

# Sunscreens Inadequately Protect Against Ultraviolet-A-Induced Free Radicals in Skin: Implications for Skin Aging and Melanoma?

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Sunscreens are employed to mitigate the adverse effects of sunlight on skin but are primarily designed to prevent ultraviolet-B-associated burning and damage. The increasingly recognized role of ultraviolet A in aging, and possibly melanoma, highlights the need to include ultraviolet A screens; however, validation remains difficult. We have used a novel method to establish the efficacy of sunscreens, by measuring ultraviolet-A-induced free-radical production (thought to contribute towards ultraviolet-A-related aging and malignant change). Electron spin resonance spectroscopy was used to detect free radicals directly in human Caucasian skin during irradiation with levels of ultraviolet comparable to solar intensities. Using this system the protection afforded by three high factor sunscreens (sun protection factor 20+) that claim ultraviolet A protection was examined. Each sunscreen behaved similarly: at recommended

application levels ( $\geq 2$  mg per  $\text{cm}^2$ ) the ultraviolet-induced free radicals were reduced by only about 55%, and by about 45% at 0.5–1.5 mg per  $\text{cm}^2$  (0.5 mg per  $\text{cm}^2$  reported for common usage). A “free-radical protection factor” calculated on the basis of these results was only 2 at the recommended application level, which contrasts strongly with the erythema-based sun protection factors (mainly indicative of ultraviolet B protection) quoted by the manufacturers (20+). The disparity between these protection factors suggests that prolonged sunbathing (encouraged by use of these creams) would disproportionately increase exposure to ultraviolet A and consequently the risk of ultraviolet-A-related skin damage. *Key words:* electron spin resonance spectroscopy/free radicals/photoaging of skin/sunlight/sunscreens. *J Invest Dermatol* 121:862–868, 2003

It is clearly established that ultraviolet (UV) wavelengths of sunlight are carcinogenic, contributing towards the formation of skin malignancy in the form of squamous and basal cell carcinoma and melanoma. There is a general consensus that basal and squamous cell carcinomas are predominantly a result of direct damage to the DNA by interaction with UVB (solar wavelengths 280–320 nm) (Linge, 1996). Epidemiologic data links melanoma to intense sunlight exposure in childhood, and provides support for a role of UVA (Moan *et al*, 1999). Although there is agreement that UV radiation is the cause, however, the precise wavelengths and mechanisms involved are not clear. Setlow *et al* (1993) showed the induction of melanoma in the fish model *Xiphophorus* by UVA, UVB, and blue-visible wavelengths; Lcy (1997) demonstrated equal effectiveness of UVA and UVB in inducing melanocytic hyperplasia in the opossum *Mono-delphis domestica*; and Noonan *et al* (2001, 2003), using combined UVB and UVA wavelengths (ratio 2:1), recently demonstrated the induction of melanoma in a transgenic neonatal mouse model. Berking *et al* (2001) showed that UVB in combination

with basic fibroblast growth factor could transform human melanocytes. The role of UVA in human melanoma is still inconclusive (Wang *et al*, 2001).

Whereas UVB is believed to interact directly with DNA to initiate signature mutations of basal and squamous cell carcinomas, UVA wavelengths (320–400 nm) are believed to interact indirectly, inducing the production of free radicals (Packer, 1994; Scharfetter-Kochanek *et al*, 1997). Free radicals may indirectly damage DNA and cause protein damage, which contributes to premature aging, or photoaging. UVA-induced p53 production and DNA damage (Burren *et al*, 1998), genomic instability (Phillipson *et al*, 2002), and immunosuppression (Dumay *et al*, 2001) have been demonstrated. Fas expression has also been shown to result from UVA as well as UVB (Bang *et al*, 2002).

Despite the extensive use of sunscreens during the last two decades, the incidence of skin cancers is still increasing, and the role of sunscreens in protecting against skin cancers is controversial. Sunscreen use has been shown to decrease the formation of actinic keratoses, which are linked to squamous cell carcinomas (Thompson *et al*, 1993; Naylor *et al*, 1995). Animal models have shown that sunscreens reduce the incidence of basal and squamous cell tumors (Sekura Snyder and May, 1975; Kligman *et al*, 1980; Forbes *et al*, 1989; Reeve *et al*, 1990), which are UVB related; however, there have been several studies to suggest that sunscreen use is associated with increased risk of melanoma (Autier *et al*, 1995; Azizi *et al*, 2000; Vainio and Bianchini, 2000). This may reflect inadequate sunscreen application (Stokes and Diffey, 1997; Wulf *et al*, 1997;

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Abbreviations: ESR, electron spin resonance; MED, minimal erythema dose; SPF, sun protection factor.

