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Non-invasive diffuse reflectance measurements of cutaneous melanin content can predict human sensitivity to UVR

Sergio G. Coelho^{1,*}, Barbara Z. Zmudzka^{2,*}, Lanlan Yin¹, Sharon A. Miller², Yuji Yamaguchi¹, Taketsugu Tadokoro¹, Vincent J. Hearing¹, and Janusz Z. Beer²

¹Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 USA

²Center for Devices and Radiological Health, Food and Drug Administration, Silver Spring, MD 20993 USA

Abstract

The diversity of human skin phenotypes and the ubiquitous exposure to ultraviolet radiation (UVR) underscores the need for a non-invasive tool to predict an individual's UVR sensitivity. We analyzed correlations between UVR sensitivity, melanin content, diffuse reflectance spectroscopy (DR) and UVR-induced DNA damage in the skin of subjects from 3 racial/ethnic groups: Asian, Black or African American and White. UVR sensitivity was determined by evaluating each subject's response to one minimal erythemal dose (MED) of UVR one day after the exposure. Melanin content was measured using DR and by densitometric analysis of Fontana-Masson staining (FM) in skin biopsies taken from unexposed areas. An individual's UVR sensitivity based on MED was highly correlated with melanin content measured by DR and by FM. Therefore, a predictive model for the non-invasive determination of UVR sensitivity using DR was developed. The MED precision was further improved when we took race/ethnicity into consideration. The use of DR serves as a tool for predicting UVR sensitivity in humans that should be invaluable for determining appropriate UVR doses for therapeutic, diagnostic and/or cosmetic devices.

Keywords

race/ethnicity; ultraviolet radiation; melanin; erythema; DNA damage

Introduction

Human skin is chronically exposed to environmental ultraviolet radiation (UVR), and many individuals are also additionally exposed to UVR from a wide range of therapeutic, diagnostic and/or cosmetic devices (1). UVR is the major cause of skin cancers and the incidences of melanoma and non-melanoma skin cancers are rapidly increasing (2,3) although they vary dramatically in different racial/ethnic (R/E) groups. At the same time, some beneficial effects of UVR exposure have been reported (4-6). The importance of modernizing public health policies in this area has been officially recognized (7,8), and there is an urgent need for rapid and reliable tools that can predict an individual's sensitivity to UVR (9).

Address for correspondence: Sergio G. Coelho Biologist, Pigment Cell Biology Section Laboratory of Cell Biology Building 37 Rm 2132 National Institutes of Health Bethesda, MD 20892, USA Phone: 301-594-9645 coelhos@mail.nih.gov.

* Authors contributed equally to this manuscript.

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It is well known that dark-skinned individuals, with high melanin content in their skin, are less sensitive to UVR than are light-skinned individuals. However, skin color, which is determined by cutaneous melanin content, can only roughly predict the UVR sensitivity of an individual (10-15). In fact, different individuals with the same cutaneous melanin content may show very different UVR sensitivities (16,17). Further, the induction of erythema and melanogenesis by UVR exposure have different wavelength-dependent mechanisms (18). Clearly, melanin content is only one of the parameters that determines an individual's sensitivity to UVR-induced sunburn, DNA damage and cancer. Fitzpatrick and coworkers considered using race as a basis for skin classification (19), and eventually they developed the widely known phototyping system, which is based on an individual's propensity to burn and/or tan following UVR exposure (19). The Fitzpatrick classification has served its purpose for several decades under clinical conditions where highly qualified personnel can make adjustments of therapeutic UVR doses by trial and error (20,21). However, phototyping cannot be used as a definitive accurate method of predicting UVR sensitivity (22).

