Received: 16 September 2022 | Revised: 25 October 2022 | Accepted: 11 November 2022

DOI: 10.1111/jocd.15528

JCD Journal of Cosmetic Dermatology WILEY

ORIGINAL ARTICLE

Applying sunscreen SPF 50 with high antioxidant capacity during fifteen days improves the dermis echogenicity and reduces the reddish skin undertone

Victor Hugo Pacagnelli Infante PhD | Patrícia Maria Berardo Gonçalves Maia Campos PhD

School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirao Preto, Brazil

Correspondence
Patrícia Maria Berardo Gonçalves Maia Campos, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Av. do Café, s/n - Vila Monte Alegre, Ribeirão Preto – São Paulo, 14040-900, Brazil.
Email: pmcampos@usp.br

Funding information
Coordenação de Aperfeiçoamento de Pessoal de Nível Superior Coordenação, Grant/Award Number: CAPES - Code 001; Fundação de Amparo a Pesquisa do Estado de São Paulo, Grant/Award Number: 2016/13705-0

Abstract

Background: Of the many effects induced by UV radiation on the skin, erythema is one of the most well-known features, which is a cutaneous inflammatory reaction correlated with acute photodamage. The utilization of sunscreen may reduce this process.

Aims: To evaluate the utilization of a sunscreen SPF50 with high antioxidant capacity during 15 days by young men without photoprotection habits.

Methods: For this, we evaluated erythema, skin hydration properties, and dermis echogenicity using skin imaging techniques. Forty male participants (aged between 18 and 28 years old), 36 without previous photoprotection habits, were recruited, and the erythema was evaluated using a visual score and skin colorimeter. Macroscopic images (VivaCam®) were also obtained. Dermis echogenicity was evaluated using high-frequency ultrasonography. All the participants received a sunscreen SPF 50 to use for 15 days.

Results: The visual score presented a strong correlation ($r = 0.8657$) with the colorimeter results. Visually and using the biophysical methodologies was possible to observe the reduction of the visual erythema. The dermis echogenicity also improved, probably correlated with the acute inflammation reduction. No alterations were observed in the skin hydration and skin barrier parameters.

Conclusions: The utilization of complementary and correlated different skin biophysical and imaging techniques in this study allows a better comprehension regarding the skin early photoaging process due the direct sun exposure. The utilization with a SPF 50 sunscreen with high antioxidant potential allows for a reduction in the erythema after 15 days of usage, a quick result, however, did not improved the skin barrier or SC hydration.

KEYWORDS
claim substantiation, photoaging, skin barrier, skin physiology, sunscreen

1 | INTRODUCTION

The skin is an important barrier against outside aggressions such as sun radiation.[1] However, it can be necessary for some reactions. Ultraviolet B (UVB) radiation, for example, is important in the process of vitamin D synthesis which occurs in the skin.[2] The problem is the excessive exposure which could change the positive effect of sun exposure in detrimental. In this sense, some skin alterations are reported to be related with excessive sun exposure. These alterations may vary form erythema until skin cancer and are related with the accumulative exposure, skin type, and other constitutional characteristics.[3,4]

Skin cancer incidence is increasing, epecially among men, that avoid the sunscreen utilization because of some masculinity norms.[5,6] The erythema (skin burn) is a risk factor for carcinomas.[3,4] Solar ultraviolet radiation (UVR, ~295–400nm) induces DNA damage, which may initiate mutations associated with different skin cancer types.[7] Besides, it is also well-known that sun radiation increases the amount of free radicals in the skin. Visible light and near infrared radiation can induce the production of free radicals in the skin, as well as UV radiation.[4,8]

There are many effects which are induced by UV radiation on the skin, however, erythema is one of the most prominent and well-known features. This alteration—which is related with an increase in the red color of the skin—is a cutaneous inflammatory reaction and is correlated with acute photodamage and higher incidence of skin cancer.[7] The acute unprotected exposure to sunlight increases the vasodilation and the blood flow to the dermis, resulting in the erythema formation.[3]

