Diffuse reflectance spectroscopy versus Mexameter® MX18 measurements of melanin and erythema in an African population

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ABSTRACT

Melanin provides protection against excess exposure to solar ultraviolet radiation (UVR) and related adverse health effects. Diffuse Reflectance Spectroscopy (DRS) can be used to calculate

cutaneous melanin and erythema, but this is complex and has been mostly used for light-to-medium pigmented skin. Handheld reflectance spectrophotometers, such as the Mexameter® MX18, can also be used. We compared DRS-calculated melanin and erythema values with Mexameter melanin and erythema index values to understand how these techniques / measurements correlate in an African population of predominantly deeply-pigmented skin. 503 participants comprised 68.5% self-identified Black African, 9.9% Indian/Asian, 18.4% White and 2.9% Coloured. The majority of Black African (45%), Indian/Asian (34%) and Coloured (53%) participants self-identified their skin as being 'brown'. Measured melanin levels increased with darker self-reported skin colour. DRS-calculated and Mexameter melanin values demonstrated a positive correlation (Spearman rho=0.87, $p<0.001$). The results from both instruments showed erythema values were strongly correlated with their own melanin values. This finding is considered spurious and may result from the complexity of separating brown and red pigment when using narrowband reflectance techniques. Further work is needed to understand melanin, erythema and colour in Black skin given sun-related health risks in vulnerable groups in Africa.

Keywords: melanin, erythema, dark skin, spectroscopy, Africa.

INTRODUCTION

Melanin affords the body some protection from excess exposure to solar ultraviolet radiation (UVR) depending on the amount and type of melanin in the skin. Excess solar UVR exposure is associated with several adverse health effects in the skin, including erythema (sunburn), light-sensitive dermatoses and skin cancers (1). Determining the amount of melanin in

the skin, and subsequently skin phototype (2) helps to inform advice on sun protection and safe sun exposure that is tailored to an individual or sub-population group with similar phenotypic characteristics.

While individuals with dark skin are considered to be better protected against solar UVR because of their relatively high melanin content when compared to individuals with fair skin, individuals with dark skin do experience adverse health effects of excess sun exposure (3). Skin cancer occurs in black people but is less common compared to people with fair skin (4). The mean age-standardized annual incidence of cutaneous melanoma per 100 000 male and female persons in Black African versus White populations in South Africa are 1.0 and 1.2 versus 20.5 and 16.5, respectively (4). The high prevalence of HIV infection in Africa may lead to higher-than-expected incidence of squamous cell carcinoma (SCC), pterygium and SCC of the cornea or conjunctiva (3).

Diffuse reflectance spectroscopy (DRS) is a technique used to objectively measure the skin's reflectance due to the melanin content of the skin (5). This technique is considered to be reasonably accurate but is complex and labour-intensive. There are simple-to-operate instruments, commercially-available, that can also be used to measure an index of melanin (6) and erythema, such as the Mexameter® MX18 spectrophotometer.

This study compares DRS-calculated values of melanin and erythema with the melanin and erythema indexes (MI and EI, respectively) derived from the Mexameter® MX18 spectrophotometer to provide an understanding of how these measurements correlate in an African population of diverse skin colour. To date, most studies of melanin content have been carried out among Caucasian (7) and Asian (8,9) populations, one has been among individuals of different biological ancestry (10) and one in a small subsample of Black Africans from South

Africa (5). We also compared the erythema measures from the Mexameter® MX18 with the DRS-calculated values of erythema, and the melanin measures from each instrument, to consider their validity for determining erythema (in non-purposively irradiated skin) among a sample with predominantly dark skin.