Due to the complexity of individual UVR sensitivity, many variables (e.g. internal and external environmental conditions, temperature and illumination) affect its determination and the observer's subjective visual assessment is a significant limitation. For sunscreen testing, guidelines for visual assessment of UVR sensitivity have been set forth (23). In previous decades, colorimetric measurements were shown to provide a level of objectivity to screen for UVR sensitivity based on skin pigmentation using CIE $L^*a^*b^*$ color space system variables and its vector representations (24-27), but since this early progress significant shortcomings with this approach have been described (28). Some authors using colorimetric measurements have gone to great lengths to avoid the confounding issue of erythema under pigmentation by using noradrenaline iontophoresis. Whether that provides biologically relevant information from a color system designed to approximate color perception is debatable (26). Consequently, quantification of erythematous responses in dark-skinned individuals has been challenging for both visual and colorimetric techniques. This inadequacy led to the development of diffuse reflectance spectroscopy (DR) to quantify skin chromophores for melanin, oxyhemoglobin and deoxyhemoglobin (28-30). In addition, there are simple diffuse reflectance instruments that evaluate specific spectral bands using light emitting diodes which have been used to evaluate UVR sensitivity (31-33), and the limitations of these instruments has been detailed previously (28).

Building on previous pioneering work, we have now created a simple non-invasive model that correlates UVR-induced erythema (as an indicator of UVR sensitivity) with cutaneous melanin content. Erythema is the main, readily-measurable acute response to UVR. In addition to the erythematous response, cyclobutane pyrimidine dimers (CPD) are one of the main forms of UVR-induced DNA damage that can trigger the carcinogenic process (34-36). Here we used data from our study on UVR responses in different R/E groups among inhabitants of the Washington, D.C. area of which some results have been previously reported (37-40). As stipulated by the FDA Research Involving Human Subjects Committee, we defined R/E groups according to accepted US standards (http://www.whitehouse.gov/omb/fedreg_1997standards), which defines 6 distinct R/E groups. For our model, we used 3 of these classifications, "Asian", "Black or African American" (hereafter called Black) and "White". Our findings show that to predict UVR sensitivity, DR serves as an objective and reliable non-invasive parameter that should be taken into consideration when considering UVR doses for therapeutic, diagnostic and/or cosmetic devices.

Materials and Methods

Study subjects

This study was conducted according to protocol #99-002-R approved by the Research Involving Human Subjects Committee of the U.S. Food and Drug Administration, and adhered to the Helsinki Guidelines. The study included 59 subjects (18 males, 41 females), who belong to one of the six R/E groups defined by the Office of Management and Budget Classification (OMB 0990-0208). CPD damage was measured for 43 of these 59 subjects. Individual R/E groups were represented by (in parentheses are the numbers of subjects for whom CPD damage was measured): Asian - 10 (9); Black - 18 (10); White - 31 (24). Eight subjects (CPD damage was measured for 5 of them) reported one or two grandparents of a different race than that dominant for them. A listing of all study subjects is given in Table S1. Before entering the study, each subject gave informed consent and was evaluated by a dermatologist. For inclusion and exclusion criteria, see (37).

UVR exposures and MED determination

An array of 8 Kodacel-filtered FS fluorescent lamps was used as the source of UVR (approximately 40% UVB and 60% UVA with a broad peak at 313 nm.) UVR output (250–400 nm) was measured in units of spectral irradiance ($\text{W}/\text{cm}^2\text{-nm}$) at 2-nm increments with an Optronic Laboratories (Orlando, FL) Model 754 portable spectroradiometer. The distance between the lamps and the skin was 22–27 cm and was adjusted to deliver approximately $100 \text{ J}/\text{m}^2\text{-eff}$ per minute (fluence rate of $1.67 \text{ W}/\text{m}^2\text{-eff}$, 250–400 nm, weighted with the CIE action spectrum for erythema (41–43)). To determine the MED of each subject, seven $2 \times 2\text{-cm}$ skin areas on one side of her/his back were exposed to a geometric series of UVR doses with 40% increments (intended range, 0.5 - 4.0 MED). Before exposure, the amount of UVR radiation delivered to each $2 \times 2\text{-cm}$ area was checked with a low profile detector that was coupled to a radiometer (IL 1700, International Light, Newburyport, MA) and, based on the reading, adjustments to the exposure time of each area were made to achieve the desired dose. One day later, the MED for each subject (defined as the lowest dose that produces pink erythema with at least one distinct border) was determined by eye. By design, the applied exposure series yields MED values that may be overestimated up to 40%. In most cases, one of the exposed $2 \times 2\text{-cm}$ areas showed pink erythema with one or more borders. However, in some cases, the lowest dose responding area showed a relatively strong pink-red reaction (in this case the lowest dose responding area is not the lowest dose given in the geometric series of UVR doses with 40% increments). In such cases, a value corresponding to approximately the middle of the interval between the “no response” and the “first and strong response” was used as the MED. However, we indicate the doses of “first and strong response” in those subjects in Table S1, which were not used as the MED in order not to overestimate the MED. We attempted to expose all areas to be biopsied to 1 MED and established the actual doses using the low profile detector mentioned above. The investigated area on the lower back usually had a history of exposure to solar or sunlamp UVR. However, these areas were not exposed to UVR for at least 6 weeks (in most cases >6 months) preceding the study. To reduce the pre-study environmental UVR exposure, this study was conducted between the months of October and May.