The unprotected sun exposure is also responsible to reduce the collagen in the dermis matrix and accelerate the photoaging process and increase in the oxidative stress.[9] UVA radiation may penetrate deeper in the skin, being correlated as an important factor in the process of skin photoaging. Such process is characterized by formation of deeper wrinkles if compared with chronological aging, loss of skin tone and reduced elasticity. In this process, the UVA radiation increases the synthesis and expression of matrix metalloproteinase-1 (MMP-1) by dermal fibroblasts. Because of that, the free radical can increase and affect the collagen in the dermal matrix.[10] All these alterations reported so far can be avoided if the population adopt photoprotection habits such as avoid direct exposure, utilization of clothes, and regular application of sunscreen.[11]

The utilization of biophysical and skin imaging techniques is important to study the skin physiology non-invasively.[12] Using these techniques, it is possible to observe the alterations caused by the unprotected sun exposure, for example. In the present work, different techniques were utilized to evaluate the skin physiology. For example, some probes can evaluate the erythema according to the absorption/reflection of a specific wavelength, thus, is possible to achieve the erythema.[13] Besides, high-resolution images can be useful to quantify the erythema formation through clinical scores.[12]

Regarding the dermis echogenicity, the utilization of ultrasonography can be useful to quantify the effects of the skin photodamage. Ultrasound is an important in vivo non-invasive technique which has been used to measure skin thickness and fluid content in the dermatological and cosmeceutical fields.[14] This technique allows to study the epidermis and dermis separately, being an advantage to understand the process of photoaging, for example, which is characterized by a subechogenic band.[15]

This study has the objective to evaluate the utilization of a sunscreen SPF50 with high antioxidant capacity by young men without photoprotection habits during 15days. For this, non-invasive biophysical and imaging skin techniques were utilized.

2 | MATERIAL AND METHODS

2.1 | Material

For the sunscreen formulations: The UV filters methylene bis-benzotriazolyl tetramethylbutylphenol and bis-ethylhexyloxyphenol methoxyphenyl triazine were provided by BASF (Germany) in a concentration of 6% each one; octyl metoxicinamate was also utilized in 6% and was provided by Symrise (distributed by Galena). PEG-75 lanolin in 2% as co-emulsifier and film-forming (ChemyUnion), corn starch in 5% as thickening agent (Maizena), tapioca starch in 5% as thickening and film-forming agent (DaTerrinha), butyl hydroxy toluene (BHT) in 0.05% as antioxidant, glycerin in 6% as humectant, phenoxyethanol combined with parabens in 0.8% as preservative, ethyelenediamine tetraacetic acid (EDTA) as chelanting agent in 0.05% and 2% of butylenoglycol were provided by Mapric Pharmaceutical and Cosmetics Products, polyglyceryl-6-distearate was utilized as emulsifier in concentration of 2% (Gattefossé).

2.2 | Casuistic

This study was approved by the Committee of Ethics in Research involving Human Subjects. After that, 40 participants with ages between 18 and 28years old were selected for this study. Thirty-six participants declared to not have previous photoprotection habits and 4 declared to apply daily the sunscreen formulation. The study was performed in June in Ribeirão Preto, (21.17°S; 47.7° W) Brazil, autumn season in south hemisphere, however, with a UV index higher than 6.[16] In this study, we measured the malar area, except the high-resolution images which were acquired from all the facial area.

The ethical standards for this study followed the Declaration of Helsinki of 1975, which was revised in 2013. To participate in this study, it was important that the participants should not be a smoker or be in treatment of oral isotretinoin. We also not include participants with diagnostic of skin diseases or with hypersensitivity of cosmetic products. Subjects which have had surgeries or aesthetic procedure in the past year were also not included. After being oriented about the research the participants which agree to participate were instructed to sign the Informed Consent Term. Participants remained from 15 to 20 minutes, for acclimatization, in a room with controlled temperature and humidity, 20–22°C and 45%–55%, respectively.

All the participants received a sunscreen formulation with SPF 50 and high radical protection factor which was previously published.[17,18] The sunscreen formulation presents a low amount of oil and/or moisturizer agents on its composition to reduce possible interferences in the clinical study. The participants were oriented to apply the sunscreen in the face at least one time during the day, before the sun exposure. We did not standardize the sunscreen weight and/or volume to simulate the real usage patterns.

