Skin Responses to Ultraviolet Radiation: Effects of Constitutive Pigmentation, Sex, and Ancestry

JENNIFER K. WAGNER, ESTEBAN J. PARRA, HEATHER L. NORTON, CELINA JOVEL and MARK D. SHRIVER

Department of Anthropology, The Pennsylvania State University, PA, USA

Present address: Esteban J. Parra, Department of Anthropology, University of Toronto at Mississauga, Mississauga, Ontario, Canada

Address reprint requests to Dr Mark D. Shriver, Department of Anthropology, The Pennsylvania State University, 409 Carpenter Building, University Park, PA 16802, USA. E-mail: mds17@psu.edu

Received 23 May 2002; in final form 20 July 2002

Constitutive skin pigmentation and skin responses to ultraviolet radiation were measured on a sample of volunteers (n = 250) living in State College, PA, USA. The sample was composed of individuals of European American (n = 190), Hispanic (n = 45), and East Asian ancestry (n = 15). Constitutive pigmentation was measured using the Adjusted Melanin Index (AMI), Erythemal Dose Response (EDR) was measured using the slope of a* at 24 h (Δa*), and Melanogenic Dose–Response (MDR) was measured using ΔAM, the slope of AMI at 7 d. The relationships between constitutive skin pigmentation, EDR, MDR, sex, age, and ancestry were investigated. European Americans showed a lower constitutive pigmentation, had a significantly higher burn response (EDR), and had a significantly lower tanning response (MDR) than Hispanics and East Asians. No significant difference is seen between Hispanics and East Asians for either constitutive pigmentation or EDR.

Constitutive pigmentation in females was slightly lower than in males in all three samples, but the difference was not significant. While no differences were observed in MDR between sexes, males had a stronger EDR than females regardless of population or constitutive pigmentation level, and this difference was significant in European Americans and Hispanics. We observed no age-related differences in any of the populations or measures investigated. We evaluated the relationship between constitutive pigmentation, EDR and MDR. There was a strong inverse correlation between constitutive pigmentation and EDR in the three samples (European Americans, \( R^2 = 0.176, P < 0.001 \); Hispanics, \( R^2 = 0.204, P = 0.009 \); East Asians, \( R^2 = 0.223, P = 0.098 \) ) and a strong direct correlation between constitutive pigmentation and MDR in European Americans and Hispanics (European Americans, \( R^2 = 0.094, P < 0.001 \); Hispanics, \( R^2 = 0.164, P = 0.012 \) ). In other words, persons with lower constitutive pigmentation both burn more and tan less than persons with higher pigmentation. However, after controlling for constitutive pigmentation, EDR and MDR were significantly correlated in European Americans (\( R^2 = 0.041, P = 0.006 \)). Thus, the general observation that persons who burn more tan less is probable because of the common link that these two phenotypes have with constitutive skin pigmentation and, in fact, once pigmentation has been adjusted for, there is a positive correlation between tanning response and burning response in European Americans.

INTRODUCTION

There has been growing interest in studying skin pigmentation and the skin’s response to ultraviolet radiation (UVR) using non-invasive procedures. The results of these studies to date, however, have been inconsistent. One main reason for this discrepancy is the lack of consensus in measuring techniques. There are a number of methods available that include self-report (e.g. Fitzpatrick phototype scale), visual assessment [e.g. minimal erythemal dose (MED)] and minimal melanogenic dose (MMD)], and less subjective assessments using reflectance technology (namely tristimulus reflectometry, narrow-band spectroscopy and diffuse reflectance spectroscopy). As shown in a companion paper (1), all measures are not created equal, and some of the commonly used measurements can lead to faulty conclusions. For example the CIElab lightness metric, \( L^* \), is affected by increases in erythema and is therefore inappropriate for use...
in measuring melanogenic dose–response (MDR). Thus, it is not surprising that while some authors have found a correlation between constitutive pigmentation (i.e. basal level of skin pigmentation found on skin which remains unexposed to UVR) and erythemal dose–response (EDR) (2–6), others have not (7, 8). Furthermore the relationship between EDR and MDR is even more uncertain. It is commonly thought that facultative pigmentation (i.e. elevated pigmentation levels in skin areas which frequently are exposed to UVR) is responsible for protection against future UVR exposures, while others argue that suppression of erythema on a second exposure is not caused by either melanin photoprotection or stratum corneum thickening (9). Selecting an appropriate method of measurement is of the utmost importance in this research, because the relationship between skin pigmentation and skin responses to UVR remain largely unexplained.