Skin biopsies

Immediately after MED determination, several $2 \times 2\text{-cm}$ areas on the contralateral side of the back were exposed to 1 MED (47 subjects) or $200 \text{ J}/\text{m}^2$ (12 subjects) of UVR. “Immediately” (7 min) after exposure, one of these areas was shave biopsied using a modified technique described in (44). The 4-mm diameter tissue sample was cut into two equal pieces; one was frozen and the other was paraffin embedded as described in (37). The frozen specimen was used to determine CPD damage while the paraffin-embedded specimen

was used for determination of melanin content by FM (37). As a control, unexposed tissue was biopsied from adjacent unexposed skin, processed, and analyzed in the same manner.

Melanin content determination by DR

Melanin content in the skin determined by DR (M_{DR}) was derived from reflectance spectra obtained with a Minolta spectrophotometer CM-2002 (Minolta Corporation, Ramsey, NJ). The Minolta CM-2002 has an integrating sphere and takes DR readings from 400 to 700 nm at 10-nm increments. The instrument also has a target mask to help with placing its 8-mm aperture precisely on the desired area and to minimize pressure of the integrating sphere aperture on the skin.

Before measurements, the subjects rested on the examination table in a prone position for several minutes or until any marks left by their clothing disappeared. DR measurements were then taken on 4 unexposed skin areas on the lower back. The spectra were taken in triplicate, $SE < 0.5\%$, of the 4 skin areas, SE for these areas was $< 2\%$. The DR data were converted into apparent absorbance (AA) spectra, where AA is the logarithm of the ratio of DR for the control “no melanin” skin ($DR_{no\ m}$) to DR for the melanin-containing skin (DR_m) ($AA = \log(DR_{no\ m}/DR_m)$). The linear part of the 630–700 nm spectrum was used to calculate melanin content according to (45) with the modifications of Jacques (46) who found that the 650–800 nm slope of DR spectra taken from the inner upper arm of extremely fair Caucasians does not significantly differ from those of the vitiligo (non-pigmented) sites. According to these modifications, for the $DR_{no\ m}$, the DR spectra were used from skin on the inner upper arm of our least-pigmented subject. As proposed by Jacques, the melanin extinction coefficient of Sarna and Swartz (47) was used to express melanin content in mg eumelanin per ml.

Determination of melanin content by FM

FM was used to determine melanin content in the biopsied skin samples as described in (37). Paraffin-embedded specimens fixed with formaldehyde were sectioned at 3- μ m thickness, mounted on silane-coated glass slides (in duplicate), and stained by the Fontana-Masson method (48). Special care was taken to stain all sections under the same conditions. Densitometry was performed using the Leica DMRB/DMLD microscope (Leica, Wetzlar, Germany) which can measure transmitted light intensity. Melanin quantity was analyzed from the integrated optical density in the epidermis in each section. Ten randomly selected areas of each specimen were photographed and quantified; SE was usually $< 5\%$. The results are expressed as the intensity of the black stain divided by the area of the epidermis.