2.3 | Clinical scoring

To provide a more detailed skin characterization, skin from the participants in this study was scored, using a five-point scale for the erythema in the face, where one means absent and five a high erythema.[12]

2.4 | High-resolution images

We utilized Visioface® equipment (Courage & Khazaka), which is a system which was created to obtain high resolution photos from the face of the participants. This device is composed of a digital camera which presents a white light of diode. A frontal angle image was obtained per participant in the basal measurement and after 15 days.

2.5 | Macroscopic images

We utilized a digital macroscopic camera (VivaCam®) for the erythema observation. The images were utilized to observe if the utilization of sunscreen for 15 days reduced the erythema in some participants and to observe the telangiectasia presence.

2.6 | Reflectance colorimeter

An area of 20 mm^2 form the skin was measured using a reflectance colorimeter. For this, a disk with a diameter of 5 mm is placed in the skin being possible to measure the skin erythema and melanin (Mexameter® MX 16, Courage & Khazaka). This device is already commercial and have 16 diodes which are positioned on the edge of the photodetector. The wavelengths emitted by the different diodes are related to 568, 660, and 880 nm. These wavelengths correspond to green, red, and infrared light, in this order. The cutaneous hemoglobin amount—which is related with erythema—is possible to be measured because of the photo-detection prevenient from the reflected light after the green and red emissions. For the melanin amount in the skin, the reflected light correspondent to red and near infrared are utilized. The values are obtained in arbitrary units.[13]

2.7 | Aqueous content of the SC (Corneometer®)

In this study, we measured the skin hydration, what means, aqueous content of the SC, from the participants. For this, a Corneometer® (Courage & Khazaka) was utilized. This equipment is based on the principle of electrical capacitance, which is related with the variation of the dielectric constant. For water molecules, this value is much higher than for other skin components. Using this device, arbitrary units (AU) are the units for our results. According to the provider, 1 AU may correspond to the range of 0.2–0.9 mg of water per gram of dry SC. We obtained five measurements and calculated the average.[19]

2.8 | Determination of transepidermal water loss (TEWL)

The skin barrier function was measured using a Tewameter® TM 210 device (Courage & Khazaka). This device is coupled to a software and is based on the diffusion principle which was described previously by Adolf Fick. Using this device, the results are obtained as function of $g.m^2.h^{-1}$. During the measurements, the probe remains 15 seconds in contact with the skin. The measures were performed in triplicate and the average value was utilized as well as the standard deviation (SD).[19]

2.9 | Dermis echogenicity

Were studied the dermis thickness and echogenicity using a 20 MHz ultrasound Dermascan C® device (Cortex, Hadsund). This device is largely applied in the dermatological field and contains a transducer focus which allows to obtain 2-dimensional images. The ultrasonic wave generated by this device has a speed of 1580 ms^{-1}. These waves are partially reflected by the different skin structures, which creates echoes of different amplitudes. These echoes can be differentiated by the software according to the intensity and amplitudes, being possible to calculate the echogenicity. For this, we measure the number of pixels with low echogenicity using the software accoupled with the device and related these values with the total number of pixels. Thus, it is possible to calculate the echogenicity ratio. This ratio means the relation between the number of low echogenic pixels and the number of total echogenic pixels (LEP/TEP). This value is proportionally inverse with the collagen density, what means, low values of echogenicity are related with better collagen density and distribution.[19]

2.10 | Perception of efficacy

After 15 days, all the participants were questioned regarding the amount of sunscreen application and to share about the experience to utilize sunscreen daily and the perception of efficacy.

2.11 | Statistics

The experimental data obtained were submitted to statistical analyzes. First, we calculated the normality distribution using the Shapiro–Wilk test. After that, a simple t-test was applied if the sample was normal. If the distribution was non-normal, we analyzed with Mann–Whitney t-test. We utilized the software Prism GraphPad 9.0 (San Diego). A Pearson's correlation test was developed between the high-resolution images score and the reflectance colorimeter values to observe the accuracy of the score.

