. However, studies of the kinetics of pigmentation following UVA exposure have included only fair-skinned persons. The aim of this study was to investigate steady-state pigmentation and fading in 12 Scandinavians and 12 Indians/Pakistanis after 6 and 12 exposures on the back using broadband UVA and UVA1 with equal sub-minimal melanogenic doses (individually predetermined). Pigmentation was measured by skin reflectance at 555 and 660 nm. The UV dose to minimal pigmentation was higher in dark-skinned persons after a single broadband UVA exposure, but independent of pigmentation/skin type after single and multiple UVA1 exposures. To elicit minimal melanogenic doses after 6 and 12 exposures, every dose is lowered by a factor of 2 and 3, respectively, but the cumulative dose increases three- and four-fold, respectively. The absolute increase in pigmentation was independent of pre-exposure pigmentation; therefore the percentage increase in pigmentation was higher in fair-skinned subjects. The absolute increase in pigmentation was higher and it took 2–3 days longer to reach steady-state after 12 UV exposures compared with 6 UV exposures. Days to steady-state pigmentation and fading were independent of pre-exposure pigmentation, and fading took 5–6 months. Comparing data from a narrowband UVB source and a Solar Simulator, it was shown that pigmentation built up faster and increased more after 12 UVA exposures (16 days) than with the Solar Simulator (21 days). Key words: pigmentation; ultraviolet radiation; UVA; multiple exposures.**

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Sun and solarium exposure with the purpose of tanning almost always consists of repeated exposures. However, few studies have investigated the pigmentation increase after multiple ultraviolet (UV) exposures, and these studies have been performed mainly on fair-skinned people (1–5). Pigmentation is generally considered to be attractive by fair-skinned people and tanning ability is therefore of great interest. Tanning in connection with UVA phototherapy may be considered undesirable by

dark-skinned patients, which also makes a study of UVA pigmentation relevant.

Fading is of interest because it represents the disappearance of natural photoprotection during winter-time, and in northern Europe pigmentation is expected to have reached a basic level by the time the level of sunshine increases in spring.

The aims of this study were: (i) to determine steady-state pigmentation and fading time after multiple UVA exposures in volunteers with a wide variation in constitutive pigmentation; (ii) to determine the doses required to reach minimal pigmentation after single and multiple exposures to both broadband UVA (bUVA) and UVA1 sources; and (iii) to determine whether the absolute increase in pigmentation, days to steady-state pigmentation and fading were dependent on wavelength (UV source), including two UVB sources (6).

## MATERIALS AND METHODS

*Subjects*

The study was approved by the local ethics committee (KF 11-057/00) and was carried out at Bispebjerg Hospital, Copenhagen, Denmark. UV exposures were given from the end of November to January. Three volunteers were UV-exposed in March and agreed not to expose their back to sunlight until their final measurement at the beginning of July. In order to include volunteers with a wide range of constitutive pigmentation, we selected both Scandinavian and Indian/Pakistani volunteers. A total of 24 healthy volunteers, 15 women and 9 men (mean age 25 years, age range 20–33 years) were enrolled in the study after giving informed consent. Skin diseases, sun-sensitizing medication, sunbathing or exposure to artificial tanning 3 months prior to the study and 4 months afterwards were exclusion criteria. The subjects' skin type distribution is shown in Table I (7). The pre-exposure skin pigmentation measured on the back ranged from 13% to 60% (8).

Table I. Minimal melanogenesis dose (MMD) measured in standard erythema doses (SEDs) for each skin type group after a single exposure to broadband ultraviolet A (bUVA) or UVA1 irradiation

Skin type	Total n (Scandinavians/ Indians-Pakistanis)	Pre-exposure pigmentation Mean pigmentation % (range)	SED to MMD	
			bUVA	UVA1
II	5 (5/0)	23.5 (15–30)	1.7	0.8
III	6 (5/1)	27.2 (16–38)	1.8	0.9
IV	4 (2/2)	30.7 (22–40)	2.2	0.9
V	9 (0/9)	47.0 (34–55)	2.4	0.9

### Study design

When erythema is considered, the dosing unit is normally minimal erythema dose (MED), expressed as number of standard erythema doses (SEDs) (9). As we were considering pigmentation, we chose to use minimal melanogenesis dose (MMD). The individual MMD was determined after a single UV exposure to six different UV doses with 25% increments. We expected the highest pigmentation to occur 7 days after exposure which was when the clinical reading of MMD was performed (10).

