

Dynamics of pigmentation induction by repeated ultraviolet exposures: dose, dose interval and ultraviolet spectrum dependence

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Summary

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Conflicts of interest

None declared.

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Background The dynamics of ultraviolet (UV)-induced melanogenesis have been well characterized for single UV exposures. However, our knowledge of the effects of repeated UV exposures on the development of new pigmentation is limited.

Objectives To characterize the dynamics and dose dependence of pigmentation induction by repeated UV exposures using two different UV sources.

Methods A total of 40 healthy subjects participated in the study: 21 were exposed to a 5% UVB/95% UVA source and 19 were exposed to a 2% UVB/98% UVA source. Skin phototypes 2–3 were represented. Subjects were exposed one to three times per week. The minimal erythemal dose and minimal melanogenic dose of all subjects were determined, and both visual and instrumental observations of the development of pigmentation and erythema were recorded.

Results Dark-brown pigmentation could be produced by a cumulative UV dose of 4200 J m⁻² given as 10 exposures over 5 weeks. However, comparable pigmentation could also be induced by a cumulative dose of 2900 J m⁻² given as eight exposures over 4 weeks. The lowest cumulative dose of 1900 J m⁻² given over 4 weeks produced moderate pigmentation. The 2% UVB source led to earlier and darker pigmentation than the 5% UVB source did for equally erythemogenic doses.

Conclusions These observations show that the dynamics of melanogenesis induced by repeated exposures depends on UV dose, dose interval and emission spectrum. They also indicate that increasing the UV dose above a certain level of cumulative exposure does not significantly increase the level of UV-induced pigmentation.

Ultraviolet (UV) radiation induces melanogenesis in human skin. However, there have been few quantitative studies of melanogenesis induced by repetitive UV exposures (as in real life). In this area, our knowledge derives primarily from the classic studies led by Parrish, Pathak and Kaidbey.^{1–4} They showed that daily suberythemogenic doses enhance pigmentation and subsequently lower the erythema threshold. Exposures given at 48-h intervals produced less erythemogenic reactions.⁴ Recent studies have shown that repeated, suberythemal exposures to UVA-rich sources are more effective in producing a tan than are those from UVB-rich sources (including solar simulators).^{1,5–7}

de Winter et al.⁸ showed that exposures given three times weekly for 3 weeks (using increasing doses) resulted in

increased pigmentation as measured by L* (measure of skin 'lightness'). However, they suggest that exposures be given less frequently than three times weekly to reduce DNA damage accumulation. Ruegeimer et al.⁹ observed an 'obvious increase in pigmentation' in 99 human subjects who used a commercial sunbed twice weekly for 6 weeks. Instrumentally, the L* values changed by a modest 2.6 chromametric units (CU) or less. Cumulative doses were between 11 300 and 14 600 J m⁻² but unfortunately they were not wavelength weighted with the erythema action spectrum and, thus, cannot be easily compared with data from other studies, including this one.

Caswell¹⁰ evaluated effects of exposures repeated according to the U.S. Food and Drug Administration (FDA) guidelines for

sunlamp manufacturers.¹¹ After 2 weeks of thrice weekly suberythemal exposures, new pigmentation became apparent. After 7 weeks of increasing exposures (up to 550 J m^{-2} – erythema effective – i.e. wavelength-weighted with the erythema action spectrum), a dark pigmentation with no visible erythema was produced. Cumulative doses were $\approx 9300 \text{ J m}^{-2}$.

Our pilot study¹² indicated that pigmentation can be induced by repeated exposures with cumulative doses much lower than those used in prior studies and commercial practice. To learn more about melanogenesis induced by repetitive UV exposures, we explored the effects of dose, dose interval, and UV source emission spectrum in a larger cohort of human subjects, the results of which are presented in this paper.

Materials and methods

Subjects

The protocol no. 01-026R was approved by the FDA Research Involving Human Subjects Committee. Forty healthy human subjects were recruited in the Washington, D.C. metropolitan area, gave written consent, and were examined by a dermatologist.

Procedures

At each visit, the subjects underwent the following procedures: (i) photography of the study area; (ii) visual assessment; (iii) instrumental skin colour measurements; and (iv) UV exposure of the study sites. Three $3 \times 3 \text{ cm}$ sites were irradiated while a fourth site served as an unexposed control. Subjects were exposed in a prone position under a canopy equipped with fluorescent UV lamps (see below) using a custom-made template with $3 \times 3 \text{ cm}$ openings. The rest of the body was covered. The instrumental measurements of skin colour were taken at all four sites on the first visit, prior to UV exposure. These values were subtracted from all subsequent measurements in each respective area to calculate changes in colour parameters. Biopsies from all four sites were taken 24 h after the final exposure. The results of the biopsy analyses are presented, in part, in Yamaguchi *et al.*¹³

Photography

A digital camera: single-lens reflex, Nikon D1, was used with a 28–105 mm lens (Nikon Corporation, Tokyo, Japan) at 2.7 million pixels (for details, see Miller *et al.*¹²) and a Canon Rebel 2000 35-mm camera with a 28–80 zoom lens was used with Kodak Royal ASA 200 film (see Coelho *et al.*¹⁴). Illumination for both cameras was provided by the Speedotron system (see Tadokoro *et al.*¹⁵).