MATERIALS AND METHODS

Procedures. Five hundred and three participants from the Council for Scientific and Industrial Research (CSIR) in Pretoria, South Africa were assessed between the 6th and 22nd October 2014 as part of a Wellness event offered by the CSIR. People attending the Wellness event were asked if they would be willing to participate in a research study. All participants provided written consent and spoke English. They answered a short questionnaire to self-identify their population group and skin colour. Skin colour options were 'very light', 'white', 'intermediate', 'olive', 'brown', 'dark brown' and 'black'. These categories were based on those defined by Del Bino and Bernerd (11) but we altered 'tan' to 'olive', and added 'dark brown' since anecdotal evidence suggested that Black Africans did not relate to the word 'tan' as a colour, and Black South Africans do not have as dark skin as Africans from other countries in Africa. The CSIR Researcher Ethics Committee approved the study protocol (certificate number 79/2013).

Instruments. The DRS probe (R200-7-UV-VIS, Ocean Optics, 830 Douglas Avenue, Dunedin, FL, 34698, USA) has a white light source, optical fibre and spectrometer, and measures the light diffusely reflected by the skin. Values for melanin and erythema are given in mol/L. The Mexameter® MX18 spectrophotometer (Courage+Khazaka , 91 Mathias-Brüggen-Str., 50829, Cologne, Germany) emits light of three defined wavelengths (568 nm, 660 nm and

870 nm) and a receiver measures the light reflected by the skin. The results correspond to the spectral absorption peaks of melanin and haemoglobin (as a proxy for erythema), respectively, providing MI and EI on a scale from 0 to 999.

Measurements. The DRS probe and the Mexameter® MX18 were applied to a clean skin area on the inner arm of the non-dominant arm to measure skin reflectance and MI/EI, respectively. We measured the inner arm skin of the non-dominant arm as this was the least inconvenient area to measure natural skin pigment and that the non-dominant arm compared to the than the dominant arm would be less likely to be affected by environmental factors that could darken natural skin pigment due to less use. We also selected this area for consistency between volunteers.

Three measurements were made at the sampled site and the average of these measurements was calculated for inclusion in the analysis. The skin site was approximately 6 cm above the elbow on the inner arm and was free of freckling, rash, dryness, bruising, birth marks or any other abnormality. When the skin at this site on the non-dominant arm did not meet these criteria, we used the other arm.

The Mexameter was calibrated daily using a standard reference. A light protection ring was used on the probe during measurements on participants to block out external light. The accuracy of measurements is ± 5%. The instrument is 130 mm long and has a diameter of 24 mm while the diameter of the measurement surface is 5 mm (area of 19.6 mm^2).

Calculations. The DRS system relies on the principle that part of the light incident on the skin will be absorbed (dependent on the absorption coefficient of the skin) while part of the non-absorbed light will be scattered multiple times and eventually exit the skin surface as diffused reflected light. Melanin and erythema concentrations were calculated from the reflectance

measured using the DRS probe, as previously described (5). We calculated total melanin in order to compare the findings to the Mexameter-measured MI.

Analysis. Data were analysed using STATA 14.0 (Statacorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP). We used a Spearman rho (ρ) to test the correlations between the measurements from the two instruments as the data were non-parametrically distributed. A natural log transformation was used on the non-normally distributed DRS erythema data. A p-value (p) of <0.05 was considered statistically significant. We further explored the relationships using linear regression models, for the total sample and also stratified by the individual population groups. Models for the total sample, adjusted for age, sex and population group, are also presented.

RESULTS

Sample description. Of the 503 participants (243 male, 260 female), 345 self-identified as Black African, 50 as Indian/Asian, 93 as White and 15 as Coloured (defined by Statistics South Africa as individuals of mixed heritage) (Table 1).

The most commonly self-identified skin colour was brown among the Black African, Indian/Asian and Coloured participants (Table 2). Melanin levels increased with darker self-reported skin colour (Table 2).

Melanin measurements. Wide variation in DRS and MI melanin concentrations was seen among self-reported Coloured participants (SD in Table 3) compared to all other population groups. DRS melanin concentrations and MI values were highly correlated (Spearman's rho=0.875, $p<0.001$) (Figure 1). The R^2 value for the regression model was 0.61 ($p<0.001$). In the stratified analysis, the R^2 values were lower than for the overall samples in all population

groups except the coloured group (Black African R^2 0.47 p <0.001; Indian/Asian R^2 0.12 p=0.02; White R^2 0.16 p <0.001; Coloured R^2 0.69 p <0.001).