Gaughan and Padilla, 1998); lack of durability of the application; the lack of, or inadequacy of, UVA filters in sunscreen preparations combined with prolonged sunbathing (Autier *et al.*, 1995); the photo-instability of sunscreen filters that results in reduced protection; or the production of reactive free radicals or mutagens within the cream (Flindt-Hanse *et al.*, 1988; Shaw *et al.*, 1992; Gasparro, 1993; Knowland *et al.*, 1993; Dunford *et al.*, 1997). A link between sunscreen use and melanoma, however, is still debated (Huncharek and Kupelnick, 2002; Rigel, 2002) and unclear (Bigby, 1999).

The sun protection factor (SPF) of sunscreens is an internationally accepted standard by which the efficacy of sunscreens is assessed: it is based solely on prevention of erythema (sunburn), which is principally induced by UVB (Cole, 2001), and it is erythema (a downstream inflammatory response to the direct damage itself) that is the criterion by which people usually limit their sun exposure (Autier *et al.*, 1999). Whereas SPF may indicate protection against UVB-induced carcinogenesis, it cannot be used as an indicator of the "indirect" damage resulting from UVA exposure, as erythema is predominantly a response to UVB.

As skin carcinogenesis is highly complex, the use of a range of markers for damage in skin itself is likely to be necessary to complement SPF (an indicator of UVB protection) for use in evaluation of total skin cancer risk. Other studies have been published that assess "direct" DNA damage, p53 formation, and protection against UV-induced immunosuppression (Freeman *et al.*, 1988; Ley and Fourtanier, 1997; Ananthaswamy *et al.*, 1998; Burren *et al.*, 1998; Bykov *et al.*, 1998). There are several methods to measure the protection afforded by sunscreens against UVA damage to skin (reviewed by Lim *et al.*, 2001); however, these methods are not validated (Cole, 2001). The protection against free radicals induced by UVA, to date, has not been measured.

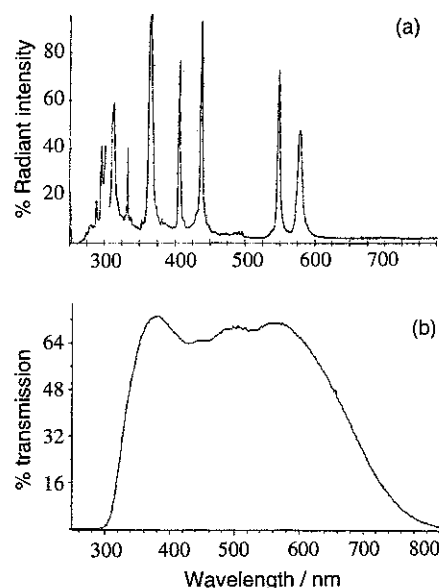
In this study we have adapted an electron spin resonance (ESR) method to measure UV-induced free-radical production in human skin, and assessed the protection against free-radical production provided by commercial sunscreens. The free radicals formed upon UV irradiation of skin (and that are associated with DNA and protein damage) are not usually directly detectable at room temperature. An exception to this, however, is the ascorbate radical, which is formed when ascorbate (vitamin C – a cellular antioxidant) reacts with free radicals. The ascorbate radical is readily detected using ESR spectroscopy in skin biopsies exposed to UV irradiation (Jurkiewicz and Buettner, 1996) and is accepted as a reliable marker for cellular free-radical production and oxidative stress (Buettner and Jurkiewicz, 1995; Jurkiewicz and Buettner, 1996). We have used the relative quantification of this radical in the same skin sample, both before and after application of sunscreen, in order to estimate the level of protection against UVA irradiation afforded by three popular sunscreens that claim UVA protection, over a range of application densities.

## MATERIALS AND METHODS

**Materials** This study using consented skin from informed patients, was approved by the West Hertfordshire NHS Trust (EC2002-20) and was conducted in accordance with the Declaration of Helsinki. Caucasian skin was obtained from consenting patients undergoing breast reduction surgery. Skin was stored in normal-saline-soaked gauze at 4°C and used within 24 h. Prior to ESR spectroscopic analysis, skin was trimmed to remove subcutaneous fat and cut to approximately 1 cm<sup>2</sup>. The surface area of the skin held flat and undeformed between the silica plates of the flat cell was measured. Sample sizes could not exceed 1 cm<sup>2</sup> due to technical limitations associated with tuning the spectrometer. Three popular brands of sunscreens, which claimed UVA protection, were randomly chosen for evaluation: brands 1, 2, and 3 were SPF 30 (containing the UVA filters titanium dioxide and terephthalylidene dicamphor sulfonic acid), SPF 25 (containing butylmethoxydibenzoyl methane), and SPF 20+ (containing octocrylene and titanium dioxide), respectively (filter concentrations were unspecified). Two of the brands chosen had a four star UVA rating (highest UK rating), and the third was obtained outside the UK and did not have this rating (although it claimed UVA protection and anti-cell-aging effects). All creams were evaluated within 3 mo of purchase.