Ultraviolet radiation sources

The UV exposure canopies (SunQuest Model SQ 2000S; ETS, Indianapolis, IN, U.S.A.) were equipped with 12 100-W fluor-

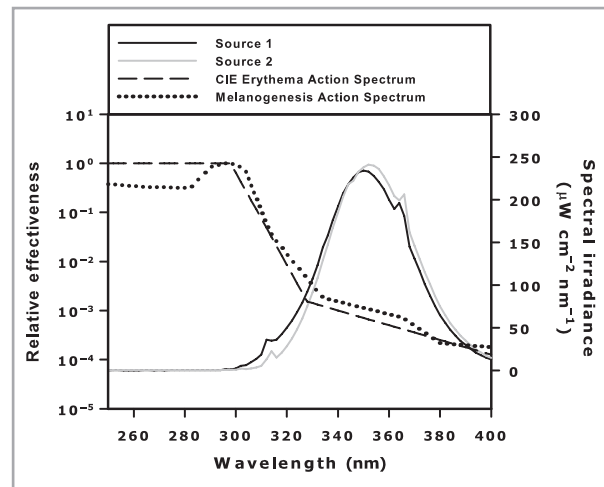


Fig 1. Emission spectra of the ultraviolet sources used in this study (canopies fitted with 12 sunlamps). Black line, source 1; grey line, source 2. For comparisons, action spectra are included: dashed line, CIE Erythema Action Spectrum;¹⁶ dotted line, melanogenesis action spectrum.² The ordinate on the right indicates spectral irradiance. The ordinate on the left indicates relative effectiveness at each wavelength for inducing erythema or melanogenesis.

escent UV lamps that are commonly used for tanning. Source 1 was equipped with Beach Sun sunlamps (Light Sources, Orange, CT, U.S.A.) and source 2 with Cosmolux VLR.T sunlamps (Cosmedico Light, Weymouth, MA, U.S.A.). The emission spectra of these lamps (Fig. 1) were measured using a double-grating spectroradiometer (Model 754; Optronic Laboratories, Orlando, FL, U.S.A.) calibrated as described in Yamaguchi *et al.*¹³

For the evaluation of the minimal erythema dose (MED), we used an array of eight Kodacel-filtered (Eastman Chemical Products, Kingsport, TN, U.S.A.) FS lamps (FSX24T12/UVB/HO; National Biological Corporation, Twinsburg, OH, U.S.A.). A low-profile detector (SSD 001A; International Light, Newburyport, MA, U.S.A.) coupled to a radiometer (IL1700; International Light) was used prior to each exposure to measure the intensity in each spot on the subjects' back and to calculate the required exposure time. This detector had previously been calibrated using the measurements made with the spectroradiometer.

Minimal erythema dose and minimal melanogenic dose determination

UV exposures were administered on one side of the back to determine each subject's MED. Eight $2 \times 2 \text{ cm}$ sites were exposed to arithmetically increasing UV doses from the FS lamps.¹² Using the CIE reference action spectrum for erythema,¹⁶ the doses were converted into erythema-effective J m^{-2} by wavelength-weighting the source emission spectrum with the erythema action spectrum, integrating the area under the resultant curve and multiplying by the exposure time. Unless otherwise specified, all UV doses reported in this paper are erythema effective.

We graded erythema on a scale from 0 for 'no reaction' to 5 for 'violaceous red'. We defined the MED as a grade of 2 (pink erythema with at least one border) 24 h after exposure. The minimal melanogenic dose (MMD) was determined using the same sites. MMD was defined as the lowest dose that produced a light brown pigmentation (grade 2, see below) 8 days after exposure.

Exposure protocols

Figure 2 shows the three UV exposure protocols. Each protocol used an initial dose of 100 J m⁻². In protocol A, doses increased by increments of 25% up to 380 J m⁻², with exposures ceasing on day 23 at a cumulative dose of 1900 J m⁻²; in protocol B, doses increased by increments of 40% up to 600 J m⁻², with exposures ceasing on day 23 at a cumulative dose of 2900 J m⁻²; and in protocol C, doses increased by increments of 50% up to 600 J m⁻², with exposures ceasing on day 30 at a cumulative dose of 4200 J m⁻². These protocols were used for 21 subjects treated with source 1 and for 19 subjects treated with source 2.

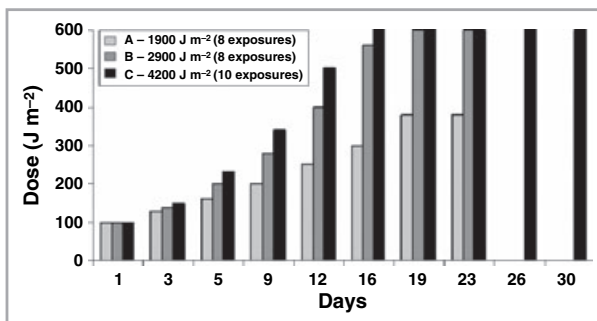


Fig 2. Ultraviolet doses and timing in the three experimental protocols.