3 | RESULTS

3.1 | Characterization and score development

In this study, all the participants without photoprotection habits presented indicatives of erythema in the face, especially in the malar and nasal areas. However, these results were not homogeneous, thus a score was developed using the high-resolutions images to semi quantify the obtained results (Figure 1).

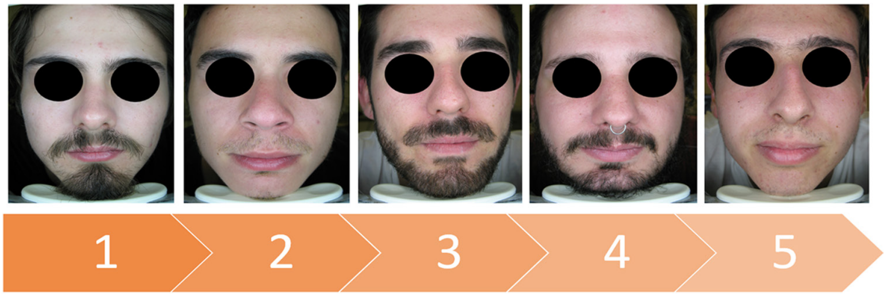

FIGURE 1 Erythema score using high-resolution images.

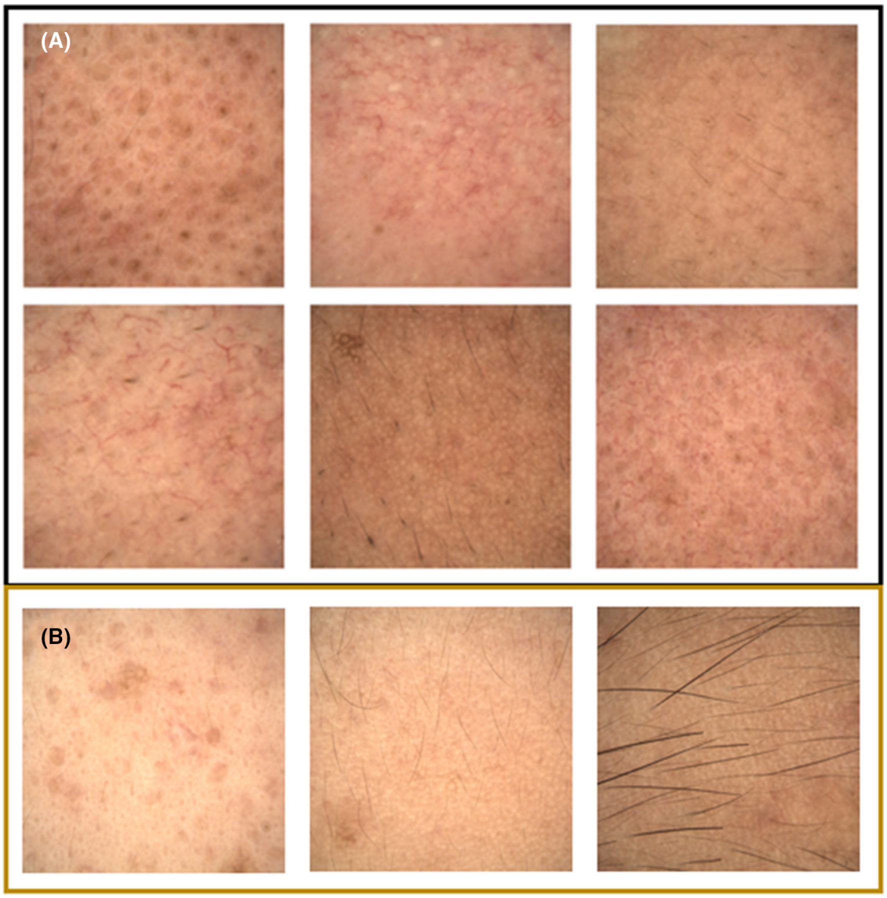

FIGURE 2 Comparation between macroscopic images for participants without photoprotection (A) and that applies sunscreen regularly (B). The group without photoprotection habits presented a more reddish skin and higher density of telangiectasia.