Table 1. Distribution of participants (n=503) by self-report questionnaire response items.

Main category	**Responses**	**Frequency (n)**	**Percentage (%)**
Gender	Male	243	48.3
	Female	260	51.6
Self-reported population group	Black	345	68.5
	Indian/Asian	50	9.9
	White	93	18.4
	Coloured	15	2.9
Age	18-25 years	57	11.3
	26-35 years	216	42.9
	36-45 years	137	27.2
	46-55 years	57	11.3
	56-65 years	32	6.3
	Older than 65 years	4	0.8
Eye Colour	Light blue/grey/green	23	4.5
	Blue/Grey/Green	34	6.7
	Hazel or Light brown	66	13.1
	Dark brown	331	65.8
	Black	49	9.7
Fitzpatrick Erythema and Tanning Reactions to First Exposure in Summer (16)	Always burn, never tan (I)	96	19.0
	Usually burn, tan less than average (with difficulty) (II) and; Sometimes mild burn, tan about average (III)	201	39.9
	Rarely burn, tan more than average (with ease) (IV)	192	38.1
	Not applicable	14	2.7

Table 2. Frequency of self-reported natural skin colour by self-reported population group. DRS melanin and erythema concentrations were multiplied by 10^2 and 10^7 (respectively) for ease of comparability with the Mexameter indices of melanin and erythema.

	Self-identified natural skin colour on inner arm of non-dominant arm							
Population group	**Very light** n **(%)**[#]	**White** n **(%)**	**Intermediate** n **(%)**	**Olive** n **(%)**	**Brown** n **(%)**	**Dark brown** n **(%)**	**Black** n **(%)**	**Total** n
Black	18 (5.2)	1 (0.2)	99 (28.7)	2 (0.5)	157 (45.6)	47 (13.6)	21 (6.1)	344
Indian/Asian	1 (2.0)	2 (4.0)	21 (42.0)	6 (12.0)	17 (34.0)	3 (6.0)	0 (0.0)	50
Coloured	0 (0.0)	2 (19.3)	4 (26.6)	1 (6.6)	8 (53.3)	0 (0.0)	0 (0.0)	15
White	23 (24.7)	49 (52.6)	20 (21.5)	0 (0.0)	1 (22.5)	0 (0.0)	0 (0.0)	93
All (n)	42	54	144	9	183	50	21	503

Note. [#] Percentages are calculated as the proportion of each population group that selected a specific self-identified natural skin colour on the inner arm of the non-dominant arm.

Table 3. Mean (± 1 Standard Deviation, SD) melanin and erythema measures by self-reported population group. DRS-calculated melanin and erythema concentrations were multiplied by 10^2 and 10^7 (respectively) for ease of comparability with the Mexameter indices of melanin and erythema.

	Melanin		**Erythema**	
Population group	**Mexameter** (MI) Mean (SD)	**DRS** (10^{-2} mol/l) Mean (SD)	**Mexameter** (EI) Mean (SD)	**DRS** (10^{-7} mol/l) Mean (SD)
Black	533.8 (137.2)	203.6 (127.9)	413.9 (63.4)	2577.2 (2350.0)
Indian/Asian	329.0 (103.6)	89.1 (83.3)	322.8 (64.0)	1203.8 (1187.1)
Coloured	282.3 (121.7)	73.3 (77.0)	295.5 (69.7)	858.6 (872.1)
White	106.1 (29.3)	11.912 (20.3)	204.5 (51.5)	378.0 (261.1)
All	426.9 (207.4)	152.9 (134.9)	362.6 (102.5)	1982.8 (2184.9)