The right and left side of the back was subdivided into two areas. One area on each side was allocated to irradiation with bUVA (Cleo Performance; Philips, Rosendaal, Holland), the other to irradiation with UVA1 (TL10; Philips). This was done by randomization in order to avoid possible anatomical differences in UV sensitivity. The right side of the back was exposed on 2 consecutive days per week for 3 weeks and the left side was exposed on 4 consecutive days per week for 3 weeks; a total of 6 and 12 exposures, respectively (Table II). Each of the four areas was divided into four squares measuring 2 cm × 2 cm, and irradiated with 0.8, 0.6, 0.4 and 0.2 MMD, respectively. In a 4-week pilot study it took 3 weeks to reach steady-state pigmentation, thus this exposure period was chosen (11).

### Assessment of pigmentation

Pigmentation and erythema in each square were assessed both visually (clinically) and objectively prior to each exposure, and one week, 2 and 4 months after the final exposure (Table II). Skin pigmentation was evaluated clinically as “no pigmentation” (the same as in the surroundings) or as “perceptible pigmentation”, and was used only in determination of MMD. The skin pigmentation, measured by reflectance spectroscopy (UV-Optimize 555; Matic, Naerum, Denmark) (8), is directly related to the melanin content of the skin (12). The same measurement quantifies erythema (12, 13). The methodology is described in detail elsewhere (14). The measured pigmentation (unit: pigmentation%) was used in all calculations, such as the absolute and percentage increases in pigmentation and days to obtain steady-state. These measurements were also related to the measured pre-exposure pigmentation on the back and to Fitzpatrick skin type.

Pre-exposure pigmentation was defined as the lowest measured value on day 1 or 2. The maximum pigmentation was set as the highest value measured in every test spot.

Pigmentation after a single high UV dose was still present after 10–12 weeks (10); thus fading was evaluated 2 and 4 months after the final UV exposure. Time to total fading was determined as the day when the pigmentation value was calculated to be equal to pre-exposure pigmentation. Methods for evaluation of fading and criteria for exclusion of fading data from analysis have been described previously (6).

All measurements were related to light source, total number of UV exposures and ethnic origin.

### Dosimetry and UV radiation sources

The emission spectra and intensity of the radiation sources were measured before the start and regularly during the study using a spectroradiometer calibrated by the manufacturer traceable to the National Physical Laboratory in the UK (Sola Tell, Sola-Hazard, 4D Controls Ltd, Redruth, UK). The erythema potential of the UV sources was measured in SED, as proposed at the 12th International Congress on Photobiology in 1996 (9, 15) and accepted by the International Commission on Illumination (abbreviated CIE from its French title) (16). One SED has the physical dimension of 100 J/m<sup>2</sup> using the CIE action spectrum (normalized at 298 nm) (17).

Each UV source consisted of a bank of six fluorescent tubes. Broadband UVA (bUVA) emits 1.3% in the UVB range and 98.7% in the UVA range (1 SED corresponds to 8283 mJ/cm<sup>2</sup>). The UVA1 tubes emit 0.1% in the UVB range and 99.9% in the UVA range (1 SED corresponds to 26729 mJ/cm<sup>2</sup>).

During irradiation the distance from the skin to the tubes was 40 cm.

### Statistical analysis

The results were compared as follows: (i) days to pigmentation build-up and fading by linear regression analysis. Linear regression analysis and covariance analysis were performed to test whether the differences between the slopes and the differences between the intercepts were significant; (ii) dose in relation to percentage and absolute increase in pigmentation.

The following data were normally distributed and therefore compared using a paired *t*-test:

- Absolute increase in pigmentation after 6 exposures vs. 12 exposures.
- UV source comparison regarding absolute increase in pigmentation.