Using the macroscopic images, we could observe an increase in the reddish skin tone for the participants without photoprotection, besides an increase in the telangiectasias, indicatives of photoaging process (Figure 2).

3.2 | Clinical efficacy

The TEWL basal measurements were 18.6 ± 5.9 g/h/m^2 and after 15 days it was 19.6 ± 4.8 g/h/m^2, evidencing no differences. For the SC water content, no differences were also observed after 15 days (45 ± 8 arb. Unit × 47 ± 10 arb. Unit.). In another hand, a significative reduction in the erythema using both, score, and reflectance colorimeter, after 15 days of sunscreen application ($p < 0.001$) was observed (Figure 3). The Pearson's correlation between the high-resolution image score and the reflectance colorimeter was considered particularly good, with a high positive correlation ($r = 0.8657$; $p < 0.001$).

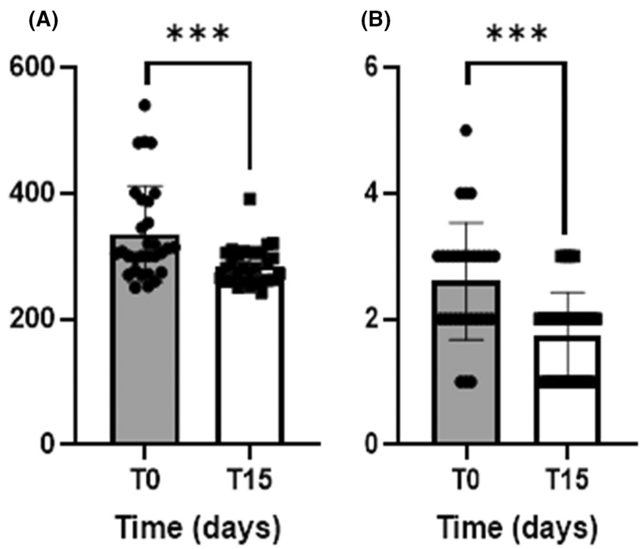

FIGURE 3 Erythema reduction after 15 days of sunscreen utilization using the colorimeter (A) and the high-resolution images score (B). Where (***) means $p < 0.001$.

In fact, the utilization of sunscreen reduced the visible erythema after 15 days according to the high-resolution images. The utilization of macroscopic images allows to observe the reduction in the reddish in both methods (Figure 4).

Besides the erythema reduction, it was possible to observe an improve in the echogenicity ratio after 15 days ($p = 0.009$) (Figure 5).

The improvement in the echogenicity was observed in most of the participants. It is possible to compare the images from one of the participants with photoprotection habits with one participant without photoprotection habits, evidencing a lower echogenicity for the second group. The echogenicity scale of the skin according to the pixels' colors is as follows: white> red> yellow> green> blue> black (Figure 6).

3.3 | Perception of efficacy

All the participants informed that applied daily the sunscreen before the direct sun exposure. They informed that applied a thin layer of sunscreen in all the facial region and did not reapply during the day. Eighty-six percent informed that felt the skin less reddish and softer.

4 | DISCUSSION

It is well-know that men use less sunscreen than women,[6,19,20] resulting in an early photoaging process and a higher incidence of skin cancer on this population. In previous studies we developed a sunscreen formulation with high SPF, dry touch, and high antioxidant capacity.[17,18] Here, we want to observe the impacts of the sunscreen application in the skin healthy of young men without photoprotection habits and early photoaging signals in a short period of time.