Visual evaluation of changes in skin pigmentation

At each visit, the skin pigmentation was graded prior to that day's UV exposure using the scale: 0, no reaction; 0.5, observer indecisive; 1, minimal perceptible pigmentation, faint or no borders; 2, light brown; 3, moderately brown; 4, dark brown.

Erythema and Melanin Indices

The Erythema and Melanin Indices (EI, MI) were measured with the DiaStron Erythema/Melanin Index meter (DiaStron, Andover, U.K.). This instrument measures the reflectance at 546, 632 and 905 nm (full width half maximum < 9 nm at each wavelength). The EI is defined as: EI = log₁₀(R_{632 nm}/R_{546 nm}) × 1000, and the MI as: MI = log₁₀(R_{905 nm}/R_{632 nm}) × 1000, where R_λ is the reflectance at the specified wavelength.

Measurements were taken in triplicate and means obtained. ΔEI and ΔMI were calculated as the difference between the mean values for exposed areas on a given day and the mean values for the same areas prior to exposure.

Diffuse reflectance spectrometry

We used the CM-2002 spectrophotometer (Minolta Corp., Ramsey, NJ, U.S.A.), which measures the diffuse reflectance from 400 to 700 nm at 10-nm increments using an integrating sphere with an 8-mm aperture and a target mask that minimizes pressure in the measured area. Measurements were taken in triplicate and the mean calculated.

The Minolta CM-2002 uses the spectral reflectance data to calculate the L*a*b* values of the CIE system of colour quantification¹⁷ as described in Coelho *et al.*¹⁴ Changes in the L* value and a* value are reported in this paper. Also, the vector quantity ΔE (combination of three parameters) was used by

Table 1 Basic parameters of ultraviolet (UV) sources used in this study compared with those of a standard solar spectrum, or 'Reference Sun' (Solar Spectral Irradiance, 1 Atmosphere Global²²)

	% UVB	% UVA	UVB (W m ⁻²)	UVA (W m ⁻²)	Erythemogenic output (W m ⁻² , effective)	Melanogenic output (W m ⁻² , effective)	Time of first exposure (s)	UVA dose per first exposure (J m ⁻²)	Melanogenic dose per first exposure (J m ⁻²)
Source 1	5	95	5.1	100	0.43	0.69	234	23 400	161
Source 2	2	98	2.3	102	0.2	0.3	486	49 572	146
Reference Sun	6	94	4.2	65	0.32	0.54	312	20 280	168

UVB and UVA irradiances were measured as described in Materials and methods and integrated over 290–320 and 320–400 nm ranges, respectively, and are expressed in W m⁻². To obtain erythemogenic and melanogenic outputs, the spectral irradiances (290–400 nm) were wavelength weighted using the International Commission on Illumination (CIE) Reference Action Spectrum for Erythema¹⁶ (Fig. 1), and the Parrish melanogenesis² (Fig. 1) action spectrum, respectively. The 'Time of first exposure' represents the time needed to deliver the initial dose. This dose equals 100 J m⁻², expressed in erythema-effective J m⁻². To obtain 'UVA dose per first exposure', the UVA output in W m⁻² was multiplied by the time required to reach 100 J m⁻². To obtain 'Melanogenic dose per first exposure', the melanogenic output in W m⁻², effective was multiplied by the time required to reach 100 J m⁻².

us to compare the results of this study with the values reported by Caswell,¹⁰ where:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}.$$

Measurements taken prior to UV exposure were used to calculate ΔL^* , Δa^* and Δb^* .

Individual typology angle based on the $L^*a^*b^*$ system

The Individual typology angle (ITA°) is a vector representation in the plane of the L^* vs. b^* values.¹⁸ ITA° is expressed

in degrees and is defined as: $ITA^\circ = [\arctan(L^* - 50/b^*)] \times 180/\pi$. The ITA° is considered an objective parameter to quantify skin colour and has been used in several studies^{19–21} to measure pigmentation development. In this study ITA° values are reported as the change in ITA° or ΔITA° .

Statistical evaluation

SAS PROC MIXED (SAS/STAT Users Guide, version 9.1, 2002: SAS Institute Inc., Cary, NC, U.S.A.), a general linear mixed model, was used to model the dependent measures (ΔMI , ΔE , ΔL^* and ΔITA°) at day 24 for protocols A and B and at day