The focus of this study was to use the most informative measures currently available to quantify constitutive pigmentation, EDR, and MDR so the relationship between these factors could be elucidated. Moreover, an additional goal of this study was to investigate the effects of constitutive pigmentation, age, sex, and ancestry on EDR and MDR.

**MATERIALS AND METHODS**

**Samples for Analysis**

We have evaluated 397 individuals living in State College, Pennsylvania for constitutive skin colour and a subset of these (n = 250) have been evaluated for solar response. All of the volunteers were adults (i.e. at least 18 yr of age), with an average age of 24.5 yr and a range of ages between 18 and 78 yr. The sample included 244 females and 153 males. The majority of this sample can be divided into three groups, European-American (n = 289), Hispanic (n = 45), and East Asian (n = 29), based on self reported stated ancestry. A subset of individuals from the larger sample were measured for responses to UVR; European-American (n = 190), Hispanic (n = 45), and East Asian (n = 15). The Hispanic group includes persons whose stated ancestry can be traced to Puerto Rico, Colombia, Mexico, Honduras, Panama, and Guatemala. Although these individuals represent a large range of cultural and biological diversity and are united primarily by language, the history of Hispanic populations is such that most have significant ancestry from Native Americans, Spaniards, and Africans (10). Each individual in this study gave informed consent prior to beginning the procedure, and this research was performed under the approval of the Pennsylvania State University IRB (IRB No. 00M0558-A4) and Penn State General Clinical Research Center General Advisory Committee.

Exclusion criteria included subjects currently taking medication that could possibly alter an individual’s sensitivity to UVR. Additionally, subjects with certain health conditions like DNA repair enzyme deficiency or collagen vascular diseases were also excluded as these conditions may alter an individual’s sensitivity to UVR. (For further details on exclusion criteria see Wagner et al. 2002).

**Clinical Methods**

The constitutive skin colour was measured on the upper inner side (medial aspect) of both arms on each subject with a Microflash 200D (Datacolor International, Lawrenceville, NJ, USA). The Microflash provides reflectance levels at 10 nm intervals along the visual spectrum and also computes standard CIE tristimulus values. Volunteers participating in the skin response phase of this study had the medial aspect of both arms exposed to six metered doses of UVR. The Solar Simulator model 16S with liquid light guide (Solar Light Co, Philadelphia, PA, USA) was used to expose each individual to doses at 16.8, 21.0, 26.2, 32.8, 41.0, and 51.3 mJ/cm². Persons not responding with visible erythema to the first series of doses were subjected to a second series of doses (i.e. 41.0, 51.3, 64.0, 80.1, 100.0, and 125 mJ/cm²). For convenience and consistency with the literature, the sites were examined for EDR 24 h after the initial exposure and for MDR 7 d after the exposure. An unexposed site on each arm was measured as a baseline at both 24 h and 7 d postexposure. Digital photographs were taken to document both the EDR and MDR.

**Statistical Methods**

Each skin site was measured three times with the Microflash and the reflectance levels of these three measures were averaged together. Apparent absorbance (AA) was calculated following the recommendations of Kollias and Baqer (11). The white standard tile provided by the manufacturer was used as the reference source instead of the baseline measurement of each individual, as it was a more efficient way of determining the AA of a large number of measures. Additionally, this approach reserves the use of the baseline measurement as the first point in a person’s dose–response curves. Least squares linear regression was used to plot the best line relating skin response to the UVR dose, and the slope of this line was taken as EDR or MDR.

The Adjusted Melanin Index (AMI) was used to estimate the melanin concentration of the skin. The AMI was calculated as the slope of AA levels from 650 to 700 nm. As we have shown in a companion manuscript (1), AMI is highly correlated with other standard measures of pigmentation [namely, L* and the Melanin (M) index]. Additionally, when determining MDR, the dose–response slope of AMI, \( \Delta AM \), is a better measure than \( \Delta L^* \), because AMI is not affected by erythema like L* is.

Erythema was measured using \( a^* \) because it has been shown to be highly correlated with the alternative more complex measure, Adjusted Erythema index (1). \( a^* \) is provided directly from the Microflash instrument using the 1976 Commission International de l’Eclairage (CIE) lab conversion standards. \( a^* \) is the measure of redness on the red-green colour scale of the CIE colour system. Erythematous dose–response was measured using the slopes of the dose–response curves summarized as the change in \( a^* \) vs. the change in dose (i.e. \( \Delta a^* \)).