In this study, the participants utilized a sunscreen with UVA, UVB protection (SPF 50), and high antioxidant capacity, which allows the reduction of free radicals' formation in the visible/NIR light.[17,18] The high protection in the UVB is fundamental to avoid the erythema formation due its inflammatory response.[13] The participants are

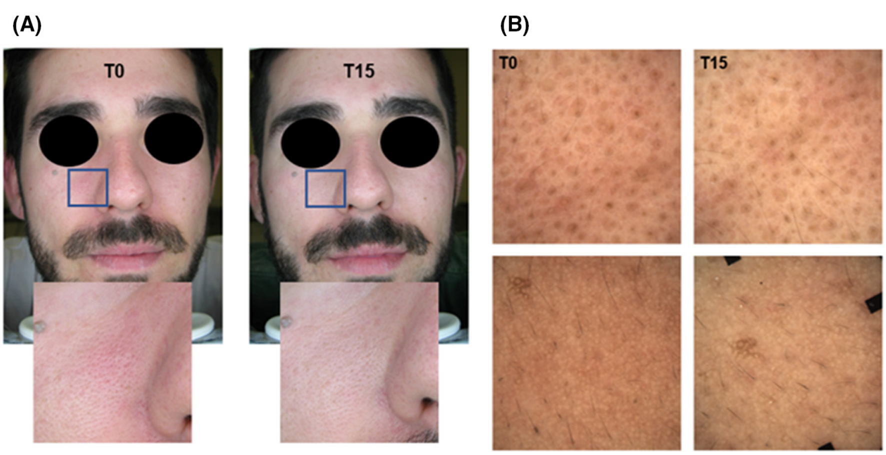

FIGURE 4 High resolution images (A) from a participant before (T0) and after 15 days of sunscreen application (T15). It is possible to observe the reduction in the redness. In the macroscopic images (B), the reddish is also perceptible after 15 days in both images.

young men from Brazil, and they avoid the utilization of sunscreen formulations during the ordinary activities, being mostly exposed for the sun radiation.[16,19,20] The study in the autumn season evidences that even in a season with lower solar incidence (UV index between 6 to 10), the erythema formation is prevalent among young men without photoprotection habits.

In other studies, comparing the skin parameters of men without photoprotection habits with young men that applies sunscreen frequently, we observed a reduction in the skin barrier function, SC hydration and dermis echogenicity for the group with unprotected exposure to the sunlight.[19,20] Thus, the utilization of a sunscreen formulation was purposed for the participants without photoprotection habits to better understand if in a short time some parameters involved in the photoaging process could be improved.

In fact, the skin barrier function and the SC hydration did not present significative alterations after 15 days of sunscreen utilization and being correlated with the skin physiology. The unprotected skin can suffer solar hyperkeratosis, because the thickened SC also leads to a stronger light scattering. This thickening effect can reduce the skin hydration parameters. Besides, the epidermis is the outermost layer of the skin, consisting of stratified keratinized pavement epithelium. The epidermal cells (keratinocytes) come from the lowest layer of the epidermis—basal layer—and in their way towards the surface they undergo a keratinization process, with cellular apoptosis, giving rise to the SC layer.[21] This process takes from 14 to 28 days and the TEWL is closely related to it since the better stratification in the SC can improve the skin barrier function.[22]

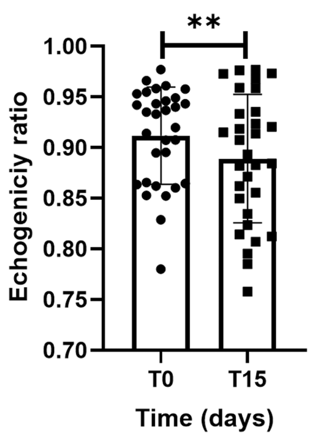

FIGURE 5 Echogenicity reduction after 15 days of sunscreen application, where (**) means $p = 0.009$.

The high and positive correlation between the high-resolution images score and the colorimeter allowed to validate a simple and efficient methodology to access the erythema formation. Thus, the perception of reduction in the reddish skin subtone after 15 days by the participants matches with the results presented by the biophysical techniques. It is clear, with all the skin biophysical and imaging techniques, that the sunscreen application daily presented positive, quick, and satisfactory results (Figure 4). The results are even more interesting when we considered that we did not standardize the amount of sunscreen application and the participants utilized just a thin layer of formulation without reapplication during the day. It is well-known that the SPF and its protection is correlated with the sunscreen amount applied in the skin surface and the reapplication during the day is necessary.[23]

The improve in the dermis echogenicity can be correlated with the other results since, with the reduction in the redness, the erythema formation due the UV exposure, may be suppressed. Since we have a probable inflammatory acute process, other classical signals may be present such as the accumulation of water in the tissues knowing as edema. The edema formation in the skin probably causes interferences in the high frequency ultrasonography analyzes since the water can interfere in the ultrasound wavelengths and its response in the basal measurement.[24] After 15 days of sunscreen usage, the acute inflammatory process is probably reduced, allowing a better accuracy of the methodology.

