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UV-Induced Lipid Peroxidation as a Biomarker of Photodamage in the Stratum Corneum of Black Skin Assayed by the HPLC–TBARS–EVSC Protocol

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Abstract

Brazil is characterized by high levels of solar exposure, which may compromise skin health due to ultraviolet radiation (UVR), particularly among individuals with darker skin tones who often underuse photoprotective measures. Although darker skin has been hypothesized to exhibit greater resistance to UVR, robust evidence remains limited. This study aimed to investigate the susceptibility of the stratum corneum (SC) of Black individuals to UV-induced lipid peroxidation using the HPLC–TBARS–EVSC protocol. SC samples were collected from the forearms of nine participants by tape stripping and subsequently exposed to an artificial UV radiation source. Lipid peroxidation was quantified through the detection of the malondialdehyde-thiobarbituric acid (MDA-TBA_2) adduct using high-performance liquid chromatography. Following UV exposure, a statistically significant increase in lipid peroxidation was observed, corresponding to an approximate 297% elevation in MDA-TBA_2 levels ($p < 0.05$). These findings demonstrate that the SC of Black skin is susceptible to UV-induced oxidative damage under the tested conditions. The results support the use of phototype-independent biochemical markers to assess photodamage and reinforce the need for effective photoprotection strategies in individuals with darker skin tones.

Keywords: black skin; lipoperoxidation; stratum corneum; ultraviolet radiation

1. Introduction

Brazil, with its extensive territorial area, experiences significant ultraviolet (UV) radiation exposure, posing public health concerns due to its potential effects on the population [1]. Most of the Brazilian population self-identifies as Black or Brown, highlighting the importance of understanding how UV radiation specifically impacts these skin types (higher Fitzpatrick phototypes), and how UV exposure affects their health [2,3].

The detrimental cutaneous effects of ultraviolet radiation (UVR), such as photoaging, sunburn, and skin cancer, are well-documented for individuals with lighter skin phototypes [3,4]. However, Black individuals are generally assumed to be less susceptible to UV-induced damage. In this population, solar exposure can lead to pigmentary disorders, photoaging, and photocarcinogenesis (melanoma and non-melanoma) [3]. The clinical manifestations of photoaging in Black skin include hyperpigmentation, dyschromia, increased skin laxity, and dermatosis papulosa nigra [3,4]. Although this group has a lower incidence of melanoma, their mortality rates are disproportionately higher compared to those with

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lighter skin, primarily due to delayed diagnosis and limited access to dermatological or preventive care [3,5].

Photoprotection remains a key strategy to prevent and mitigate UV-induced damage on Black skin; however, its full impact on Black skin and its specific cutaneous attributes are not fully elucidated. While the use of sun protection is recommended for Black individuals to reduce melanoma risk, the precise relationship between UVR exposure and melanoma development in Black skin is not yet fully established [3]. High-SPF (sun protection factor) sunscreens are recommended to minimize UVR damage [4–6]. Due to higher melanin content, Black skin often does not exhibit visible erythema, and current standardized efficacy trials do not adequately represent individuals with higher phototypes [3,7]. Consequently, there is ongoing debate regarding whether Black skin is inherently more protected against UVR and if current sunscreens meet the specific needs of this population [8].

The selection of the HPLC–TBARS–EVSC (high-performance liquid chromatography/thiobarbituric acid reactive species/ex vivo stratum corneum) protocol was based on its enhanced analytical specificity. By using HPLC to separate and quantify the malondialdehyde-thiobarbituric acid adduct (MDA-TBA_2) [9], this approach provides an objective and accurate bioanalytical readout. While in vivo methods require UV exposure of the participants and trained professionals to evaluate erythema formation, our protocol uses tape stripping to collect the stratum corneum (SC), causing minimal discomfort to participants, and quantifies photodamage using a robust analytical tool [8,10,11].

These considerations raise critical questions about the necessity for photoprotection in Black skin and highlight the need for studies that rigorously characterize and acknowledge the distinctive attributes of Black skin [8]. In this investigation, we attempted to ex vivo elucidate the SC differential response of Black subjects (phototypes IV to VI) to artificial UVR exposure. In this context, we investigated the susceptibility of the SC of individuals with Black skin (phototypes IV to VI) to lipid peroxidation induced by artificial UVR, quantified by the HPLC–TBARS–EVSC protocol.