In order to improve the normality and homoscedasticity of the distributions of several of the quantitative measurements employed here, transformations were applied prior to
Regarding constitutive pigmentation, we used AMI. For ease of interpretation, the values of AMI are reported as \((\text{AMI} \times 10^5)\). To transform AMI, a constant was added to make all scores positive, and the square root was extracted. A similar transformation was carried out for the slope of AMI after 7 d (\(\Delta \text{AM}\)), which was used to measure MDR. The slope of \(a^*\) at 24 h (\(\Delta a^*\)) was used untransformed as a measure of EDR. Differences in pigmentation, MDR, and EDR between samples were tested by conventional ANOVA analysis, and the effect of relevant independent variables was evaluated by regression analysis using the SPSS 10.0 statistical package (SPSS Inc., Chicago, IL, USA).

**RESULTS**

**Differences of Pigmentation, MDR and EDR Between Population Samples**

Table 1 shows the average untransformed values of constitutive pigmentation as measured by AMI, EDR measured by \(\Delta a^*\) at 24 h, and MDR measured by AAM at 7 d in people of European-American, Hispanic, and East Asian ancestry. As a measure of dispersion, standard deviations are also indicated in parentheses. As expected, European Americans show the lowest level of constitutive skin pigmentation (mean AMI = 12.71, SD = 7.85). The highest value of constitutive pigmentation is observed in Hispanics (mean AMI = 27.40, SD = 23.52), and East Asians show intermediate levels (mean AMI = 21.53, SD = 15.86). Regarding EDR, the strongest burning response is observed in European Americans (mean EDR = 3.36, SD = 1.21), with lower responses in Hispanic (mean EDR = 2.49, SD = 1.07) and East Asians (mean EDR = 2.74, SD = 0.79). Finally, East Asians show the largest tanning response of the three samples (mean MDR = 18.13, SD = 8.74), almost doubling the value observed in Hispanics (mean MDR = 9.79, SD = 4.05), and not surprisingly, European Americans had a decreased capacity to tan with respect to the other two groups (mean MDR = 6.82, SD = 3.78).

Using conventional ANOVA analysis, we tested if the observed differences in constitutive pigmentation AMI, EDR and MDR among the samples of European-American, Hispanic and East Asian individuals were significant. In the analysis of constitutive pigmentation, sex was considered a covariate. In the EDR and MDR analysis, the analysis was carried out in two alternative ways in order to evaluate differences in EDR and MDR between groups: first considering sex as the only covariate and second considering both sex and constitutive pigmentation as a covariate. These results are presented in Table 2. We only include here results for EDR and MDR using both sex and constitutive pigmentation as a covariate, because the results are similar when controlling only for sex. It is important to note that the European-American sample is much larger than the Hispanic and East Asian samples, so the power of the test is significantly reduced when comparing the latter two samples. European Americans are significantly lighter than Hispanics (\(P < 0.001\)), and the difference in constitutive pigmentation between European Americans and East Asians borders significance (\(P = 0.05\)). No significant difference in constitutive pigmentation is observed between East Asians and Hispanics (\(P = 0.371\)). Regarding EDR, European Americans burn significantly more than both Hispanics (\(P = 0.001\)) and East Asians (\(P = 0.04\)), and there is no difference in EDR between Hispanics and East Asians (\(P = 0.849\)). European Americans have a significantly lower MDR than either Hispanics (\(P = 0.002\)) or East Asians (\(P < 0.001\)). Finally, despite the fact that there is no difference in either constitutive pigmentation or EDR, East Asians have a significantly higher tanning response than Hispanics (\(P < 0.001\)).