ESR experiments were carried out using a Bruker EMX spectrometer (Rheinstetten/Karlsruhe, Germany) equipped with an ER 4103TM cavity and a Wilmad Glass tissue cell (WG 806-B-Q) (Buena, NJ). Typical ESR settings were 40 mW microwave power, 0.075 mT modulation amplitude,  $2 \times 10^5$  receiver gain, and sweep time 20 s with repeated scanning (five scans) unless otherwise indicated. UV irradiation was carried out *in situ* in the spectrometer (with the cavity completely shielded by black plastic sheeting) using a super high pressure 100 W Nikon mercury lamp (model LH-M1100CB-1) focused on the cavity transmission window. The emission spectrum of the lamp is shown in Fig 1(a). A 5 cm water filter was used to remove infrared radiation together with two optical glass filters having a combined thickness of 0.7 cm Barr and Stroud filtering (available from Andover Corporation, Salem, NH) wavelengths below 300 nm and having a 1% transmittance of UVB radiation at 300 nm and 19% at 320 nm (transmission spectrum shown in Fig 1(b)). Visible wavelengths were not filtered. The UV fluence incident upon the sample within the spectrometer was measured using a potassium ferrioxalate actinometer (Valenzeno *et al.*, 1991), which was slightly modified as follows: 0.006 M stock actinometer solution (0.25 mL) was irradiated (5 min) directly in the flat cell held in the cavity of the spectrometer and then washed out of the flat cell into a 5 mL flask with distilled water; 2 mL of this solution was added to 0.4 mL 1% 1,10-phenanthrene, 1 mL pH 4.5 buffer (Sigma), and diluted to 5 mL with d-H<sub>2</sub>O. The optical densities of irradiated and unirradiated solutions were compared at 510 nm. The UV fluence is within levels of solar irradiation (the UVA component of the total UV radiation penetrating the earth's atmosphere is 90%) measured between 11.00 a.m. and 3.00 p.m. with the same UV actinometer (irradiated with natural sunlight through an aperture cut in black card to the same dimensions as the ESR cavity window), June–September, London, UK (direct sunlight). The photon flux was calculated in mol quanta per second (Calvert and Pitts, 1966, page 781, equation 7.6) and converted to radiant flux in joules per second by multiplying by the Avogadro constant, Planck's constant, and the radiation frequency at 350 nm (mean frequency 300–400 nm over which the actinometer absorbs radiation). The molar absorptivity of potassium ferrioxalate ( $1.1 \times 10^3$  per mol dm<sup>3</sup> cm) is also required for this calculation. The radiant flux incident upon the actinometer was calculated to be 3 mW (3 mJ per s) and the irradiance at the cavity window (area 2.3 cm<sup>2</sup>) was then calculated to be 1.3 mW per cm<sup>2</sup> (mJ per s per cm<sup>2</sup>). The irradiance is lower than levels of UVA that have been employed for solar-simulated irradiation (reported levels of solar-simulated UVA are 35 and 60–80 mW per cm<sup>2</sup>) (Ley *et al.*, 1997; Burren *et al.*, 1998).

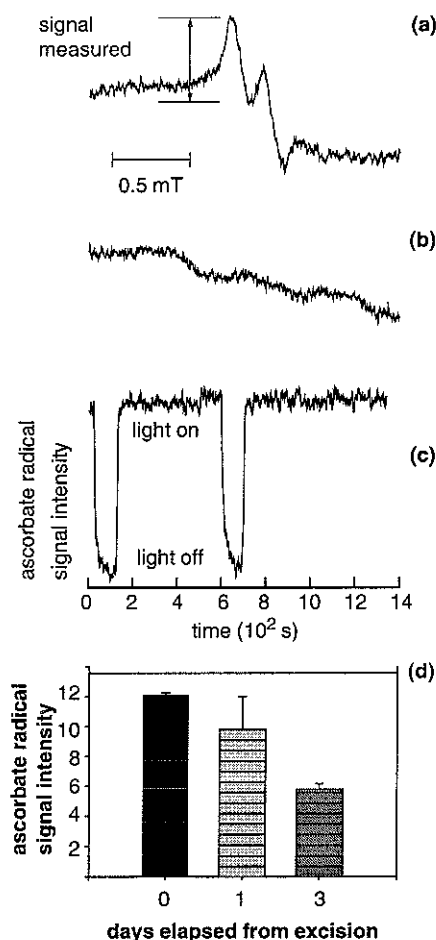
**Adaptation and validation of the method to quantify free-radical formation** ESR methodology was used to detect the ascorbate radical



**Figure 1. Irradiation.** (a) Typical emission spectrum of the mercury lamp used for irradiation experiments. Supplied with permission by the manufacturers (Osram). (b) Transmittance (percent of incident irradiation) measured between 250 and 820 nm of the two glass filters used in irradiation experiments.