2. Materials and Methods

2.1. Material

All chemicals used in this study were of analytical grade and were employed without additional purification. Transparent 750 polypropylene adhesive tape 3M (São Paulo, SP, Brazil), and sample filtration was performed using 0.22 µm syringe filters Millipore (Burlington, MA, USA). Ultrapure water was generated using a Milli-Q Simplicity UV system Millipore (Burlington, MA, USA) and was used for all experimental procedures. Stratum corneum collection was carried out with Scotch® (São Paulo, SP, Brazil). Thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropane, tetrahydrofuran, methanol, butylated hydroxytoluene (BHT), sulfuric acid, and phosphoric acid were purchased from Sigma–Aldrich (Saint Louis, MO, USA). N-butanol and 1,4-dioxane were obtained from Merck (Darmstadt, Hesse, Germany).

2.2. Legal and Ethical Aspects, and Study Population

Nine healthy subjects (males and females), aged between 18 and 60 years, as detailed in Table 1, with skin phototypes IV, V, and VI, and no history or clinical evidence of skin disease, were enrolled in the study. The participants exhibited phenotypic characteristics consistent with individuals of sub-Saharan African descent. Individuals with systemic or dermatological conditions were excluded from the study. The protocol was previously approved by the Ethics Committee of the Faculty of Pharmaceutical Sciences, University of São Paulo (CAAE 86055024.0.0000.0067; protocol approval code: 7,411,856; approval date 26 February 2025), in accordance with the principles of the Declaration of Helsinki.

All study procedures were explained orally and in writing to the participants through an Informed Consent Form, ensuring anonymity and the right to voluntary withdrawal at any time [8,9,11,12].

Table 1. Participants' characteristics, considering gender, age, and skin phototype (n = 9).

Subjects	Age	Phototypes	Gender
P1	24	VI	Male
P2	24	VI	Female
P3	38	V	Female
P4	21	IV	Male
P5	53	IV	Female
P6	42	V	Female
P7	48	V	Female
P8	26	V	Female
P9	50	VI	Male

2.3. Preparation of Samples and SC Extraction

The SC extraction was performed after the participants had been acclimatized under the experimental conditions for 15–20 min before the start of the procedure at an ambient temperature of 22 ± 2 °C and relative humidity of 40–60%. Adhesive tapes measuring 2.0 cm × 2.0 cm were applied with constant pressure to previously cleansed skin, using gauze, at adjacent sites on the dorsal forearm for SC removal. The selected areas were randomized. Six consecutive tape strips were collected, with the first strip being discarded [8–10,12].

2.4. HPLC–TBARS–EVSC Protocol

The HPLC–TBARS–EVSC protocol used in this study was previously developed and analytically validated by our Research Group, as described by Sauce et al. (2021) [8]. The tape strips were transferred to 50 mL Falcon tubes and extracted with 10 mL of methanol using mechanical agitation (vortex) for 1 min, followed by an ultrasonic bath for 15 min. In capped glass tubes, 2.0 mL of the resulting methanolic extract was mixed with 288 µL of 0.2% BHT and 800 µL of 0.44 M. Subsequently, a 1200 µL aliquot of 0.6% TBA (prepared in 0.44 M) was added. The samples were heated in a thermostated water bath at 90 °C for 45 min. After cooling to room temperature, the MDA-TBA_2 adduct was extracted into 1200 µL of n-butanol, followed by centrifugation at 3000 *g* for 10 min. The upper organic layer was collected, filtered through a 0.22 µm membrane, and 40 µL aliquots were injected into the HPLC system. Chromatographic quantification was performed using a C18 reverse-phase column (4.6 mm × 25.0 cm, Shimadzu) maintained at 30.0 ± 1.0 °C. The mobile phase consisted of methanol and potassium phosphate buffer (35:65, *v*/*v*, pH 7.0) at a flow rate of 1.0 mL/min, with detection at 532 nm. Data were expressed as the percentage increase in lipid peroxidation [8–10,12], calculated according to Equation (1) [8].

$$\text{Increase in lipid peroxidation } (\%) = 100 \times \frac{SCirr - SCn}{SCn} \quad (1)$$

where *SCn* and *SCirr* represent non-irradiated and irradiated stratum corneum samples, respectively.

2.5. Simulation of Solar Radiation

A photostability chamber (Atlas Suntest CPS+) equipped with a xenon lamp (1500 W) was used. Irradiation was performed at a total spectral irradiance of 765 W/m^2 using a solar simulator filter allowing the transmission of wavelengths above 290 nm, and

the temperature was controlled at 35 °C. Samples were exposed until reaching a final dose of 5506 kJ/m^2, with the exposure time adjusted as required to achieve the target dose. This irradiation level corresponds to approximately 35–55% of the typical daily global solar radiation in winter (June) in São Paulo (~10–15 MJ/m^2/day), according to the Brazilian Solar Energy Atlas. After irradiation, the samples were subjected to the previously described procedure [1,8,9,11–13].