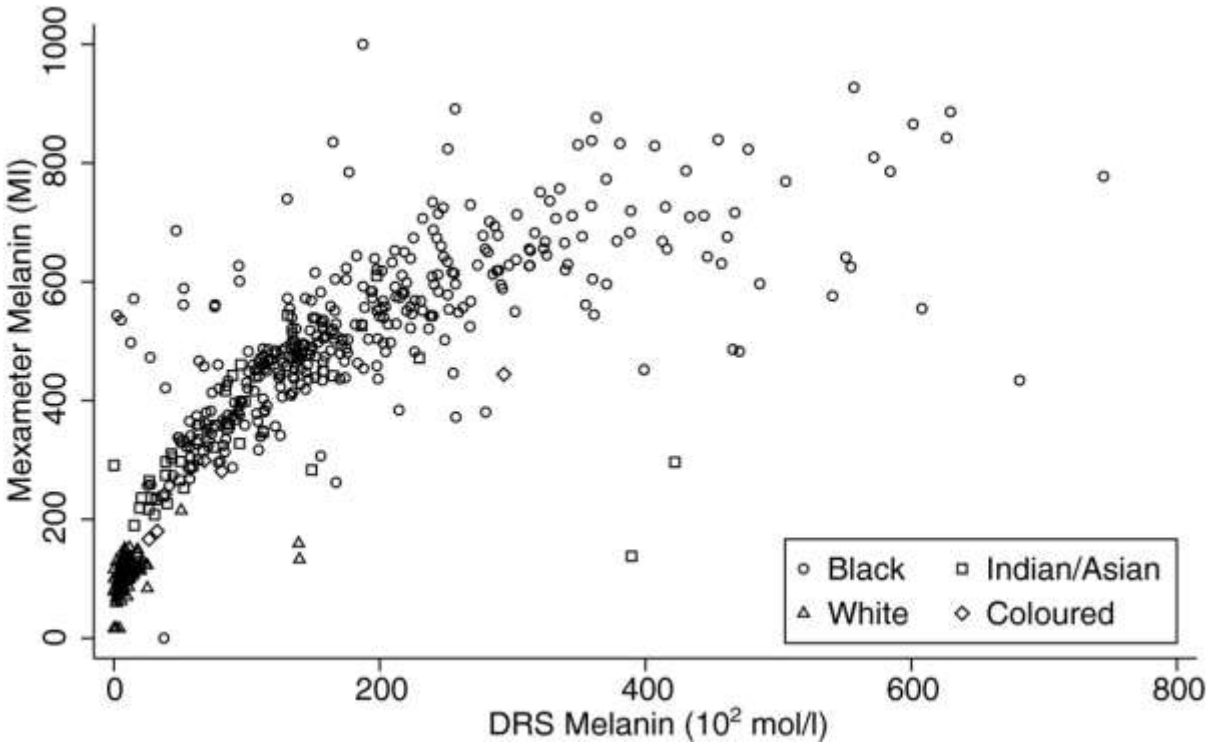

Figure 1. Participants' (n=503) DRS melanin concentration (mol/L) and Mexameter melanin index (MI) values. DRS melanin concentrations were multiplied by 10^2 for comparison purposes.

Erythema measurements. DRS erythemal measurements exhibited a wide range from 88.63 to 19 802.00 10^7 mol/L (mean 1 982.82 SD 2 184.91). EI values from the Mexameter ranged from 20.20 to 560.66 EI units (mean 362.61 SD 102.51).

Erythema compared to melanin. Mexameter MI and EI values were strongly correlated (Spearman rho=0.94, p<0.001) (Figure 2). A strong correlation (Spearman rho=0.86, p<0.001) was also observed between MI and EI for self-reported Black African participants. Spearman correlations and p-values for the remaining population groups were: Indian/Asian (Spearman rho=0.79, p<0.001); Coloured (Spearman rho=0.92, p<0.001); and White (Spearman rho=0.61, p<0.001). In the linear regression model, EI explained 87% of the variance in MI values (R^2 0.87 p<0.001); this was slightly stronger in a model including adjustment for age, sex and population

group (Adjusted R^2 0.89, p<0.001). The R^2 values for the models stratified by individual population groups were: Black African (0.70 p<0.001), Indian/ Asian (0.74 p<0.001), White (0.49 p<0.001) and Coloured (0.88 p<0.001).