Determination of CPDs

The methodology used to measure CPDs was as previously described (49,50). Briefly, we used a monoclonal antibody (TDM-2) which is specific for CPD, and we detected specifically bound TDM-2 antibody with a secondary antibody conjugated to fluorescein isothiocyanate (FITC). Nuclear DNA was counterstained with propidium iodide (PI). The FITC fluorescence was then superimposed over the PI fluorescence to check co-localization. CPDs induced by UVR exposure were obtained by subtracting the ratio of TDM-2-FITC/PI fluorescence for the unexposed (control) biopsy from that ratio for the UVR-exposed biopsy.

Statistical analyses

MED and CPD per 100 J/m² were log transformed prior to conducting parametric analysis. Due to saturation of M_{FM} , the relationship between M_{FM} and M_{DR} was explored by negative exponential regression ($y = a \cdot (1 - \exp(-b \cdot x))$). The dependence of UVR sensitivity (MED) or the yield of UVR-induced DNA damage (CPD per 100 J/m²) on melanin content

(M_{DR} , or M_{FM}) was assessed by simple linear regression. Associations between R/E and MED or UVR-induced damage (CPD per 100J/m²) were explored by ANOVA. P-values <0.05 are considered statistically significant. A generalized linear model was employed to predict MED by M_{DR} and R/E (since the interaction term between MDR and R/E is not significant, only the main effects are kept in the predictive model). Two subjects (S2 and S43) had negative M_{DR} values, and therefore were excluded from the predictive model. Residual plots were used to verify the model assumptions of normality and homoscedasticity, and a calibration plot was used to explore the agreement of predicted MED and observed MED. All statistical analyses were conducted using SAS 9.2 (SAS Institute, Cary, NC).

Results

Melanin content measured by DR and by FM

Melanin content measured in the skin of the study subjects by DR and by FM (M_{DR} and M_{FM} , respectively) are compared in Fig. 1 and Tables 1 and S1. The differences between M_{DR} and M_{FM} derive from the fact that the two methods are based on very different principles: DR utilizes reflectance of the skin to measure melanin content, whereas FM relies on densitometry of the silver stain in skin sections. With the exception of subjects S43 and S2 for whom the M_{DR} values were below zero, the M_{DR} varied from 0.1 ± 0.1 to 22.9 ± 0.2 , while M_{FM} varied from 1.3 ± 0.2 to 20.5 ± 1.3 . There was a strong correlation between the two methods indicated by the negative exponential regression ($p < 0.001$, correlation coefficient = 0.800) in Fig. 1a. When comparing the two methods, it should be noted that DR, unlike FM, is a non-invasive method.

The distribution of melanin stained by FM in the epidermis of the 3 R/E groups is localized predominantly in the basal and lower spinous layers of the epidermis (Fig. 1b). Substantial differences in the melanin content among the 3 R/E groups are obvious and occur in all layers of the epidermis. Images such as those in Fig. 1b were quantified as described in the Methods. Table 1 shows that the total melanin content varied significantly over the 3 R/E groups. Black skin contained several-fold (~6x or ~3x, depending on the method used) times more melanin than White skin, with Asian skin showing intermediate levels. However, all White subjects showed <4.7 mg/ml or 8.3 AU of melanin. In the Black group, all subjects except for one (S28 of mixed ancestry) had melanin content 6.7 mg/ml and 9.0 AU. Hence, melanin content ranges for Black and White skin showed little overlap.