Finally, this study was important to validate a scale of erythema according to objective measures using a skin reflectance colorimeter. This find can be useful for future studies and clinical evaluations of erythema. Thus, the short-time study using young men without

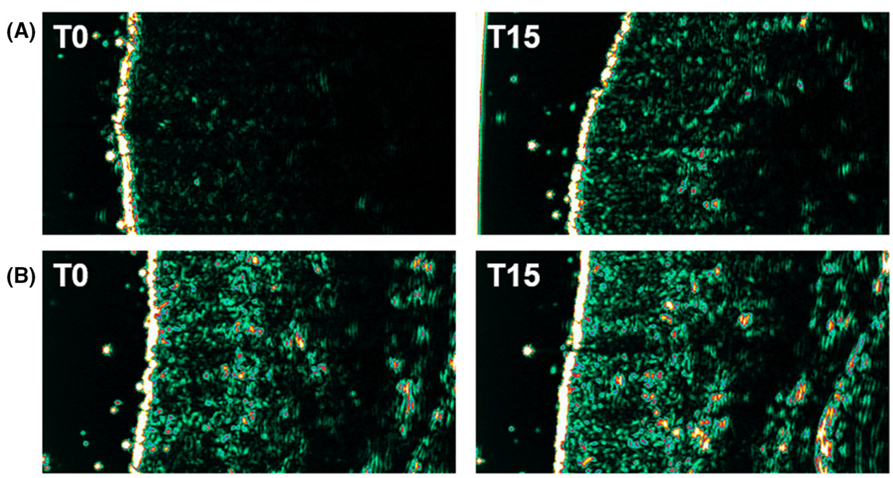

FIGURE 6 Dermis echogenicity accessed with high-frequency ultrasound. The brighter the pixels, the most echogenic is the dermis. The participants with erythema (A) presented a low density of bright pixels in the initial time and after 15 days the images improved. For the participants with photoprotection habits (B), a high echogenicity dermis was always observed.

photoprotection habits is also important evidence regarding to the importance of sunscreen daily utilization, which was able to improve the overall skin healthy. In a future perspective we hope to evaluate the impacts of sunscreen application using other population such as older men and/or women.

In conclusion, young men that did not apply sunscreen daily presents an increase in the reddish skin subtone. The utilization of a SPF 50 sunscreen with high antioxidant potential allows for a reduction in the reddish skin subtone and improve the dermis echogenicity after 15 days of usage. We observed a quick improve in some skin parameters, however, it was not enough to improve the skin barrier or SC hydration. According to our finds, the sunscreen application needs to be reinforced by dermatologists, especially for the young masculine population, which present some resistance for the adherence of photoprotection habits.

AUTHOR CONTRIBUTIONS

Dr. Victor Infante was responsible for the conceptualization, data collection, formal analysis, and paper structuration. Prof. Dr. Patrícia Campos was responsible for supervision, paper structuration, and formal analysis.

ACKNOWLEDGMENTS

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP (grant number: 2016/13705-0) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior Coordenação – CAPES – Code 001.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study was approved by the Committee of Ethics in Research involving Human Subjects (CEP/FCFRP 58368416.6.0000.5403).

ORCID

Victor Hugo Pacagnelli Infante https://orcid.org/0000-0001-7151-5755
Patrícia Maria Berardo Gonçalves Maia Campos https://orcid.org/0000-0001-6678-1207

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How to cite this article: Infante VHP, Maia Campos PMBG. Applying sunscreen SPF 50 with high antioxidant capacity during fifteen days improves the dermis echogenicity and reduces the reddish skin undertone. *J Cosmet Dermatol*. 2023;22:872-879. doi:10.1111/jocd.15528