2.6. Statistical Analysis

Statistical analysis was performed using Jamovi version 2.6. Data distribution was assessed using the Shapiro–Wilk test. Given the sample size (n = 9) and the non-normal distribution of area under the curve (AUC) values, paired comparisons between non-irradiated (*SCn*) and irradiated (*SCirr*) stratum corneum samples were performed using the Wilcoxon signed-rank test. Results were presented as median (interquartile range, IQR) and mean ± standard deviation (SD). A p-value < 0.05 was considered statistically significant.

3. Results and Discussion

The racial/ethnic classification of the Brazilian population is based on self-identification and includes the following categories: White, Yellow (Asian), Indigenous, Black, and Brown (Mixed race). Approximately 10% of the population self-identifies as Black and 45% as Brown [14]. White skin, also referred to as Caucasian skin, generally includes individuals with Fitzpatrick skin phototypes I to III [3,15], corresponding to approximately 44% of the Brazilian population [14].

In Brazil, the term "Black individual" has been adopted to encompass both Black and Brown individuals, recognizing a shared racial identity shaped by phenotypic characteristics, including skin color, and by common experiences of racial discrimination [16]. This sociocultural framework differs from that commonly used in international scientific literature, which often classifies highly pigmented skin under the broad category of "skin of color" without considering the specific biological and dermatological attributes of Black skin within the Brazilian population [2,3,8,16]. Such generalized classifications may group populations with distinct intrinsic skin properties, including individuals of Asian ancestry (phototypes II–V) and Black individuals (phototypes IV–VI), while overlooking important differences beyond pigmentation alone. These considerations highlight the importance of investigating Black skin as a distinct biological entity in dermatological research and of proposing tailored photoprotective strategies.

Our Research Group developed the HPLC–TBARS–EVSC protocol as an innovative approach to assess lipid peroxidation using ex vivo SC samples obtained by tape stripping, a minimally invasive or non-invasive technique that does not induce photodamage to the subject [9,11,12]. Lipid peroxidation results from oxidative stress generated by UVR-induced free radical reactions with polyunsaturated fatty acids, leading to the formation of secondary products such as MDA, which originates from the cleavage of endocyclic polyunsaturated fatty acids. The assay quantifies MDA through its reaction with TBA, resulting in the formation of the MDA-TBA$_2$ adduct, a biomarker of SC oxidative stress [9,11,12].

The information generated through the HPLC–TBARS–EVSC protocol for Black skin is particularly relevant, as this model represents one of the first approaches to quantify oxidative biomarkers in SC, independently of erythema formation following UVR exposure [8]. Conventional in vivo photoprotection efficacy assessments are primarily based on erythema endpoints, which are visually detectable and quantifiable in lighter phototypes. Nevertheless, erythema response is less perceptible and more difficult to standardize in highly pigmented skin, such as Black skin, limiting the inclusion of individuals with higher phototypes in these evaluations [7]. Indeed, regulatory frameworks in Europe, the United

States, and Brazil rely on in vivo SPF methodologies that typically recruit participants with phototypes I-III for standardization [10,17,18]. Consequently, individuals with darker skin phototypes are frequently underrepresented in efficacy testing protocols [8]. In this context, the HPLC–TBARS–EVSC protocol offers a phototype-independent alternative by directly quantifying a lipid peroxidation biomarker [9,11]. Such an approach may broaden the scientific basis of photoprotection research to encompass diverse skin phototypes [8].

Following UVR exposure in the photostability chamber, SC lipid peroxidation in Black participants increased by approximately 297% (Table 2) through the markedly elevated levels of the MDA–TBA_2 adduct (Figure 1). We found that the average areas under the curve (AUC) were 7217 for non-irradiated SC and 27,653 for irradiated SC, yielding an irradiated/non-irradiated ratio of 3.97. A statistically significant difference was observed between irradiated and non-irradiated conditions ($p < 0.05$), despite considerable inter-sample variability. The *SCirr*/*SCn* ratios for each participant are illustrated in Figure 2.

Table 2. Lipid peroxidation parameters in the stratum corneum (SC) of Black participants before and after UVR exposure ($n = 9$).

Parameter	Values
Ratio (*SCirr*/*SCn*)	3.97
Increase in lipid peroxidation (%)	297%
SCn	7217 ± 5251 [1]
SCirr	27,653 ± 24,787 [1]

[1] AUC (average ± standard deviation); *n* = non-irradiated; *irr* = irradiated.