Table 2 Study subjects and their basic characteristics

Subject	Age (years)	Sex	Phototype	MED ($J\ m^{-2}$, erythema-effective)	MMD ($J\ m^{-2}$, erythema-effective)
Source 1 n = 21					
T7	32	F	2	385	435
T8	40	F	2	205	330
T9	33	M	2.5	185	415
T10	28	F	2.5	330	> 430
T11	23	F	2.5	345	> 520
T12	24	M	2.5	285	> 420
T13	50	F	3.5	355	> 540
T14	40	M	3	170	285
T16	45	M	2.5	335	390
T17	38	F	2.5	330	465
T18	36	F	2.5	290	> 435
T19	34	M	2	195	320
T20	30	F	3	185	370
T21	38	F	2.5	320	> 480
T22	24	M	2.5	330	> 490
T24	22	F	3	615	> 760
T25	29	M	2.5	200	300
T26	26	F	2.5	295	> 445
T27	41	F	3.5	220	435
T28	31	M	2.5	210	360
T30	27	F	2	225	350
Source 2 n = 19					
T31	41	M	2.5	250	315
T32	26	M	2	300	450
T33	28	F	3	225	375
T35	26	F	3	450	> 675
T36	32	M	2	190	315
T37	57	M	3	300	450
T38	39	M	2.5	250	375
T40	34	F	2.5	315	440
T41	62	F	3	450	675
T42	24	F	2	300	400
T43	35	M	2.5	300	375
T44	65	F	2.5	375	525
T45	25	F	2.5	225	375
T47	47	M	3	200	400
T48	47	M	2.5	375	450
T49	45	F	2.5	250	375
T50	35	F	3.5	300	300
T51	29	F	3.5	300	375
T52	33	F	3.5	225	450

MED, minimal erythema dose; MMD, minimal melanogenic dose. Cases where the MMD was not found at the maximum dose given are indicated by '>'.

31 for protocol C, by source 1 and 2, as linear functions of protocols A, B and C.

Results

Evaluation of ultraviolet sources

We used two different UV sources (see Materials and methods). Their emission spectra are shown in Figure 1. Source 1 emitted 5% UVB/95% UVA, while source 2 emitted 2% UVB/98% UVA. Table 1 shows that although the proportion of UVB in both UV sources was lower than that of the Reference Sun,²² source 1 was more powerful and source 2 was less powerful than the Reference Sun in terms of both erythemogenic and melanogenic output. The UVA component of the dose per first exposure (100 J m^{-2}) was > 2 times higher for source 2 than for source 1, although the melanogenic-effective dose was similar for both sources and the Reference Sun. These differences in emission spectra significantly affected the efficacies of these lamps in producing pigmentation.

Study subjects

The age, sex, skin phototype, MED and MMD for all study subjects are listed in Table 2. The phototype of study subjects was

determined according to Fitzpatrick.²³ When subjects did not exactly fit the criteria of phototype 2 or 3, intermediate values, 2.5 and 3.5, were used. The mean \pm SD age of the 40 subjects was 35.5 ± 6.7 years. They were divided into two groups (1 and 2), and each group was treated with a different source (see above). Group 1 comprised 13 women and eight men, and group 2 comprised 11 women and eight men. The MEDs of the subjects ranged from 170 to 615 J m^{-2} and did not correlate well with skin phototype (Table 2). The MMD ranged from 285 to $> 760 \text{ J m}^{-2}$ and, in all but one case (T50, phototype 3.5) the MMD was higher than the MED.

Pigmentation development: photographic documentation and visual observations

The photographic series in Figure 3 shows an example of the gradual development of pigmentation. Overall, protocol A produced a light to moderate pigmentation while protocols B and C led to a dark pigmentation by day 24. Importantly, from that day onwards there was little difference between the effects of protocols B and C, indicating saturation of visible pigmentation with protocol B. According to protocol C, additional exposures were given on days 26 and 30. However, these exposures produced little further darkening of the skin as seen by eye. The observations were continued after the final



Fig 3. An example of pigmentation development (subject T13). The $3 \times 3 \text{ cm}$ areas exposed according to protocols A, B and C, and of the unexposed control X are marked on the image taken before exposures (day 1). The last exposures in protocols A and B occurred on day 23 and in protocol C on day 30.

exposures ceased for an additional 3 weeks with protocols A and B, and an additional 2 weeks with protocol C. During that time, the pigmentation levels in all study areas barely decreased by visual assessment.

Pigmentation development: quantification

To monitor pigmentation development, we used five approaches (see Fig. 4) as described in Materials and methods:

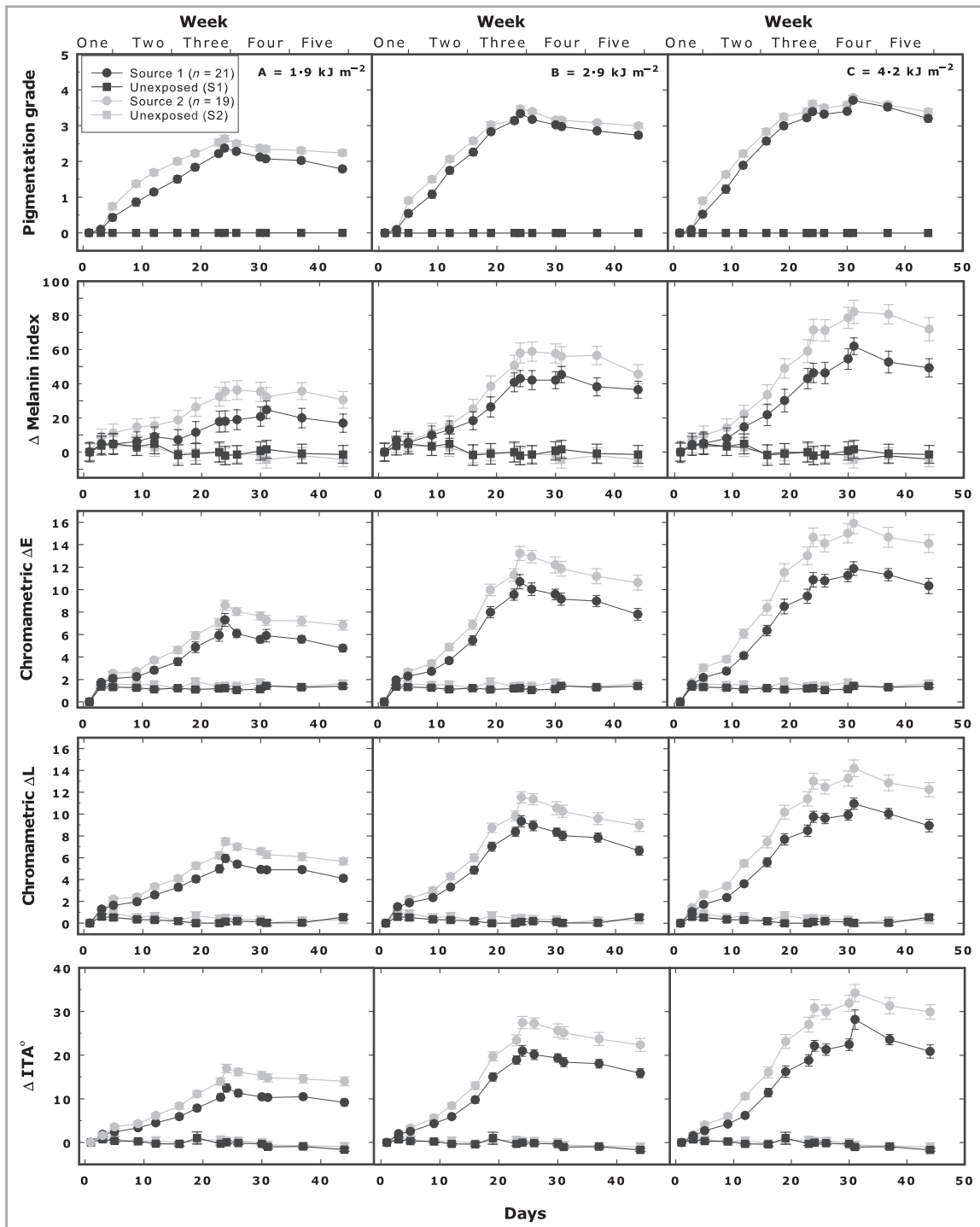


Fig 4. Skin colour changes during and after repeated ultraviolet exposures according to protocols A (left column), B (centre column) and C (right column). Data points and lines are in black and grey for sources 1 and 2 (S1 and S2), respectively. First row: results of visual grading (see Materials and methods; mean values; SEM bars are smaller than the data points). Second row: Melanin Index measured with the DiaStron Erythema/Melanin Index Meter. Third to fifth rows: ΔE , ΔL and ΔITA° , derived from the spectrophotometric measurements. Second to fifth rows: mean values \pm SEM. The last exposures were given on day 23 in protocols A and B, and on day 30 in protocol C. ITA° , individual typology angle.

(i) visual semiquantitative assessment of pigmentation using a grading scale; (ii) reflectance spectrometry at three wavelengths to obtain MI,²⁴ and diffuse reflectance spectrometry across the visible range with the capacity to obtain; (iii) ΔL^* ; (iv) ΔE ; and (v) ΔITA° parameters using the CIE $L^*a^*b^*$ colour system.^{18,25}

For protocol A, (Fig. 4, upper, left-hand box), the maximum mean pigmentation grade did not reach a value of 3 (moderately brown) at any time throughout the experiment, for either UV source. Using protocol B, a grade 3 pigmentation was achieved by day 23 for both UV sources, and using protocol C, this pigmentation level was achieved by day 19 for both UV sources. Using protocol C, some, but not all, subjects reached a pigmentation grade of 4 (dark brown) for both UV sources. Protocols B and C produced very similar final results for both sources: the least squares means for protocols B and C were 3.3 and 3.7 for source 1, and 3.5 and 3.8 for source 2, respectively.

Instrumental measurements showed that the maximum pigmentation was achieved on day 24 for protocols A and B, and day 31 for protocol C. At days 24 and 31, the least square means for protocols A, B and C, for ΔMI , ΔE , ΔL^* and ΔITA° and both sources, were statistically significantly different ($P < 0.01$). The one exception was for protocols B and C, with dependent variables ΔE and ΔL^* and source 1, which were not statistically significantly different ($P > 0.2$). Source 2 showed higher and more rapid melanogenic effectiveness than source 1 ($P < 0.005$).