directly in human skin on UV irradiation as previously published (Jurkiewicz and Buettner, 1996). The skin specimen (unprotected) was held in a Wilmad tissue cell, placed directly into the ESR cavity, and subjected to 100 s UV irradiation to establish the background levels of ascorbate radical. The skin sample, when mounted in a tissue cell, was held flat and undeformed between two silica plates comprising the tissue cell. The UV source was then blocked and the skin area was marked precisely on the covering silica slide and measured. The measured area is that of the skin held flat, and undeformed, between the silica slides. The sunscreen was applied to the measured area at a range of application levels (quantified by weighing) centered around that recommended in the sunscreen industry (2 mg per  $\text{cm}^2$ ). The slide was then placed with the cream side directly against the skin and again UV irradiated, and the free-radical signal intensity measured. The skin area was restricted to 0.5–1  $\text{cm}^2$  and the amounts of cream that were applied to the skin were not lower than 0.5 mg to minimize errors due to weighing. This set a lower limit for application of approximately 0.5 mg per  $\text{cm}^2$ . Given the results of preliminary experiments and that the manufacturers' recommended application level is 2 mg per  $\text{cm}^2$ , an upper limit for the quantitative analysis of 4 mg per  $\text{cm}^2$  was chosen. Nine measurements performed in this way were taken for each sunscreen across this range of application.

Quantification of the ascorbate radical spectrum was by measurement of the height of the low field absorbance peak relative to the midpoint (marked on Fig 2a). To verify that the signal of any radical species either



**Figure 2. Validation of the method.** (a) ESR spectra of human Caucasian breast skin immediately upon irradiation. (b) Comparable irradiation of factor 30 suncream alone (brand 1) at 2 mg per  $\text{cm}^2$  application (spectra are centered by  $g$ -value and are directly comparable). (c) The low field absorbance peak of the ascorbate radical detected in skin immediately after excision (marked in Fig 1a) monitored with time. The lamp iris was opened and closed during the scan to determine the response of the radical signal. (d) Mean ascorbate radical signal intensity at 0 d ( $n=3$ ), 1 d ( $n=9$ ), and 3 d ( $n=3$ ) after excision (error bars represent standard deviations).

already present in the sunscreen or formed as a result of UV irradiation of the sunscreen alone did not interfere with the signal of the ascorbate radical formed in the skin, comparison with the ESR spectra obtained from illumination of sunscreen alone was made.

Two methods were used to measure protection: (1) using the same skin sample (reducing possible intersample variation in ascorbate levels) or (2) comparing two different samples of the same skin both unprotected and protected with cream. Method (1) was used for the majority of the study, not only to reduce intersample variation of ascorbate, but also to ensure adequate levels of ascorbate in each skin sample studied (which occasionally could be low in some specimens, believed to be for dietary reasons). This method required that ascorbate (1) is sufficiently stable in the skin over the 100 s UV irradiation period taken to obtain a measurement, and (2) is stable over the period between the measurements when skin was removed from the spectrometer, coated with cream, and then reinserted in the spectrometer. Stability over the 100 s irradiation period was verified using fresh skin by continuous measurement of the free-radical signal intensity over approximately 1400 s UV irradiation (Fig 2c). Stability between measurements was also verified (not shown).

As skin was used up to 24 h post surgical excision, it was necessary to verify that ascorbate was sufficiently stable with refrigerated storage over this time period. Ascorbate levels in the skin were compared by comparison of the ascorbate radical signal intensity upon irradiation at different time periods of storage: at 0 (in practical terms approximately 2 h post excision) ( $n=3$ ); 1 d ( $n=9$ ); and 3 d ( $n=3$ ) (Fig 2d).

To verify that the ascorbate radical signal did not originate from irradiation scattered through the tissue cell (whose etched lower surface decreases light transmittance considerably compared to that through the transparent cover slip) skin was irradiated both protected at the front, and then at the back, by black tape of similar dimensions to the skin sample. The ascorbate radical signal was abolished when protective tape (greater than the skin area) was between the incident irradiation and the skin, but when the tape was placed behind the skin (to prevent entry into the skin of scattered radiation) the ascorbate radical signal was clearly detected at a similar signal intensity to skin not shielded by tape at the dermal aspect (not shown). If the tape was the exact dimensions of the skin sample, and placed in front of the skin, then a weak signal due to the ascorbate radical could occasionally be observed (up to about 10% the unprotected signal), suggesting entry of irradiation through the cut edge of the skin.

As a further check of the primary method used (method (1)), the protection at 2 mg per  $\text{cm}^2$  application was also determined using method (2) for one brand of sunscreen. Paired samples of skin were cut from the same piece of skin ( $n=5$ , separate patients) to similar dimensions. One of these samples was mounted in the tissue cell, as described above, and irradiated for 100 s to determine the free-radical signal intensity in unprotected skin. This sample was then removed from the cell and replaced by the second skin sample, which was then covered with a film of factor 25 sunscreen applied to the upper silica slide, to an application density of 2 mg per  $\text{cm}^2$ , to an area that exceeded that of the skin sample (to ensure blocking of the edge effect). This sunscreen-protected skin was irradiated for 100 s and the free-radical signal intensity was measured. Both sunscreen-protected and unprotected samples, in this experiment, were shielded at the dermal aspect with black tape. The protection provided by a commonly used dermatologic cream, lacking UV filters, was also measured.