The following data were not normally distributed and were therefore compared using non-parametric tests:

- Days to reach total fading after 6 exposures vs. 12 exposures (Wilcoxon signed-rank test).
- UV source comparison regarding days to reach steady-state pigmentation (pigmentation build-up and fading, respectively) (Wilcoxon signed-rank test).
- Days to reach total fading for Scandinavian vs. Indian/Pakistani subjects (Mann-Whitney *U* test).
- To test if there was a constant ratio between UV-dose (SED) to minimal pigmentation after one exposure vs. multiple (6 and 12) exposures for all skin type groups (Kruskal-Wallis test).
- Skin type in relation to days to steady-state pigmentation (Kruskal-Wallis test).
- Skin type in relation to percentage increase in pigmentation (Kruskal-Wallis test).

Table II. Study design outline time of exposure and measurements

	Week 1							Week 2							Week 3							Week 4											
	Day							Day							Day							Day											
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7					
UV exposures (total of 12)	×	×	×	×				×	×	×	×					×	×	×	×	×	×												
UV exposures (total of 6)				×	×						×	×										×	×										
Pigmentation measurement <sup>a</sup>	×	×	×	×				×	×	×	×				×	×	×	×														×	

<sup>a</sup>Clinical and instrumental evaluation of the pigmentation.

<sup>b</sup>Fading was evaluated 2 and 4 months after the final UV exposure (the 6<sup>th</sup> and the 12<sup>th</sup> UV exposure, respectively).

Table III. Pigmentation increase and days to steady-state pigmentation in relation to exposure frequency, ultraviolet (UV)-dose and pre-exposure pigmentation using the combined source

No. of exposures	MMD	n <sup>a,c</sup>	Mean pre-exposure pigmentation <sup>d</sup>	Absolute pigmentation increase (percentage increase) <sup>d</sup>	Days to steady-state (n <sup>b,c</sup> )
6	0.8	41	32.4	7.7 (27.1)	15.0 (30)
	0.6	40	32.6	7.1 (25.6)	15.9 (29)
	0.4	34	33.7	6.8 (25.2)	17.3 (26)
12	0.8	46	34.2	10.5 (33.9)	16.4 (38)
	0.6	47	34.2	9.2 (29.7)	17.9 (36)
	0.4	44	34.9	7.4 (24.6)	19.2 (33)

MMD: minimal melanogenesis dose.

<sup>a</sup>Number of measurements that could be analysed for absolute and percentage increase in pigmentation.

<sup>b</sup>Number of measurements that could be analysed for days to steady-state pigmentation.

<sup>c</sup>Ideally it should have been 48 (24 volunteers, each irradiated by 2 UVA-sources).

<sup>d</sup>Measuring unit: pigmentation%.

For each skin type tested against another skin type *p*-values were afterwards corrected according to Bonferroni.

*p*-values less than 0.05 were considered to be significant.

## RESULTS

After a total of 6 exposures to the lowest dose, 0.2 MMD, none of the 24 volunteers developed tanning. After 6 exposures to 0.4 MMD 11 persons did not develop tanning and after 0.6 and 0.8 MMD no visual tanning appeared in five and four persons, respectively. We therefore could not analyse further the 0.2 MMD group. For the three other doses (0.4, 0.6 and 0.8 MMD) thus a total of 20 persons did no visually tan (distributed as 17 test squares from Scandinavians and 3 from Indians/Pakistanis without a tan). A further 9 measurements were not taken. Thus, a total of 29 measurements could not be analysed. Ideally 144 measurements should be analysed (3 doses  $\times$   $n=24 \times 2$  UV sources). Thus a total of 115 measurements could be analysed after a total of 6 exposures.

After a total of 12 exposures to 0.2 MMD, likewise none of the 24 volunteers developed tanning. After 12 exposures to the three higher doses 7 volunteers (all Scandinavians) did not develop pigmentation, this occurred mainly after 0.4 MMD. Thus, a total of 137 measurements could be analysed after 12 exposures.

Days to steady-state pigmentation were difficult to evaluate, as pigmentation build-up was not linear and predominantly followed an S-curve. A total of 107 measurements remained after 12 exposures and 85 measurements after 6 exposures (Table III).

### Absolute increase in pigmentation in relation to pre-exposure pigmentation and dose

After multiple UV exposures all the volunteers obtained the same absolute increase in pigmentation after the same MMD, respectively, 0.8, 0.6 and 0.4 MMD, independent of pre-exposure pigmentation (Fig. 1A).

This proves that the MMD at the pre-test (single UV exposure) was determined correctly.