We used a statistical software package (SAS PROC MIXED) to model the following parameters: ΔMI , ΔITA° and chroma-

metric ΔE and ΔL^* . Separately, the potential independent variables sex and phototype were not statistically significantly different from zero ($P > 0.06$). However, we found that all of the dependent variables (ΔMI , ΔE , ΔL^* and ΔE) depended on MED ($P < 0.04$).

Erythematous component of the pigmentation

No significant erythema was visible during the development of pigmentation for most subjects. However, instrumental measurements detected increases in the a^* parameter of the CIE $L^*a^*b^*$ system and in the EI measured using the DiaStron Meter. Figure 5 shows that for source 1, the maximum mean Δa^* reached a value of ≈ 3 for protocol A and ≈ 4.3 for protocols B and C. With source 2, these values were slightly higher: 3.5, 5.3 and 5.6 for protocols A, B and C, respectively. These maximum values were recorded on days 24 and 31, which were the only two instances when measurements were made 24 h after exposure. The changes in EI were similar to the changes in the a^* parameter.

Discussion

Different approaches were used in the few prior studies of the melanogenic efficacy of repeated UV exposures. Ruegemer *et al.*⁹ found that doses of 1.13 or 1.46 J m⁻², twice weekly for 6 weeks, were insufficient to induce significant increases in pigmentation. Bech-Thomsen *et al.*⁵ used two 'UVB' and four 'UVA' sources to administer 10 doses of 234 J m⁻² (erythema-effective) over 4 weeks, cumulatively 2340 J m⁻².

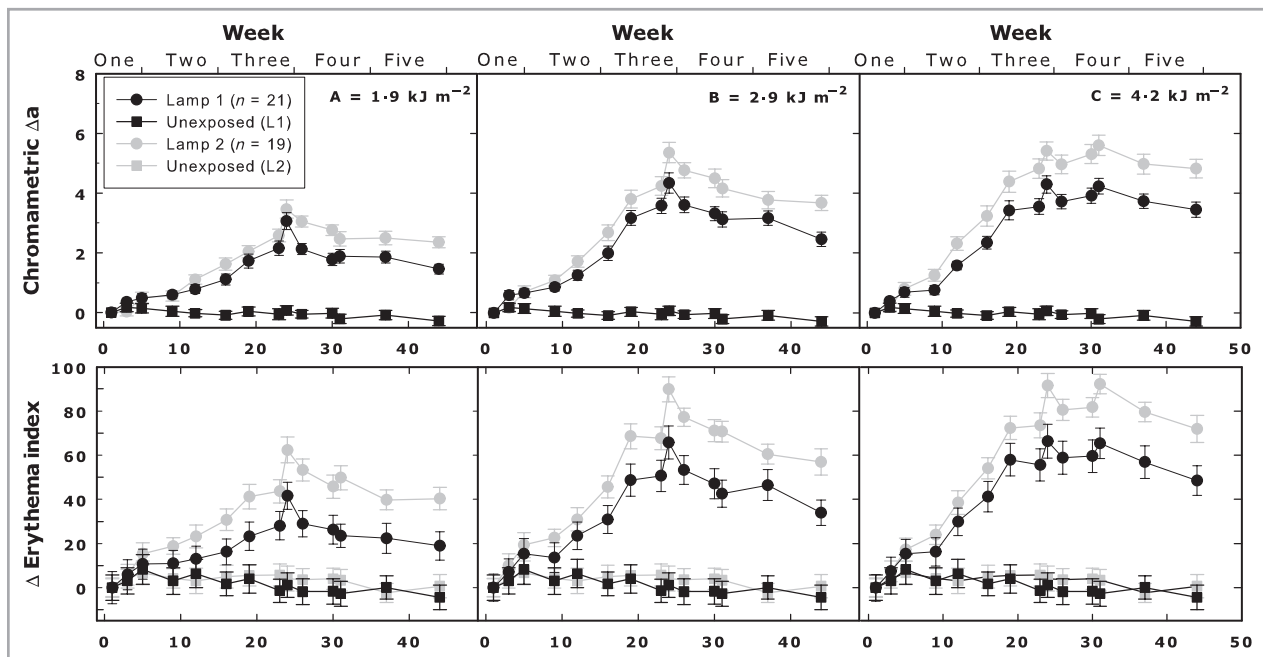


Fig 5. Changes in erythema-related parameters during and after repeated ultraviolet exposures according to protocols A (left column), B (centre column) and C (right column). Data points (mean values \pm SEM) and lines are in black and grey for sources 1 and 2, respectively. Top row: chromametric Δa^* . Bottom row: Δ Erythema Index measured with the DiaStron Erythema/Melanin Index Meter. The last exposures were given on day 23 in protocols A and B, and on day 30 in protocol C.

No tan was produced with the UVB-rich sources, but the UVA-rich sources enhanced pigmentation. Seite *et al.*⁶ induced a strong pigmentation with UVA (330–400 nm) exposures three times weekly for 13 weeks using increasing doses.