**The Role of Age and Sex in Constitutive pigmentation, EDR and MDR**

We evaluated if age was a factor affecting pigmentation, EDR and MDR. The results are not significant in any of the groups or quantitative measures considered in this study. We also tested if there were differences between sexes in constitutive pigmentation, EDR and MDR. The analysis was carried out independently in the three population samples (Table 3). When evaluating sex differences in EDR and MDR, the analysis was conducted first without including constitutive pigmentation as a covariate and again including constitutive pigmentation as a covariate. We report the results of the analysis using skin pigmentation as a covariate. Males are darker than females in the three samples, but the differences in constitutive pigmentation between sexes are not significant in any of the groups. With respect to EDR in the European-American sample, males burn significantly more than females. This result was observed both when constitutive pigmentation was not included as a covariate (\(P < 0.001\)) and when it was considered as a covariate (\(P < 0.001\)). Thus, even after controlling for differences in...
constitutive pigmentation, males have a significantly higher EDR than females in this population. In Hispanics, males also have a higher EDR than females, and the difference is also significant when controlling for constitutive pigmentation ($P = 0.04$). The same trend is observed in East Asians, but the differences are not significant. Finally, no differences in MDR were observed between sexes in any of the samples.

Effect of Constitutive Pigmentation on EDR and MDR and Relationship of EDR and MDR

In Fig. 1, we show the relationship between constitutive pigmentation and EDR (Fig. 1A), constitutive pigmentation and MDR (Fig. 1B), and EDR and MDR (Fig. 1C). We carried out regression analysis to test the significance of the relationships of these phenotypes independently in the three population samples. These results are presented in Table 4. There is a very strong inverse relationship between constitutive pigmentation and EDR in the three samples. In other words, persons with higher the constitutive pigmentation levels have lower burning responses. This effect is significant in European Americans ($\beta = -0.436$, $P < 0.001$) and Hispanics ($\beta = -0.452$, $P = 0.003$), and, although the slope is steeper in East Asians, the result is not significant given the small sample size ($\beta = -0.472$, $P = 0.098$). A significant positive correlation is found between constitutive pigmentation and MDR in European Americans ($\beta = 0.307$, $P < 0.001$) and Hispanics ($\beta = 0.405$, $P = 0.012$). Persons in these two groups with higher constitutive pigmentation levels show higher tanning responses. No significant effect of pigmentation on MDR was observed in East Asians ($\beta = -0.266$, $P = 0.340$).

We also explored the relationship between EDR and MDR in the three samples. When constitutive pigmentation is not considered in the model, there is no significant correlation between EDR and MDR in any of the samples. However, when controlling for constitutive pigmentation, there is a significant positive correlation between EDR and MDR in the European-American sample ($\beta = 0.214$, $P = 0.006$). Thus, when differences in constitutive pigmentation are controlled for, those with higher burning responses also have higher tanning responses.

DISCUSSION

The average constitutive skin pigmentation of each group studied, as well as each of the average EDR and MDR are interesting to examine in the context of previous anthropological works studying pigmentation variation in populations from around the globe. Of particular interest is the relationship, highlighted in such recent works as Robins (12), Relethford (13) and Jablonski and Chaplin (14), between...
skin pigmentation, latitude, and UVR, reinforcing the view that skin pigmentation, at least in certain environments, such as the tropics, is under strict control by natural selection. In keeping with this relationship it should not be surprising that the European-American sample has the lowest average constitutive pigmentation, followed by East Asians and Hispanics. That Hispanics show the largest range of pigmentation values (Table 1) is not surprising given that this sample of Hispanics comes from diverse countries in Latin America where individuals trace their ancestry to Native American, European and African populations (see 10, 15). These results indicate that European Americans burn significantly more and tan significantly less than the other two groups (Table 2). A very interesting finding is that although there are no significant differences in constitutive pigmentation levels or EDR between Hispanics and East Asians, East Asians do show a significantly higher tanning response than Hispanics. The ΔAM at 7 d in East Asians is almost twice that observed in Hispanics (Table 2). Further studies with larger sample sizes and replicate population samples will be necessary to confirm this finding, but a previous study has also indicated differences in solar response between East Asians and Europeans (16). Furthermore, the findings of Stanford et al. (17) suggest that East Asians have a uniform tanning capacity regardless of skin type or constitutive pigmentation but also have no explanation for this apparent population difference in MDR.

Although the age of the participants of this study ranged between 18 and 78 yr, it is important to note that this sample was mostly comprised of persons between 18 and 30 yr old. Thus, these data do not allow for investigations of the effects of age either in the first two decades of life or the late decades of life, times at which it has been reported that some changes in pigmentation and skin responses are known to occur (18). Given the low variance in age, it is also not surprising that we did not observe any significant effect of age on pigmentation or skin response. Further investigation into the effect of age on pigmentation and skin response to UVR is necessary. Ideally the study should be designed longitudinally, given the amount of variation between individuals and populations in these phenotypes.