There was a positive linear correlation between doses and absolute increase in pigmentation; the higher the dose the higher the absolute increase in pigmentation (Table III), although this was significant only after 12 exposures to both bUVA ( $p < 0.0001$ ) and UVA1 ( $p = 0.0199$ ). The absolute increase in pigmentation was higher after 12 exposures than after 6 exposures for 0.8 bUVA (0.8 of MMD using bUVA) (mean 10.8 vs. 7.8,  $n = 19$ ,  $p = 0.0005$ ), 0.6 bUVA (mean 8.8 vs. 7.2,  $n = 21$ ,  $p = 0.015$ ) and 0.8 UVA1 (mean 10.6 vs. 7.6,  $n = 22$ ,  $p = 0.003$ ), 0.6 UVA1 (mean 9.5 vs. 7.4,  $n = 18$ ,  $p = 0.017$ ).

### Percentage increase in pigmentation in relation to pre-exposure pigmentation and dose

There was a negative linear correlation between percentage increase in pigmentation and pre-exposure pigmentation, which means that the lower the pre-exposure pigmentation the higher the percentage increase in pigmentation (Table IV) (UVA1, 12 exposures, Fig. 1B).

When comparing linear regression lines for the different MMD doses only the intercepts were significantly different (bUVA,  $p = 0.013$ , Fig. 1B and UVA1,  $p = 0.038$ ), therefore the percentage increase in pigmentation after 12 exposures to both bUVA and UVA1 was dose-dependent.

For the combined UVA sources the percentage increase in pigmentation after 6 exposures also showed a trend to be dose-dependent (Table III).

### Absolute and percentage increase in pigmentation in relation to skin type

Generally, there was no correlation between absolute and percentage increase in pigmentation and skin type. However, a significant correlation was found in 5 out of 12 doses ( $p = 0.02-0.05$ ); 0.8 bUVA (6 exposures), 0.6 bUVA (6 and 12 exposures), 0.6 UVA1 (6 exposures)

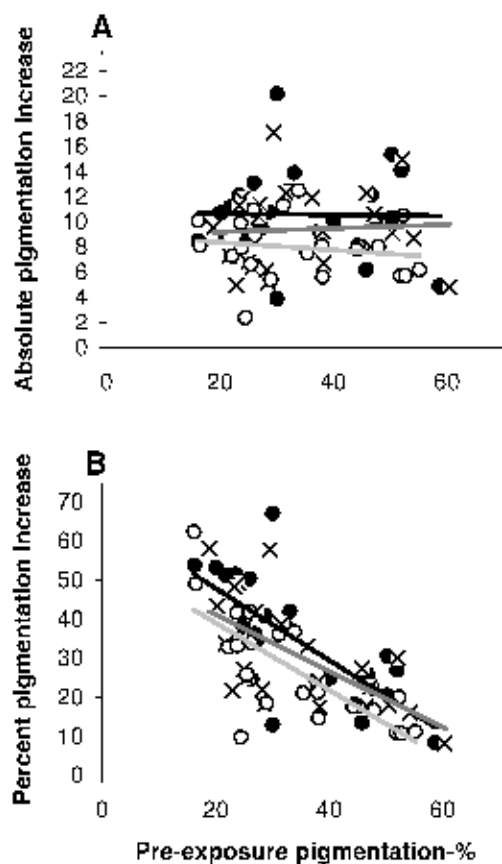


Fig. 1. (A) Absolute increase in pigmentation after 12 exposures to UVA1 associated with pre-exposure pigmentation on the back. It shows that the volunteers, despite different pre-exposure pigmentation, obtained the same absolute increase in pigmentation after 12 exposures to the same minimal melanogenesis dose (MMD) (0.8, 0.6 and 0.4 MMD) and that the absolute increase in pigmentation was higher the higher the MMD.

Equations for the linear regression lines for the three doses are as follows: ●, black line: 0.8 MMD:  $y = -0.005x + 10.763$ ,  $r^2 = 0.0003$  ( $p = 0.94$ ),  $n = 23$ ; ×, dark-grey line: 0.6 MMD:  $y = 0.015x + 8.8947$ ,  $r^2 = 0.003$  ( $p = 0.79$ ),  $n = 24$ ; ○, light-grey line: 0.4 MMD:  $y = -0.0308x + 8.9951$ ,  $r^2 = 0.03$  ( $p = 0.47$ ),  $n = 22$ .