Melanin content and MED

The ratios between the highest and the lowest M_{DR} and M_{FM} values were not similar compared to the corresponding ratios in MED values (Table S2). This observation illustrates the complexity of the relationships between melanin content in the skin and UVR sensitivity (51). The fact that MED tends to increase with an increase in constitutive melanin content measured by M_{DR} and by M_{FM} is as expected (Fig. 2). MED values tend to be lowest for White subjects and highest for Black subjects; however, there is some overlap between MED values for these two R/E groups and there is a considerable overlap between MED values for Asian subjects and those for Black and for White subjects. The data suggest that small changes in melanin content in fair skin can substantially affect UVR responses (Fig. 2a and 2b). The correlation between MED and M_{DR} ($R^2 = 0.659$) was far better than the correlation between MED and M_{FM} ($R^2 = 0.431$) which emphasizes the rationale for using the non-invasive DR method to predict UVR sensitivity (Table S3).

Melanin content and induction of DNA damage

To compare the DNA damage-inducing effectiveness of the same physical dose in skins with different melanin contents, we evaluated CPD damage per 100 J/m² of UVR. The amount of CPDs induced by exposure to 100 J/m² of UVR and their distribution in the epidermis is different in the three R/E groups. The CPD damage per 100 J/m² is lower in more pigmented skin as expected (Fig. 1b, Tables 1 and S1). While the CPD distribution is uniform throughout the epidermis in less pigmented skin, the CPD damage in dark skin declines with increasing distance from the skin surface. In this study, we analyzed the effects of total melanin content on the induction of DNA damage without adjusting for the spatial distribution of the melanin content within the epidermis.

The relationships between the CPD damage and melanin content for 43 subjects in different R/E groups is complex (Fig. 2c and 2d). CPD damage was measured “immediately” (7 min) after UVR exposure. These observations show that a UVR dose induces more DNA damage in human skin with low melanin content than in skin with high melanin content (regardless of the method used to determine melanin content) (Fig. 2c and 2d). Simple linear regression results (Table S3) indicate that in addition to MED ($p < 0.001$), the CPD damage per 100 J/m² ($p = 0.012$) is significantly associated with M_{DR} .

The MED predictive model

In addition, ANOVA analysis shows that the relationships between R/E and MED or CPD damage are statistically significant (Table S4). This led us to consider R/E as an additional variable in UVR sensitivity determination. Clearly, cutaneous melanin content can be used to determine skin sensitivity to UVR, but our data also indicate that R/E may improve the accuracy of such a determination. Using experimental data (Table S5), we developed a predictive model for the relationships between melanin content, R/E and MED: $\ln(MED) = M_{DR} + R/E$ (where $R/E(A) = 0.22$, $R/E(B) = 0.43$, or $R/E(W) = 0$); with an $R^2 = 0.708$, $p < 0.001$ (for the effect of M_{DR}), and $p = 0.017$ (for the effect of R/E). This linear regression model was developed to predict UVR sensitivity (MED) using the non-invasive M_{DR} method. A residual plot and a normal probability plot (Q-Q plot) were employed to justify the assumptions of linear regression models: homoscedasticity (constant variance) of the errors and normality of errors. The residual plot shows constant variance of errors along M_{DR} , and the Q-Q plot shows no significant difference between residual distribution and normal distribution (Fig. S1). Both the residual plot and the normal probability plot, together with the scatter plot showing the linear relationship between MED and M_{DR} , validate the usage of the linear regression model. Using the parameters defined in Table S5 and the individual's M_{DR} value, one can then get a reliable estimate of an individual's UVR sensitivity in MEDs when comparing the predicted MED vs the observed MED (Fig. 3). Points along the diagonal line of the plot indicate perfect predictions with our model.

Discussion

We investigated the effects of cutaneous melanin on UVR responses in human subjects of various R/E origins. Melanin content and MED were measured in all 59 volunteers, and in 43 of those 59 volunteers, the CPD damage caused by UVR exposure was determined (Table S1). Melanin content was measured using a non-invasive technique (DR) and an invasive biopsy method (FM). As reported previously (10,15,17,51), our data show that melanin strongly affects UVR responses of the skin: our analysis of combined data for all R/E groups indicates that the correlation between melanin content and MED or CPD damage per 100J/m² are statistically significant (Fig. 2, Table S3). The difference in these relationships may be related to the nature of the DNA damage/erythema dependence. It has been shown that UVR-induced DNA damage precedes erythema (34,52), and that CPD