Caswell¹⁰ evaluated pigmentation development in phototype 3 and 4 subjects exposed 24 times over 8 weeks using a protocol based on the FDA guidance for tanning equipment manufacturers.¹¹ The doses were increased significantly each week, and the cumulative doses were 9240 and 9438 J m⁻² for phototype 3 and 4, respectively. Table 3 provides details and shows that such a protocol produced a ΔE of 11.5 CU. Our protocols were similar to those used in phototherapy, i.e. starting with a suberythemal dose and increasing each subsequent dose by 25–50%. Table 3 shows that protocol C (cumulative dose 4200 J m⁻²) led to ΔE values of 11.9 and

15.9 CU for source 1 and 2, respectively. Note that our cumulative doses were a factor of 2–3 lower than those in Caswell.¹⁰

We previously found that once weekly exposures were ineffective (ΔE of 2.7 CU¹²). Twice weekly exposures up to 380 J m⁻² produced light to moderate pigmentation. It appears that full activation of melanogenesis requires ≥ 2 exposures (to > 380 J m⁻²) per week. In this study, we saw significant differences between the effects of protocols A and B, and those of protocols A and C, but not those of protocols B and C, for both lamps. This result was confirmed in the analysis of melanin content from the biopsies as well.¹³ This occurred despite the fact that protocol C resulted in a 50% higher cumulative dose than did protocol B. Hence, during repetitive UV exposures, melanogenesis may reach a threshold.

Week	Number of exposures	Doses, J m ⁻² (erythema-effective)	Cumulative dose, J m ⁻² (erythema-effective)	ΔE for source 1	ΔE for source 2
Protocol A					
1	3	100–160	390	2.1	2.5
2	2	200–250	840	2.8	3.7
3	2	300–380	1520	4.9	5.9
4	1	380	1900	6.1	8
5				5.9	7.3
6				5.6	7.2
7				4.8	6.8
8					
Protocol B					
1	3	100–200	440	2.3	2.7
2	2	280–400	1120	3.7	4.9
3	2	560–600	2280	8	10
4	1	600	2900	10	12.9
5				9.2	11.9
6				9	11.2
7				7.8	10.6
8					
Protocol C					
1	3	100–230	480	2.2	3
2	2	340–500	1320	4.1	6.1
3	2	600	2520	8.5	11.5
4	2	600	3720	10.8	14.1
5	1	500	4200	11.9	15.9
6				11.3	14.7
7				10.3	14.1
8					
Caswell				ΔE at end of week	
1	3	66	198	2	
2	3	154	660	2.7	
3	3	330	1650	4	
4	3	440	2970	6.8	
5	3	440	4290	8.5	
6	3	550	5940	9	
7	3	550	7590	10.7	
8	3	550	9340	11.5	

Table 3 Ultraviolet doses and mean ΔE (chromametric units) in the three experimental protocols compared with the data of Caswell¹⁰

Higher UVA/lower UVB output (Table 1) led to earlier appearance of new pigmentation and a darker final pigmentation (Fig. 4). The melanogenic potential of UVA radiation has been known for a long time;³ nevertheless, the significant differences in pigmentation caused by relatively small shifts in the emission spectrum deserve attention. Interestingly, the differences in melanogenic effectiveness of our two UV sources would not be expected based on the melanogenesis action spectrum, as the melanogenic-weighted doses per 100 J m⁻² were similar for both sources (Table 1). Thus, the UVA dose per unit of erythemal-effective dose may be a better indicator of the melanogenic efficiency than is the melanogenic-weighted dose.

Visually, we observed little redness in study sites repeatedly exposed to UV. However, the erythema-related parameters Δa^* and EI increased gradually during repeated exposures (Fig. 5). It has been suggested that the specificity of these parameters can be compromised in the presence of pigmentation.²⁶ The changes in the Δa^* parameter reported in this study were similar to those reported in Caswell.¹⁰

UV-induced pigmentation of the skin, once established, is relatively stable. Figure 4 shows that the pigmentation did not diminish appreciably for at least 3 weeks after the final exposure (protocols A and B). In fact, in these two protocols, the pigmentation diminished by < 10% between day 23 (when exposures ceased) and day 37. These observations indicate that once a moderate to dark pigmentation is established, subsequent exposures at 2-week intervals should be sufficient to maintain pigmentation.

In summary, our observations indicate that for repetitive UV exposures of skin phototypes 2 and 3: (i) dependence of UV-induced pigmentation on cumulative dose reaches a threshold; i.e. increasing the dose above this threshold produces little or no additional pigmentation; (ii) once established, pigmentation of the skin is relatively stable; and (iii) the efficiency of the melanogenic process can be markedly enhanced by selection of scientifically based values of dose per exposure, exposure frequency and UV spectrum.

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References

- 1 Parrish JA, Zaynoun S, Anderson RR. Cumulative effects of repeated subthreshold doses of ultraviolet radiation. *J Invest Dermatol* 1981; **76**:356–8.