(B) Percentage increase in pigmentation after 12 exposures to UVA1 associated with pre-exposure pigmentation on the back. It shows that there was a negative linear correlation between percentage increase in pigmentation and pre-exposure pigmentation. It also shows that the percentage increase in pigmentation was dose-dependent.

Equations for the linear regression lines for the three doses are as follows: ●, black line: 0.8 MMD:  $y = -0.9181x + 66.317$ ,  $r^2 = 0.52$  ( $p = 0.0001$ ),  $n = 23$ ; ×, dark-grey line: 0.6 MMD:  $y = -0.7213x + 55.661$ ,  $r^2 = 0.42$  ( $p = 0.0007$ ),  $n = 24$ ; ○, light-grey line: 0.4 MMD:  $y = -0.08625x + 56.357$ ,  $r^2 = 0.57$  ( $p < 0.0001$ ),  $n = 22$ .

and 0.4 UVA1 (6 exposures). Among these 5 exceptions multiple comparisons between individual skin types gave a significant difference in only 3 cases: a higher percentage increase in pigmentation for skin type III or IV vs. skin type V.

#### Minimal melanogenesis dose in relation to skin type and number of UV exposures

The average MMD after a single exposure to bUVA and UVA1 are shown for each skin type group in Table I. These results show that MMD after bUVA was slightly higher in darker skin types (not significant). However, the linear correlation between pre-exposure pigmentation and MMD was significant ( $p = 0.003$ ). After 6 and 12 exposures to bUVA the MMD was independent of pre-exposure pigmentation, but was significantly different comparing skin type IV to skin type II, III and V (Table V).

For UVA1 the MMD was similar for all skin types after 1, 6 and 12 exposures, respectively (Table V).

Table V furthermore shows that the dose fractions to elicit MMD decrease with number of exposures. There was a constant reduction factor of 2 (bUVA 2.29, UVA1 2.31) between MMD after one exposure and 6 exposures independent of skin type ( $p = 0.37$ ) and UV source ( $p = 0.89$ ). The reduction factor was 3 (bUVA 3.24, UVA1 3.20) between one exposure and 12 exposures independent of skin type ( $p = 0.30$ ) for UVA1, but for bUVA skin type IV was significantly higher than II, III, V ( $p < 0.005$ ). Combining data from bUVA and UVA1 there was no significant correlation with skin type ( $p = 0.91$ ).

There was a three-fold increase in the cumulative dose to MMD (bUVA 2.74, UVA1 2.79) when giving 6 exposures compared with one exposure and a four-fold increase (bUVA 4.10, UVA1 4.26) when giving 12 exposures compared with one exposure. This means that the cumulative dose to MMD is higher the more UV exposures are given, even though the individual exposure doses are lower.

#### Days to steady-state pigmentation

The number of days to reach steady-state pigmentation had no significant correlation to the pre-exposure pigmentation or skin type, except after 6 exposures to

Table IV. Association between percentage increase in pigmentation and pre-exposure pigmentation calculated by linear regression for each minimal melanogenesis (MMD) dose and each of the ultraviolet (UV) sources and 6 and 12 exposures, respectively. Given are  $p$ -values and  $r^2$  values of the individual regression lines. Equation lines for UVA1, 12 exposures, see Fig. 1B

MMD	bUVA			UVA1								
	6 exposures	12 exposures		6 exposures	12 exposures							
	$n$	$r^2$	$p$	$n$	$r^2$	$p$	$n$	$r^2$	$p$	$n$	$r^2$	$p$
0.8	19	0.56	0.0002	23	0.33	0.004	22	0.31	0.007	23	0.52	0.0001
0.6	22	0.45	0.0008	23	0.35	0.003	18	0.64	<0.0001	24	0.42	0.0007
0.4	19	0.53	0.0005	22	0.52	0.0002	15	0.58	0.001	22	0.57	<0.0001

Table V. Individual minimal melanogenesis dose 7 days after last exposure in skin type II–V for a total of 1, 6 and 12 exposures (cumulative dose)