The dose-response of the ascorbate radical signal intensity to the UVA irradiation was verified using neutral-density filters to reduce the UVA dose to the skin by increasing amounts. Neutral-density filters were added sequentially to the water and two glass filter combination of the UV lamp, and for each filter system the absorbance at 510 nm of UV-irradiated actinometer solution was measured, relative to unirradiated solution. For each filter system, the ascorbate radical signal intensity, upon 100 s UV irradiation, was also measured in different samples of skin, cut to similar dimensions.

**Statistics** Comparison between two groups of data was undertaken using a Student's  $t$  test. All analyses were performed using Sigma Stat statistics software, version 2.0 (Jandel Corporation, Chicago, IL).

## RESULTS

**Validation of method** UV-irradiated skin produced the recognizable ESR spectrum (Fig 2a) of the ascorbate radical (characterized by hyperfine splitting  $a(\text{H})=0.17$  mT (Jurkiewicz and Buettner, 1996)), which was either undetectable, or detected to very low levels, in nonirradiated skin (not shown). In contrast

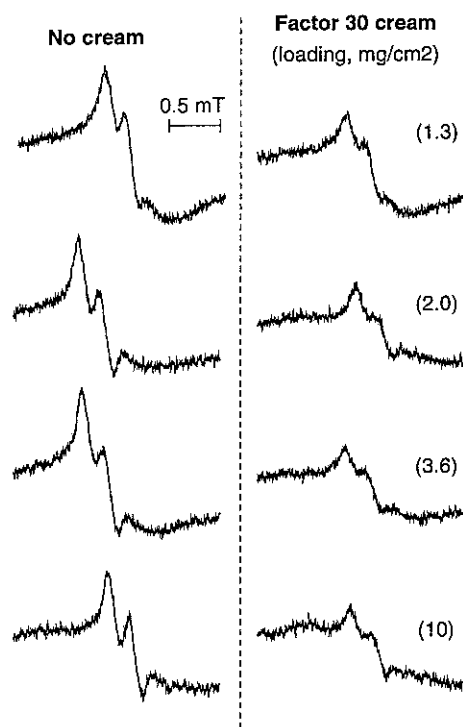
to previous reports (Jurkiewicz and Buettner, 1996), the ascorbate radical was not consistently detected in nonirradiated skin in our experiments. This may be because the skin in our experiments was shielded from room light, believed previously (Jurkiewicz and Buettner) to have a small effect on the radical signal, or it may have reflected the use in our experiments of fresh rather than frozen skin samples (cellular degradation and metal-ion release could have stimulated oxidative stress within these frozen skin specimens). The radicals formed in sunscreen alone (Fig 2*b* shows UV-irradiated sunscreen alone at 2 mg per cm<sup>2</sup>) did not interfere with the ascorbate radical at application levels up to about 4 mg per cm<sup>2</sup>; however, at applications much greater than this ( $\geq 10$  mg per cm<sup>2</sup>), radical production in the sunscreen itself becomes appreciable (brands 1 and 3).

The ascorbate radical is detected immediately in skin upon irradiation and responds rapidly to a change in radiation levels as shown in Fig 2(*c*), being restored to original levels after closing and reopening the lamp iris. Over the whole period of UV irradiation (up to 1400 s) the ascorbate depletion is very slight (Fig 1*c*). The ascorbate radical signal decreased very slowly with prolonged irradiation (when studied over a period of approximately 30 min); however, the rate of decrease varied according to the time of skin storage prior to experimentation. Skin studied immediately after removal from the patient exhibited very low rates of ascorbate depletion (Fig 2*c*) (probably due to dehydroascorbate reductase activity in intact viable cells that are still present in skin after 24 h refrigeration), and UV-induced depletion rates were found to increase with storage, becoming significant after 3 d refrigeration.

The stability of ascorbate, during refrigerated storage, was verified by measurement of the ascorbate radical signal intensity (upon immediate UV irradiation) in different samples of the same piece of skin at different times of refrigerated storage (0, 1, and 3 d), as shown in Fig 2(*d*). The mean signal intensity is slightly lower at 1 d after excision compared to that recorded immediately after excision, but is broadly comparable, reflecting similar levels of ascorbate in the skin. At 3 d after excision, the mean ascorbate radical signal intensity is significantly lower, being approximately half that after excision, and thus ascorbate levels are depleted at 3 d. Therefore, whereas every effort was made to use skin samples as soon as was practicable after excision, a cut-off period of 24 h was chosen.