- 2 Parrish JA, Jaenicke KF, Anderson RR. Erythema and melanogenesis action spectra of normal human skin. *Photochem Photobiol* 1982; **36**:187–91.
- 3 Pathak M, Fanselow DL. Photobiology of melanin pigmentation: dose/response of skin to sunlight and its contents. *J Am Acad Dermatol* 1983; **9**:724–33.
- 4 Kaidbey KH, Kligman AM. Cumulative effects from repeated exposures to ultraviolet radiation. *J Invest Dermatol* 1981; **76**:352–5.
- 5 Bech-Thomsen N, Ravnborg L, Wulf HC. A quantitative study of the melanogenic effect of multiple suberythemal doses of different ultraviolet radiation sources. *Photodermatol Photoimmunol Photomed* 1994; **10**:53–6.
- 6 Seite S, Moyal D, Richard S *et al.* Effects of repeated suberythemal doses of UVA in human skin. *Eur J Dermatol* 1997; **7**:204–9.
- 7 Ravnbak MH, Wulf HC. Pigmentation after single and multiple UV-exposures depending on UV-spectrum. *Arch Dermatol Res* 2007; **299**:25–32.
- 8 de Winter S, Vink AA, Roza L *et al.* Solar-simulated skin adaptation and its effect on subsequent UV-induced epidermal DNA damage. *J Invest Dermatol* 2001; **117**:678–82.
- 9 Ruegamer J, Schuetz B, Hermann K *et al.* UV-induced skin changes due to regular use of commercial sunbeds. *Photodermatol Photoimmunol Photomed* 2002; **18**:223–7.
- 10 Caswell M. The kinetics of the tanning response to tanning bed exposures. *Photodermatol Photoimmunol Photomed* 2000; **16**:10–14.
- 11 U.S. Food and Drug Administration. Policy on Maximum Timer Interval and Exposure Schedule for Sunlamps, August 21, 1986. Rockville, MD: Department of Health and Human Services, Food and Drug Administration, Center for Devices and Radiological Health, 1986.
- 12 Miller SA, Coelho SG, Zmudzka BZ *et al.* Reduction of the UV burden to indoor tanners through new exposure schedules: a pilot study. *Photodermatol Photoimmunol Photomed* 2006; **22**:59–66.
- 13 Yamaguchi Y, Coelho SG, Zmudzka BZ *et al.* Cyclobutane pyrimidine dimer formation and p53 production in human skin after repeated UV irradiation. *Exp Dermatol* 2008 Epub ahead of print.
- 14 Coelho SG, Miller SA, Zmudzka BZ *et al.* Quantification of UV-induced erythema and pigmentation using computer-assisted digital image evaluation. *Photochem Photobiol* 2006; **82**:651–5.
- 15 Tadokoro T, Yamaguchi Y, Batzer J *et al.* Mechanisms of skin tanning in different racial/ethnic groups in response to ultraviolet radiation. *J Invest Dermatol* 2005; **124**:1326–32.
- 16 CIE Standard S 007/E-1998. Erythema Reference Action Spectrum and Standard Erythema Dose, ISO edn. Vienna, Austria: CIE, 1999; 17166.
- 17 Fullerton A, Fischer T, Lahti A *et al.* Guidelines for measurement of skin colour and erythema. A report from the Standardization Group of the European Society of Contact Dermatitis. *Contact Dermatitis* 1996; **35**:1–10.
- 18 Chardon A, Cretois I, Hourseau C. Skin colour typology and sun-tanning pathways. *Int J Cosmet Sci* 1991; **13**:191–208.
- 19 Del BS, Sok J, Bessac E *et al.* Relationship between skin response to ultraviolet exposure and skin color type. *Pigment Cell Res* 2006; **19**:606–14.
- 20 Jo SJ, Yoon HS, Woo SM *et al.* Time course of tanning induced by narrow-band UVB phototherapy in Korean psoriasis patients. *Photodermatol Photoimmunol Photomed* 2006; **22**:193–9.
- 21 Park S-B, Huh C-H, Choe Y-B *et al.* Time course of ultraviolet-induced skin reactions evaluated by two different reflectance spectrophotometers: DermaSpectrophotometer and Minolta Spectrophotometer CM-2002. *Photodermatol Photoimmunol Photomed* 2002; **18**:23–8.

- 22 CIE Technical Committee 2-17. *Solar Spectral Irradiance*. CIE Technical Report No. CIE 85. Vienna, Austria: Commission Internationale de l'Eclairage (CIE) Central Bureau, 1989.
- 23 Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. *Arch Dermatol* 1988; **124**:869–71.
- 24 Diffey BL, Robson J. The influence of pigmentation and illumination on the perception of erythema. *Photodermatol Photoimmunol Photomed* 1992; **9**:45–7.
- 25 Weatherall IL, Coombs BD. Skin color measurements in terms of CIELAB color space values. *J Invest Dermatol* 1992; **99**:468–73.
- 26 Alaluf S, Atkins D, Barrett K *et al.* The impact of epidermal melanin on objective measurements of human skin colour. *Pigment Cell Res* 2002; **15**:119–26.