We also analysed the effect of sex on constitutive pigmentation, EDR and MDR (Table 3). Sex-based differences in constitutive skin pigmentation have been reported in numerous anthropological studies, using a variety of skin reflectance measurement techniques. In the majority of these reports females display higher skin reflectance than males, this result being consistent across many different cultural groups (e.g. 19–23). This trend was observed in this study, however, the results were not significant, as was observed by Nordlund and Ortonne (24) and Lock-Andersen et al. (18). Contrarily, Rohet al. (16) reported that the sex differences in pigmentation were significant in a sample from Korea, with males being darker. Some authors (e.g. 25), argue, however, that such sex-based differences are not representative of biological differences between the sexes, and instead reflect differing patterns of activity or clothing between males and females. For example, in American society it may be socially acceptable for males to remove their shirts while outside on a hot day, while such behaviour is uncommon among females.

In our study, MDR was similar in both sexes in the three populations; however, we observed a significant difference in EDR with males displaying a higher EDR than in females. In European Americans the result is significant irrespective of whether the analysis was conducted with or without constitutive pigmentation as a covariate. Thus, in European Americans the differences between males and females in burning response remain even after controlling for any effect of constitutive pigmentation on EDR ($P < 0.001$). The same is true in the smaller Hispanic sample ($P = 0.042$), although differences in EDR between males and females are not significant when constitutive pigmentation is not used as a covariate. Likewise, the trend is observed in East Asians although, not surprisingly given the small sample size ($n = 15$) of this group, the differences are not significant. Thus, it appears that irrespective of the population group, males show a higher burning response (but not a higher tanning response) than females. We are not aware of any previous reports on male/female differences in skin response and expect that if these results are confirmed, it is plausible that there might be either hormonal or physiological cause for these differences in skin response by sex. For example, it has been shown that oestrogen enhances the epidermal barrier of the stratum corneum by increasing the water-holding capacity (26).

Finally, we investigated the relationships among the three variables: constitutive pigmentation, EDR, and MDR (Fig. 1 and Table 4). Constitutive pigmentation shows a clear correlation with both EDR and MDR. Populations and persons with darker skin have both lower EDR and higher MDR levels. In other words, individuals with higher pigmentation tend to burn less and tan more than lighter individuals. Biologically these relationships are expected if we consider that melanin can act as a sun screen protecting the skin from burning and if the amount of melanin in a generally unexposed skin site is also an indication of the relative ability of the skin to produce more melanin.

Despite the strong correlation of each with pigmentation level, there is no significant correlation between EDR and MDR in any of the population samples. This lack of a relationship can be clearly visualized in Fig. 1(c); however, in the European-American sample, there is a positive and
significant (P = 0.006) correlation when we control for the effect of constitutive pigmentation. Thus, the general observation that persons who burn more than less is probable because of the common link that these two phenotypes have with constitutive skin pigmentation and, in fact, once pigmentation has been adjusted for, there is a positive correlation between burning and tanning responses in European Americans where individuals who burn more also tan more. This relationship makes biological sense if we consider that the intercellular signals that the immune system is acting on to initiate the inflammatory response (erythema) may also be responsible for signalling the tanning response.

In the companion manuscript (1), we evaluated the usefulness of different quantitative variables obtained by reflectometry to measure skin pigmentation and skin response to UVR. We emphasized the importance of using appropriate measures to effectively test skin pigmentation response. In this study, we have explored the effect of relevant variables, such as ethnicity, age, sex, and constitutive pigmentation in EDR and MDR. We have made some interesting observations that introduce new questions to explore in dermatological research. Of particular interest are the observations of an increased burning response in males, an increased tanning response in people with East Asian ancestry, and a positive relationship between EDR and MDR in European Americans.

Acknowledgements – We would like to thank the volunteers who gave their time and skin for this research. We also acknowledge the support of the Pennsylvania State University General Clinical Research Center (M01RR10732). This work was supported in part by a grant from NIH (M01RR10732). This work was supported in part by a grant from the Pennsylvania State University General Clinical Research Center (M01RR10732). This work was supported in part by a grant from NIH (M01RR10732). This work was supported in part by a grant from NIH (M01RR10732). This work was supported in part by a grant from NIH (M01RR10732).

REFERENCES

6. Park BS, Youn JI. Topographic measurement of skin color by narrow-band reflectance spectrophotometry and minimal erythema dose (MED) in Koreans. Skin Res Tech 1998;4:14–17