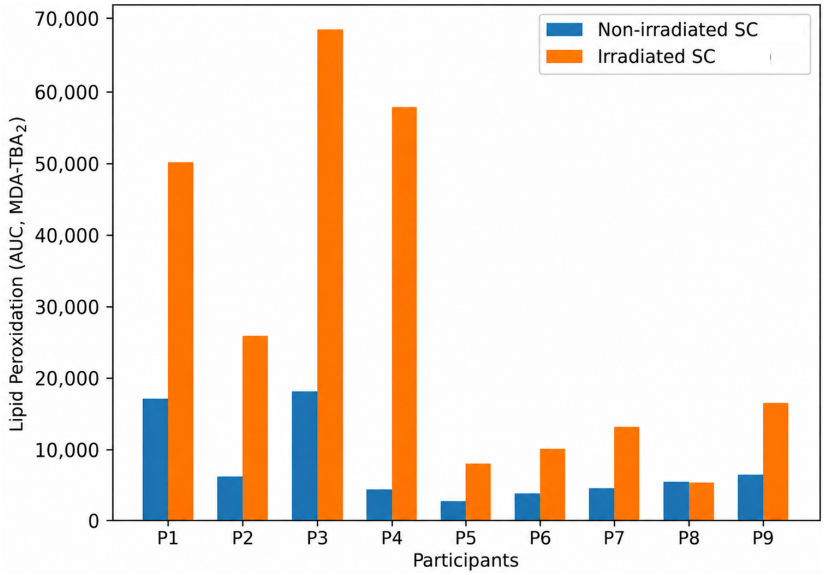


Figure 1. Lipid peroxidation of the stratum corneum (SC) of Black skin, expressed as the area under the curve (AUC) of MDA-TBA_2 quantified by HPLC–TBARS–EVSC protocol. Data are shown individually for each participant (P1–P9).

These findings, which demonstrated the behavior of the SC of Black skin before and after UVR exposure, became particularly meaningful when compared with our previous results using the same protocol; however, those earlier investigations involved mixed groups (phototypes II–V), thereby underscoring the distinct population profile represented by the entirely Black sample evaluated in the present study, as described by Marques et al. (2023) and Ruscinc et al. (2024) [11,12].

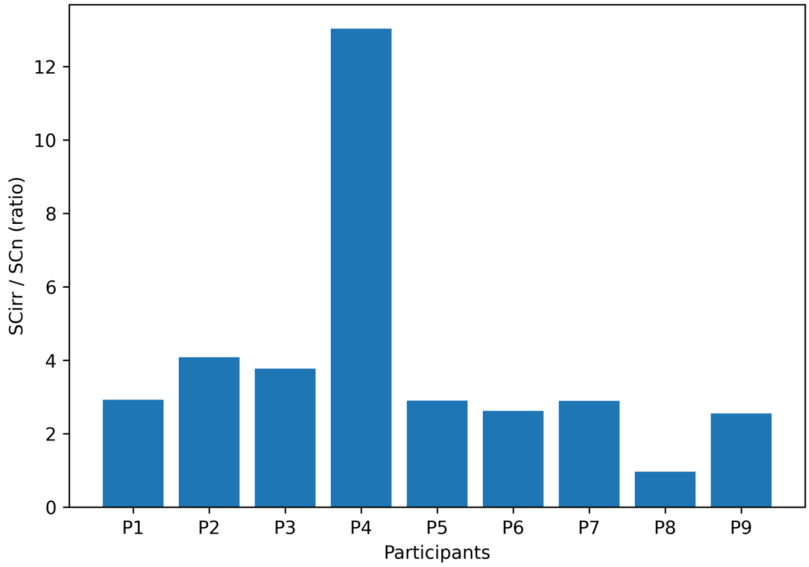


Figure 2. Individual lipid peroxidation ratios (*SCirr*/*SCn*) in the stratum corneum of Black skin, expressed as the ratio between irradiated (*SCirr*) and non-irradiated (*SCn*) conditions. P1–P9 are the participants.