induction solely in the superficial layer of the epidermis is sufficient to produce erythema (52). Hence, while the CPD analysis measures DNA damage across the epidermis, the MED data may reflect the effect of only a fraction of the induced CPD. Our results are consistent with previous reflectance analysis on phototypes I-IV, which showed that constitutive pigmentation correlated well with MED both in healthy individuals and in individuals with skin cancer (33). In addition, a subsequent study by the same group indicated that reflectance data in phototypes I-V again showed good correlations with MED. However, the authors noted problems with erythema evaluation at higher pigmentation levels (32).

Total cutaneous melanin content is insufficient to adequately explain all the variations in UVR sensitivity of human skin. This is strikingly illustrated by the data presented in Table S1. Selected matched pairs of subjects with similar melanin content, e.g., S5 (Asian) and S80 (White) (melanin content 2.27 and 2.10 mg/ml by DR and 3.4 AU for both by FM) or S37 (Black) and S61 (Black/ American Indian or Alaska Native) (melanin content 10.04 and 10.15 mg/ml by DR and 14.9 and 14.4 AU by FM) show very different erythematous responses (MED: 300 vs. 460 and 600 vs. 1175 J/m², respectively). Similar examples can be found among the CPD data. Differences in UVR sensitivity can be readily found when pairs of subjects with the same melanin content belong to two different R/E groups. This observation indicates that R/E is an important covariate in the melanin/UVR response relationships (Table S4).

Even with a R/E classification system based on self-determination with all its shortcomings incorporated as an additional factor in the model, it is a statistically significant factor involved in both the sensitivity of human skin to induction of erythema and the DNA damage caused by UVR exposure. It is expected that the correlations would improve if analogous analyses can be performed with a larger database of additional R/E groups to further improve the reliability with darkening skin type. At the same time, besides R/E and total melanin content, there are other factors affecting UVR responses, which are discussed below.

There was a good correlation between the determination of melanin content by DR and by FM, especially for skin with low melanin content (Fig. 1). However, the two methods “see” melanin differently as indicated by the data in Fig. 2a and 2b, and Table S3. At the present time, race is not readily definable in scientific terms (53-55). In the case of the skin, however, the links between melanin and race are obvious (Table 1) albeit not simple (Fig. 2). The complexities here may be related to a range of melanin attributes such as those listed in Table S6. Further, they may be related to the fact that the absorption of optical radiation by melanin involves different, wavelength- and molecular weight-dependent mechanisms (56,57). In addition, the distribution of melanin in skins of different R/E origin plays a significant role in determining visible color and also in the relative photoprotective nature of that melanin. All these factors combined are responsible for the different appearance of the skin (one of the main aspects of the R/E-related phenotype) and may affect cutaneous UVR responses. In addition, some melanin-controlled functions, such as the recently reported pigment-dependent apoptosis (39,58), may have different effects on UVR responses in different races.

To make use of findings of considerable importance for public health policies regarding UVR exposure, we need to improve our understanding of UVR responses in different R/E groups and, importantly, to improve the prediction of such responses in individuals so that they may be valuable to researchers and clinicians (59,60). As proposed by us, our non-invasive approach would serve as an asset to those researchers and clinicians to predict UVR sensitivity in future datasets at both a personal and/or population level. This approach could be incorporated directly into diffuse reflectance equipment or as a post-processing step using

already existing data. Future work using this tool would facilitate diversification and modernization of guidelines in the area of human UV exposure. Our results show that the non-invasive DR method, in conjunction with R/E, is a quantifiable approach to predict UVR responses of human skin and should be an important parameter when determining UVR doses to be used in clinical, therapeutic and cosmetic devices.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CPD	cyclobutane pyrimidine dimer(s)
DR	diffuse reflectance spectroscopy
FM	Fontana-Masson staining
M_{DR}	melanin content determined by diffuse reflectance spectrometry
MED	Minimal Erythema Dose
M_{FM}	melanin content determined by Fontana-Masson staining plus densitometry
ND	not done
R/E	race/ethnicity
SD	standard deviation
SE	standard error