**Quantification of the protection provided by high factor sunscreens** Figure 3 shows ESR spectra obtained before and after sunscreen (brand 1, factor 30) application to breast skin. These data are typical of those used for subsequent quantitative analysis of the protection provided by high factor sunscreens. There was generally only a small (<50%) observable reduction in the signal intensity of the ascorbate radical compared to unprotected skin. This reduction appeared to peak at approximately 2 mg per cm<sup>2</sup>, with little further reduction at greater application levels.

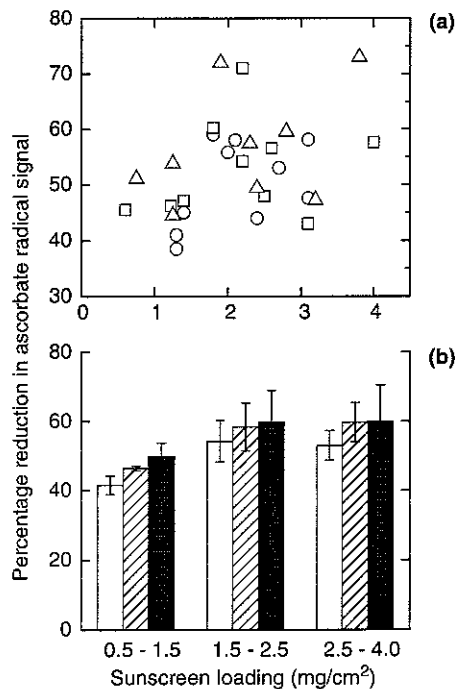
Figure 4(*a*) shows a plot of the percentage reduction in signal intensity of the ascorbate radical (taken as the height of the low field absorption relative to the midpoint of the spectrum, Fig 2*a*) at different applications of high factor sunscreens (all three brands). The application density was calculated as the measured weight of sunscreen applied to the upper silica slide relative to the measured skin area of skin held flat and undeformed in the tissue cell. The percentage reduction in signal intensity is similar for all three brands studied and increases with application up to approximately 2 mg per cm<sup>2</sup> with no further protection apparent at higher application. Despite use of skin from one anatomic region to cut down on intersite variation, there is still variability in the protection measured at constant application: although this could result from unavoidable errors associated with the experimental procedure and analysis, it could also result from individual variations in skin architecture that affect the protection provided by the sunscreen.



**Figure 3.** Typical ESR spectra of UV-irradiated human Caucasian skin without and with sunscreen protection. Each horizontal row is a different skin sample unprotected (first vertical column) and coated (second vertical column) with factor 30 sunscreen (brand 1 with UVA protection); applications range from 1.3 to 10 mg per cm<sup>2</sup>.

A rank plot of the data in Fig 4(*a*) is shown in Fig 4(*b*): the mean percentage reduction in signal intensity of the ascorbate radical was calculated for levels of application 0.5–1.5, 1.5–2.5, and 2.5–4 mg per cm<sup>2</sup>, and the standard deviation was calculated for  $n=3$  for each sunscreen at each level of application. These levels of application were defined: the mean application of sunscreen (for all the brands) for the 0.5–1.5 group is actually about 1.2 mg per cm<sup>2</sup>; 2.2 mg per cm<sup>2</sup> for the 1.5–2.5 group; and 3 mg per cm<sup>2</sup> for the 2.5–4 mg per cm<sup>2</sup> group. It was found that the level of protection at each application is comparable for the three brands studied: the protection at 1.5–2.5 mg per cm<sup>2</sup> and above is approximately 50%–60% reduction in signal intensity; however, at 0.5–1.5 mg per cm<sup>2</sup> protection is less, between 40% and 50% for the three brands. Statistical analysis of the data showed that for brands 1 and 2 the protection at 0.5–1.5 mg per cm<sup>2</sup> application is significantly lower ( $p=0.034$  and  $0.024$ , respectively) than the protection at 1.5–2.5 mg per cm<sup>2</sup>. For brand 3, although there is the same trend, this does not quite reach statistical significance ( $p=0.243$ ). Data at 2.5–4.0 mg per cm<sup>2</sup> application are not significantly different from those at 1.5–2.5 mg per cm<sup>2</sup> (for all three brands). The differences between the protection measured at 0.5–1.5 and 1.5–2.5 mg per cm<sup>2</sup> are slightly lower than might be expected; however, this is believed to reflect the actual mean applications of 1.2 and 2.2 mg per cm<sup>2</sup> (which are not precisely doubled). The mean application in the 2.5–4 mg per cm<sup>2</sup> groups is actually 3 mg per cm<sup>2</sup> and may explain the lack of a statistically significant difference between the 2.5–4 and 1.5–2.5 mg per cm<sup>2</sup> groups. Due to the small sample size, interindividual variations could be contributing here.