Marques et al. (2023) aimed to streamline the HPLC–TBARS–EVSC methodology using a mixed population of participants (phototypes II–V), comprising 9 males and 9 females ($n = 18$), with emphasis on quantitative outcomes derived from AUC and the irradiated/non-irradiated SC ratio. In males, the AUC increased from 12,493.7 in non-irradiated SC to 22,959.2 in irradiated SC, yielding a ratio of 2.06. In females, a more pronounced difference was observed, with AUC values rising from 9251.7 (non-irradiated SC) to 29,939.61 (irradiated SC), corresponding to a ratio of 3.22. These results highlighted a clear UVR-induced increase in lipid peroxidation, with the magnitude of change captured by both AUC and ratio metrics, indicating a higher relative susceptibility in females compared to males [11]. Similarly, Ruscinc et al. (2024) applied the HPLC–TBARS–EVSC protocol in Caucasian and Black participants (phototypes II–V) to assess polyphenol-mediated protection against UVR-induced oxidative stress. The study reported an irradiated/non-irradiated SC ratio of 2.0, consistent with a doubling of lipid peroxidation following irradiation. Although absolute AUC values were not detailed, the reported ratio reinforced the robustness of this parameter as a comparative indicator of oxidative damage across different study designs and populations [12].

Our results demonstrated high oxidative stress in the SC of Black participants, as evidenced by both an elevated irradiated-to-non-irradiated SC ratio and a pronounced increase in lipid peroxidation. To the best of our knowledge, this is the first study in which the HPLC–TBARS–EVSC protocol was applied exclusively to Black skin.

The lipids of the SC are organized between corneocytes according to the "brick-and-mortar" model. This architecture is essential for barrier integrity, flexibility, and mechanical resistance [19]. Lipid peroxidation occurs when free radicals react with unsaturated lipids, triggering oxidative chain reactions that propagate damage and form lipid peroxides, which may further amplify ROS production [9], and elevated ROS levels have been associated with skin aging, inflammatory skin disorders, and carcinogenesis [20].

4. Strengths and Limitations

The strengths of our investigation include the exclusive evaluation of Black participants (phototypes IV–VI), which addresses an important gap in photobiology research. In addition, the application of the HPLC–TBARS–EVSC protocol enables the objective

quantification of UV-induced lipid peroxidation in the SC using robust analytical parameters that are independent of phototype-related visual endpoints. The use of a minimally invasive ex vivo approach based on tape stripping further strengthens the methodological design by avoiding interferences associated with erythema-based assessments. In fact, most internationally accepted in vivo sunscreen efficacy tests rely on erythema formation as a biological endpoint, which is not a reliable indicator in darker skin due to higher constitutive pigmentation and reduced visible erythemal response. Moreover, such in vivo protocols involve direct UV exposure to subjects, whereas the present ex vivo approach allows the assessment of UV-induced damage through a controlled analytical measurement of a validated biomarker, without inducing clinical photodamage.

However, as limitations, the sample size may constrain the generalizability of the findings and contribute to interindividual variability. In addition, no comprehensive lipidomic profiling was performed, which limits the analysis to lipid peroxidation endpoints rather than a broader characterization of SC lipid composition. Furthermore, although the ex vivo model provides a highly controlled experimental environment, it does not fully reproduce in vivo physiological conditions. Finally, inherent interindividual variability among participants may have influenced the magnitude of the observed responses, reflecting biological differences in skin composition and susceptibility to oxidative stress.

5. Conclusions

Our study demonstrated the applicability of the HPLC–TBARS–EVSC protocol to assess UVR-induced oxidative stress in the SC of Black individuals, representing, to our knowledge, one of the first investigations to apply this methodology exclusively to this population. By employing objective parameters, this approach addresses important limitations associated with erythema-based assessments in darker phototypes.

Our findings suggested that the SC of Black skin was susceptible to UVR-induced oxidative damage. In this context, the MDA–TBA_2 adduct emerges as a relevant biomarker of lipid peroxidation, reflecting alterations in lipids associated with barrier function. The ex vivo tape-stripping approach further enables a minimally invasive assessment of the outermost skin layer.

However, these findings should be interpreted considering the study limitations. Overall, our investigation supports the use of phototype-independent biomarkers to evaluate the effects of UVR, contributing to advances in photobiology research in underrepresented populations.

Ongoing and future studies will investigate the mitigation of lipid peroxidation by UV filters and explore the relationship between oxidative stress and key cutaneous parameters.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

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Abbreviations

The following abbreviations are used in this manuscript:

UVR	ultraviolet radiation
SC	stratum corneum
ROS	reactive oxygen species
HPLC–TBARS–EVSC	high-performance liquid chromatography-thiobarbituric acid reactive species-ex vivo stratum corneum
MDA-TBA_2	malondialdehyde-thiobarbituric acid
SPF	sun protection factor
BHT	butylated hydroxytoluene
TBA	thiobarbituric acid
SCn	non-irradiated stratum corneum
SCirr	irradiated stratum corneum
AUC	area under the curve

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