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Figure 1. Constitutive melanin content and UVR-induced DNA damage responses

(a) Comparison of melanin content in the skin as measured by DR (M_{DR} , mean values from 3 measurements on 4 skin areas $\pm$ SE [horizontal bars]) and FM (M_{FM} , mean values from 10 randomly selected areas of each biopsy specimen $\pm$ SE [vertical bars]). Fitted line indicates a negative exponential regression ($p < 0.001$, correlation coefficient = 0.800). (b) Upper - Examples of FM-stained skin showing melanin in the epidermis of “White” (W, subject S31), “Asian” (A, subject S21), and “Black or African American” (B, subject S37) subjects. Lower - Examples of CPD abundance and distribution as revealed by immunohistochemistry in biopsies taken immediately (7 min) after exposure to 1 MED in the same subjects as indicated above. Dashed lines indicate the border between the epidermis and dermis. Scale bar = 50 μ m.

Figure 2. Relationships between melanin content and MED and CPD damage following UVR
 (a, b) The MED data corresponding to the melanin content as measured by DR (M_{DR}) and by FM (M_{FM}) was graphed for each subject. (c, d) The CPD yield represents the difference between CPD level measured in the skin “immediately” (i.e., ~7 min) after UVR exposure and CPD level measured in unexposed skin. CPD damage was normalized to 100 J/m² based on actual doses (1 MED or 200 J/m²) delivered to each biopsy site. Notes: SE for melanin content data are as shown in Figure 1. Curve fit for all data indicated by black lines. Horizontal axes are all square root transformed. The data are presented for n=10 in the Asian, n=18 in the Black, and n=31 in the White groups.

Figure 3. Determining UVR sensitivity: Predicted vs Observed MED

To test the accuracy of our prediction model for UVR sensitivity, the calibration plot explores the agreement between our observed MED outcomes and our MED predictions. When points fall along the fitted line, this indicates perfect MED predictions with our model. Note: Horizontal and vertical axes are all log transformed.

Table 1

Melanin content, UVR sensitivity and DNA damage in R/E groups.

R/E	N (m/f)	M _{DR} , mg/ml		M _{FM} , AU	
		Mean ± SD	Range	Mean ± SD	Range
White	31(13/18)	1.9 ± 1.5	-0.3 - 4.7	3.8 ± 1.5	1.3 - 8.3
Asian	10(4/6)	4.2 ± 2.7	1.7 - 10.3	8.3 ± 4.7	2.7 - 16.7
Black	18(2/16)	13.0 ± 5.7	3.1 - 22.9	13.0 ± 3.4	6.0 - 20.5

R/E	Age	MED, J/m ²		CPD per 100 J/m ²	
		Mean ± SD	Range	Mean ± SD	Range
White	48 ± 11	307 ± 95	149 - 498	0.20 ± 0.22	-0.03 - 1.10
Asian	47 ± 14	427 ± 112	280 - 601	0.09 ± 0.08	0.01 - 0.23
Black	37 ± 8	850 ± 430	448 - 1987	0.06 ± 0.05	0.03 - 0.23

Abbreviations: CPD, cyclobutane pyrimidine dimer(s); M_{DR}, melanin content determined by diffuse reflectance spectrometry; MED, Minimal Erythema Dose; M_{FM}, melanin content determined by Fontana-Masson staining plus densitometry; n (m/f), number of subjects (males/females); ND, not done; R/E, race/ethnicity; SD, standard deviation

¹Since FM yields arbitrary units (AU), any resemblance of numerical values obtained by the two methods is coincidental.

²M_{DR} is an apparent absorbance concentration of melanin determined from the linear part of the 630-700 nm spectrum according to Kollias and Baqer (45) with the modifications of Jacques (46) and the melanin extinction coefficient of Sarna and Swartz (47) to express melanin content in mg eumelanin per ml.