The ascorbate radical signal intensity is significantly lower in sunscreen-protected skin ( $p=0.002$ ,  $0.004$ , and  $0.001$  for brand 1 at 0.5–1.5, 1.5–2.5, and 2.5–4 mg per cm<sup>2</sup> application, respectively, and similar values for brands 2 and 3) compared to the control



**Figure 4. Quantitation of sunscreen protection at different applications.** (a) Plot of the percentage reduction in ascorbate radical signal intensity against application ( $\text{mg per cm}^2$ ) for UV-irradiated skin covered with high factor sunscreens (three brands;  $\circ$ , brand 1;  $\square$ , brand 2;  $\triangle$ , brand 3, SPF 20+ and claiming UVA protection). (b) Rank plot of percentage reduction in ascorbate radical signal intensity ( $n=3$  for each brand at each level of application) for UV-irradiated skin covered with high factor sunscreens (open, hashed and shaded bars, brands 1, 2, and 3, respectively) at different levels of application (0.5–1.5, 1.5–2.5, and 2.5–4  $\text{mg per cm}^2$ ). Error bars represent standard deviations, and the protection at 0.5–1.5  $\text{cm}^2$  application (brands 1 and 2) is significantly lower ( $p=0.034$  and  $0.024$ ) than the protection at 1.5–2.5  $\text{cm}^2$  application. The ascorbate radical signal intensity, in sunscreen-protected skin, is significantly lower ( $p=0.002$ ,  $0.004$ , and  $0.001$  for brand 1 at 0.5–1.5, 1.5–2.5, and 2.5–4  $\text{mg per cm}^2$  application, respectively, and similar values for brands 2 and 3) compared to untreated skin.

skin, which was subjected to 100 s irradiation, removed from the spectrometer, replaced unprotected (i.e., in the absence of sunscreen) in the spectrometer, and re-irradiated for 100 s.

Method (2) was found to give a comparable result to the first method of 58% ( $\pm 10\%$ ) reduction in signal intensity of the ascorbate radical in sunscreen-protected skin (at 2  $\text{mg per cm}^2$  application) relative to unprotected specimens. A dermatologic cream, without any UV filters, applied at an application density of 2  $\text{mg per cm}^2$  resulted in a 3.7% reduction in the ascorbate radical signal intensity ( $n=3$ ). The reduction in ascorbate radical signal intensity was found to correlate directly with the reduction in the UV dose to the skin when irradiation was carried out through neutral-density filters (not shown). In addition, the glass filters used to filter UVB wavelengths were then removed to increase the UV dose to the skin: when the filters were removed, the signal intensity of the ascorbate radical increased, and it was possible to lower the microwave power of the spectrometer to observe the radical. The ascorbate radical signal intensity therefore is not at maximum levels in the UVA system, and this suggests that the sensitivity and usefulness of this method is likely to improve at higher irradiation intensities than used in our experiments.

It is concluded therefore that this ESR method is a sensitive method for measuring free-radical protection by sunscreens and

might be used at higher UVA intensities than the irradiation used for this study. For precise quantitative measurements, both methods are potentially useful; however, the application of sunscreen to a larger area than the skin (to prevent edge effects) and the protection of the skin at the dermal aspect are recommended, particularly if the experiments are to be performed at higher irradiation intensities than we have used (where scattering of irradiation to the dermal aspect of the skin within the ESR spectrometer might become significant). It is concluded that the use of different skin samples, as opposed to the same skin sample, does not significantly affect the quantitative results.

## DISCUSSION

It is concluded from this study that the signal intensity of the ascorbate radical, in UVA-irradiated skin, is directly proportional to the UVA dose to the skin, and therefore it can be used to measure the protection, by the reduction of the ascorbate radical signal intensity, of a sunscreen applied as a barrier to the skin epidermis. We find, using this method, that Caucasian skin is only protected to about 55% against free-radical production by high factor sunscreens (which provide the maximum available UVA protection in the UK), applied at the recommended application of 2  $\text{mg per cm}^2$ , using an irradiance comparable to relatively weak solar irradiation (within direct sunlight measurements 11.00 a.m.–3.00 p.m. June–September, London). At 0.5–1.5  $\text{mg per cm}^2$  (actual mean application 1.2  $\text{mg per cm}^2$ ) this protection is only about 45% reduction (statistically significant) in the UV-induced radical concentrations and is likely to be less at lower applications.

The protection currently provided by sunscreens is indicated by the erythema-based SPF measurement, i.e., the factor by which the dose for minimal erythema (MED) is increased with protection under standard solar-simulated conditions (or the factor by which sunlight exposure time is increased, before burning, with sunscreen compared to without protection). The irradiation delivered to the skin in these experiments is 1.3  $\text{mJ per cm}^2$  per s UVA for 100 s. One MED is about 20–30  $\text{mJ per cm}^2$  UVB for a Caucasian (type I skin). As UVB is 10% of the total UV component (UVA and UVB) of sunlight penetrating the earth's atmosphere, 1 MED UVB will be associated with 180–270  $\text{mJ UVA}$  (which causes 0.001 of the erythema response of UVB) (McKinlay and Diffey, 1987). The radiation used in these experiments (1.3  $\text{mJ per cm}^2$  per s UVA) is equivalent to 130  $\text{mJ per cm}^2$  UVA (for 100 s irradiation). Thus the UVA radiation dose in our experiments is estimated to be equivalent to sunlight of MED equal to about 0.6, i.e., to be suberythema. It would also be of great interest therefore to determine the behavior and efficacy of these sunscreens at higher UVA intensities, i.e., solar irradiation equivalent to that of hotter climates; however, current limitations of equipment have delayed this.