	<i>n</i>	Number of UV exposures					
		bUVA			UVA1		
		1	6	12	1	6	12
II	5	1.7 (1.7)	0.74 (4.4)	0.68 (8.2)	0.7 (0.7)	0.33 (2.0)	0.31 (3.7)
III	6	1.8 (1.8)	0.93 (5.6)	0.72 (8.6)	0.9 (0.9)	0.43 (2.6)	0.32 (3.8)
IV	4	2.2 (2.2)	0.85 (5.1)	0.43 (5.2)	0.9 (0.9)	0.38 (2.3)	0.25 (3.0)
V	9	2.4 (2.4)	1.02 (6.1)	0.73 (8.8)	0.9 (0.9)	0.38 (2.3)	0.28 (3.0)
All	24	2.1 (2.1)	0.91 (5.5)	0.67 (8.0)	0.84 (0.84)	0.38 (2.3)	0.29 (3.5)
% of 1 exposure	24	100 (100)	46 (262)	34 (410)	100 (100)	47 (279)	36 (426)

UV: ultraviolet; bUVA: broadband UVA.

0.8 bUVA ( $p=0.018$ ). Multiple comparisons between individual skin types gave no significant difference for days to steady-state pigmentation.

Overall, 2.0 more days were needed to reach steady-state pigmentation after 12 than 6 exposures (Table III), although this was significant only for 0.4 bUVA with 3.4 days (mean 19.7 vs. 16.3,  $n=11$ ,  $p=0.024$ ), 0.8 UVA1 2.3 days (mean 16.5 vs. 14.2,  $n=11$ ,  $p=0.0098$ ) and 2.2 days after 0.6 UVA1 (mean 17.6 vs. 15.4,  $n=11$ ,  $p=0.042$ ).

### Fading

No significant difference in days to total fading of pigmentation was found between Scandinavian (median 165 days) and Indian/Pakistani subjects (median 123 days), except for 12 exposures to 0.6 bUVA, which faded more rapidly in Scandinavians (median 106 days) than in Indians/Pakistanis (198 days) ( $n=15$ ,  $p=0.019$ ). Likewise, after 12 exposures to 0.8 UVA1 tanning faded faster in Scandinavians (median 131 days) than in Indians/Pakistanis (197 days) ( $n=12$ ,  $p=0.048$ ).

There was no significant difference in days to reach total fading after 6 and 12 exposures (Table VI). Fading took a mean of 173 days after 6 exposures and 185 days after 12 exposures.

### UV-source comparison: UVB vs. UVA

Our UVA-sources, bUVA and UVA1, were compared with previously published data on a narrowband UVB

Table VI. Time to total fading in relation to number of exposures and dose

Number of UV exposures	MMD	Days to total fading	
		bUVA Median (range) [ <i>n</i> ]	UVA1 Median (range) [ <i>n</i> ]
6	0.8	149 (76–339) [12]	182 (94–319) [9]
	0.6	136 (76–239) [9]	153 (81–234) [8]
	0.4	138 (76–249) [6]	150 (82–432) [7]
12	0.8	161 (110–258) [12]	162 (86–228) [12]
	0.6	172 (76–602) [15]	113 (94–675) [12]
	0.4	118 (63–751) [11]	167 (91–307) [8]

UV: ultraviolet; MMD: minimal melanogenesis dose; bUVA: broadband UVA.

source (nUVB) (Philips TL01) and a Solar Simulator (SS) (Solar Light Co., Philadelphia, PA, USA) (6). Pigmentation built up faster after 12 exposures to 0.8 bUVA and UVA1 (mean 16 days) compared with 0.8 SS (21 days) ( $p<0.05$ ).

After 6 exposures the absolute increase in pigmentation was the same for all UV sources. After 12 exposures the pigmentation was lower for 0.8 SS compared with 0.8 nUVB, 0.8 bUVA and 0.8 UVA1 ( $p<0.05$ ). We found no significant difference in days to total fading between the UV sources.

### DISCUSSION

UVA1 phototherapy is used for treatment of conditions such as atopic dermatitis, scleroderma and graft-versus-host disease. The treatment is connected with often undesired pigmentation. However, for UVA1 there was no correlation between tanning ability (absolute increase in pigmentation) and dose to minimal pigmentation (MMD). This means that the pigmentation achieved is independent of skin type, and thus is the same for all, independent of the pre-exposure pigmentation and only dependent on the UV dose in SED. The (absolute) increase in pigmentation during a clinical treatment series is thus independent of the patient's pre-exposure pigmentation for both UVA1 and bUVA using MMD as a dosing unit. By using equal sub-doses of individually predetermined MMD instead of the usual equal MED, different parameters related to pigmentation may be determined more precisely.