On the basis of the results in this study it is possible to calculate a "free-radical protection factor" as the length of UVA exposure time with sunscreen protection, compared to without protection, to achieve the same amount of UVA penetrating the skin. As the radical signal intensity is approximately halved at the test level of application (2  $\text{mg per cm}^2$ ), the results suggest that, using this test method, the same UV dose will be achieved in twice the length of duration of exposure, thus suggesting a free-radical protection factor of about 2 (and less than 2 at typically used levels of application). It is possible, with further development, that a free-radical protection factor might be a useful measure of UVA protection afforded by sunscreens. Our results are also consistent with previous reports (Azizi *et al*, 2000) that, at typical levels of application (measured to be 0.5  $\text{mg per cm}^2$ ) (Bech-Thompsen and Wulf, 1992–93), the UVB protection (measured by SPF) is less than that measured at the recommended level of application of 2  $\text{mg per cm}^2$ . A free-radical protection factor of 2 is substantially less than the erythema-based protection factors for these

creams (20–30) and suggests that users of these creams will be disproportionately exposed to UVA.

Although there are published methodologies to measure UVA protection (reviewed by Lim, 2001), these have yet to be applied to directly quantify the protection provided by sunscreens that are currently commercially available. These sunscreens (available in Europe) often do not provide data for the percentage composition of filters they contain. A study to develop a methodology to quantify UVA protection in commercial creams (Takeuchi *et al*, 1998) suggests that a UVA protection of 2 is afforded by a factor 15 UVA sunscreen, compared to a protection factor of 3 by a UVA filter (3% butylmethoxydibenzoyl methane) in a base cream (using transgenic mice containing the human elastin promoter linked to a chloramphenicol acetyl transferase reporter gene). These results, although obtained using a lower SPF rated sunscreen, and mouse rather than human skin, are broadly comparable to our results. Ley and Fourtanier (1997) measured the protection against UVA-induced DNA damage in mouse skin afforded by a UVA filter in a base cream: using a 5% filter (terephthalylidene dicamphor sulfonic acid) they found an 8-fold reduction in DNA damage; however, the sunscreen preparation may have included a higher percentage of filter than that used in commercial preparations. It is also interesting that a sunscreen formulation providing low UVA protection provided 57% protection against UVA1 immunosuppression (Dumay *et al*, 2001).

As our irradiation source contains visible light, it is possible that the protection against free-radical production is low because of a visible light contribution to damage; however, the results of Jurkiewicz and Buettner (1996) suggest that the visible light contribution is minor compared to that of UVA, and the former's contribution may reflect a tail of the UVA chromophore (unidentified), which extends into the blue-visible region of the spectrum, rather than a separate visible chromophore. The importance of the visible light contribution is a separate subject worthy of further investigation, as sunscreens are not designed to extend beyond the UV region of the solar spectrum.

In conclusion, the results of this study to investigate free-radical protection by certain currently available high factor sunscreens suggest they provide approximately 55% protection when applied directly to the skin at the recommended amount of 2 mg per cm<sup>2</sup>, and less at lower application (at an irradiance comparable to solar UVA). The use of ESR to measure directly the UVA-induced free-radical production in human skin is a rapid and useful method to measure the free-radical/UVA protection. The results suggest that sunscreen users are little protected against UVA free-radical production and the damaging effects of UVA. In particular, as the existing sunscreen SPF provides a measure of the protection principally against UVB-induced erythema, users of high factor sunscreens may have an artificial sense of security that they are protected similarly against UVA. Thus, as already suggested by Autier *et al* (1995), use of high factor sunscreens may paradoxically be associated with increased skin cancer risk. The role of UVA in melanoma development, however, and the role of sunscreens in protecting against skin cancer, is still inconclusive and controversial (Urbach, 1992–93) and further work will be necessary to clarify these issues.

There is therefore an urgent need for clarification of the role of UVA in skin malignancy; for the validation of UVA SPF measurements of commercial sunscreens; for the UVA protection to be reassessed by sunscreen manufacturers to reflect the protection at typical levels of application; and also for the production of more effective UVA sunscreens at these levels of application. Our results suggest that the UVA/free-radical protection currently provided by sunscreens is inadequate, and it may be a precautionary measure that Caucasians (especially those with fairer skin types and at higher risk of skin cancer) avoid prolonged sunbathing, even though "protected" by sunscreens, as the use of sunscreens could increase their risk of UVA-induced free-radical damage.

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