Only a few studies have investigated the pigmentation increase after multiple exposures, and these have been mainly in fair-skinned persons (1–5). It is debatable whether these studies were able to determine a person's tanning ability, as steady-state pigmentation was not reached due to the use of increasing UV doses (3) or too few exposures (1, 2, 4, 5).

Kollias et al. (18) found that the MMD after a single exposure at 365 nm was the same in skin types I, II and V. We confirm this and find it true also after multiple UVA1 exposures.

We observed that UVA was more melanogenic than erythemogenic in skin types II–V for both single and multiple UV exposures; as evidenced by  $MMD < MED$ , and we thereby confirm and extend what Parrish et al. (19) observed in skin types II and III. Since there was a wider range of skin types represented in our study (skin types II–V) and a wider variation in constitutive pigmentation, the earlier results by Parrish et al. can now be generalized to skin types II–V. In contrast to other studies (2, 19), we used equal sub-melanogenic doses (sub-MMD), which means that our doses also were sub-erythemogenic.

Our reflectance instrument, the UV-Optimize, measures pigment specifically, thus independently of erythema (as if redness was zero) (14). However, if the skin optics change due to oedema it will lead to some uncertainty in the measurements, but this concern goes for all reflectance-measuring systems and not only for the UV-Optimize.

The melanogenic effect of UV sources is highly dependent on wavelength (2, 19). A comparison between four examined UV sources has shown that SS was the least melanogenic, then nUVB, bUVA and, finally, UVA1 was the most melanogenic (11, 20). The absolute increase in pigmentation was significantly lower for SS compared with nUVB, bUVA and UVA1 after 12 exposures to 0.8 MMD.

Pigmentation was built up faster with UVA sources than with SS, but the differences were significant only after 12 exposures to the maximum dose of 0.8 MMD. However, a similar trend was seen for the lower doses and after 6 exposures.

In general, the absolute increase in pigmentation was higher after 12 than after 6 exposures. However, this was not significant for 0.4 MMD, which might reflect the higher number of exclusions of mainly Scandinavians due to lack of pigmentation after this dose. This exclusion of fair-skinned persons could induce a bias towards higher initial pigmentation in the test areas of the lowest dose 0.4 MMD, as is also seen in Table III. However, differences are small when comparing the average pre-exposure pigmentation. In addition, concerning 6 exposures, the pre-exposure pigmentation was generally higher in the test areas of 0.8 and 0.6 MMD compared with the pre-exposure pigmentation in the test areas of 0.4 MMD.

Fading is of interest because it represents the disappearance of natural photoprotection during wintertime. The overall fading time was 5–6 months and should thus be expected already to have reached a basic level in northern Europe by the time the level of sunshine increases in the spring. In the course of fading the number of data that could be analysed was generally the same for Scandinavian and Indian/Pakistani volunteers, we therefore consider our results to be unbiased. Fading was investigated by measuring at only three time-points;

1 week, 2 and 4 months after the final UV exposure, although this is obviously a very rough scale.

In conclusion, due to the use of equal MMD exposures, tanning ability, expressed as absolute pigment increase, was independent of skin type and pre-exposure pigmentation and linear with sub-MMDs, the higher the dose the higher the absolute increase in pigmentation. SED to minimal pigmentation was higher in dark-skinned persons after a single exposure to bUVA, but independent of pigmentation/skin type after single and multiple exposures to UVA1. MMD determined at a single UV exposure worked well when used at repetitive UV exposures in volunteers with a wide variation in constitutive pigmentation.

We have shown for UVA, as for UVB (6), that going from a single exposure to 6 and 12 exposures spread over 3 weeks, the required dose fraction to minimal pigmentation was lowered by a factor of 2 and 3, respectively. This was independent of pre-exposure pigmentation. Thus the risk of sunburn is lowered, but the cumulative dose increases three- and four-fold, respectively. Fading to pre-exposure pigmentation level took 5–6 months.

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