DRS-calculated melanin concentrations versus erythema concentrations were also positively correlated (Figure 3) with the strongest correlation in Black Africans (Spearman rho=0.87, p<0.001) compared to White (Spearman rho=0.41, p<0.001), Indian/Asian (*p*=0.86, p<0.001) and Coloured (*p*=0.83, p<0.001) population groups. In the linear regression model, erythema concentration explained 72% of the variation in melanin concentration (p<0.001). This was improved slightly with adjustment for age, sex and population group (Adjusted R^2 0.79, p<0.001). The individual R^2 values for the stratified analysis were: Black Africa (0.67 p<0.001), Indian/ Asian (0.66 p<0.001), Coloured (0.78 p<0.001) and White (0.19 p<0.001).

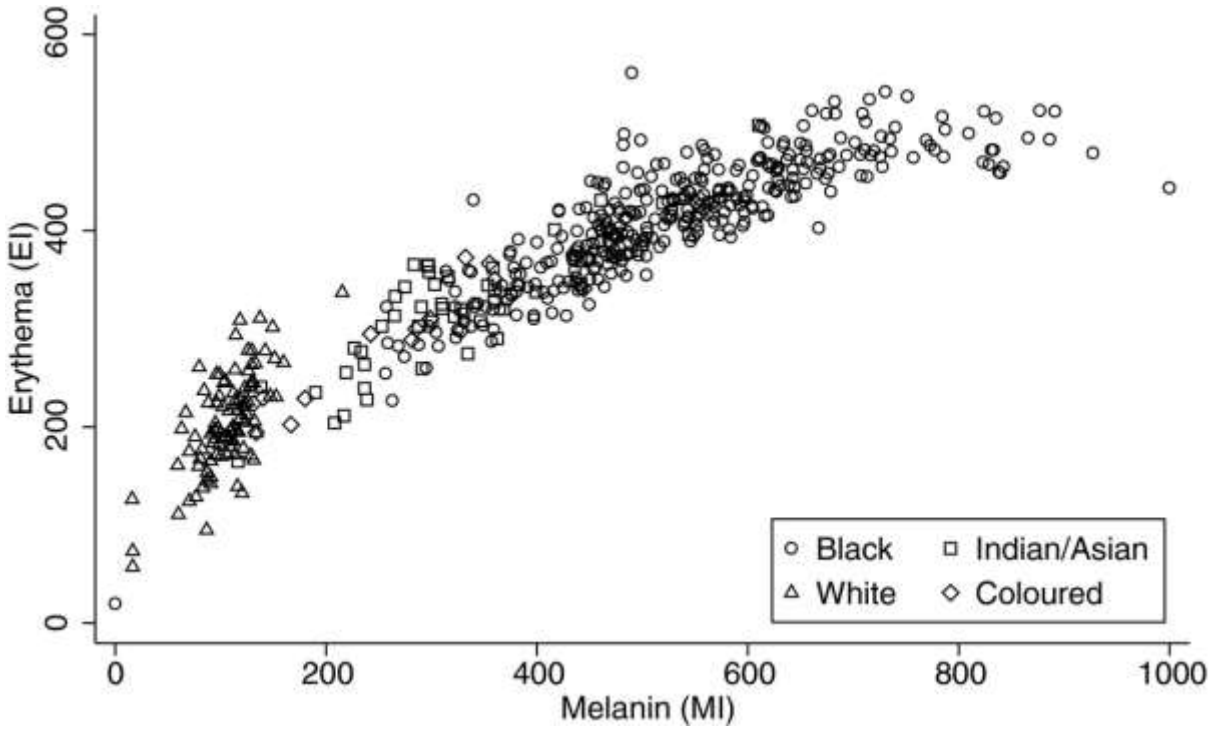

Figure 2. Mexameter melanin index (MI) and erythema index (EI) values demonstrate a positive correlation that is likely to be a measurement artefact (n=503).

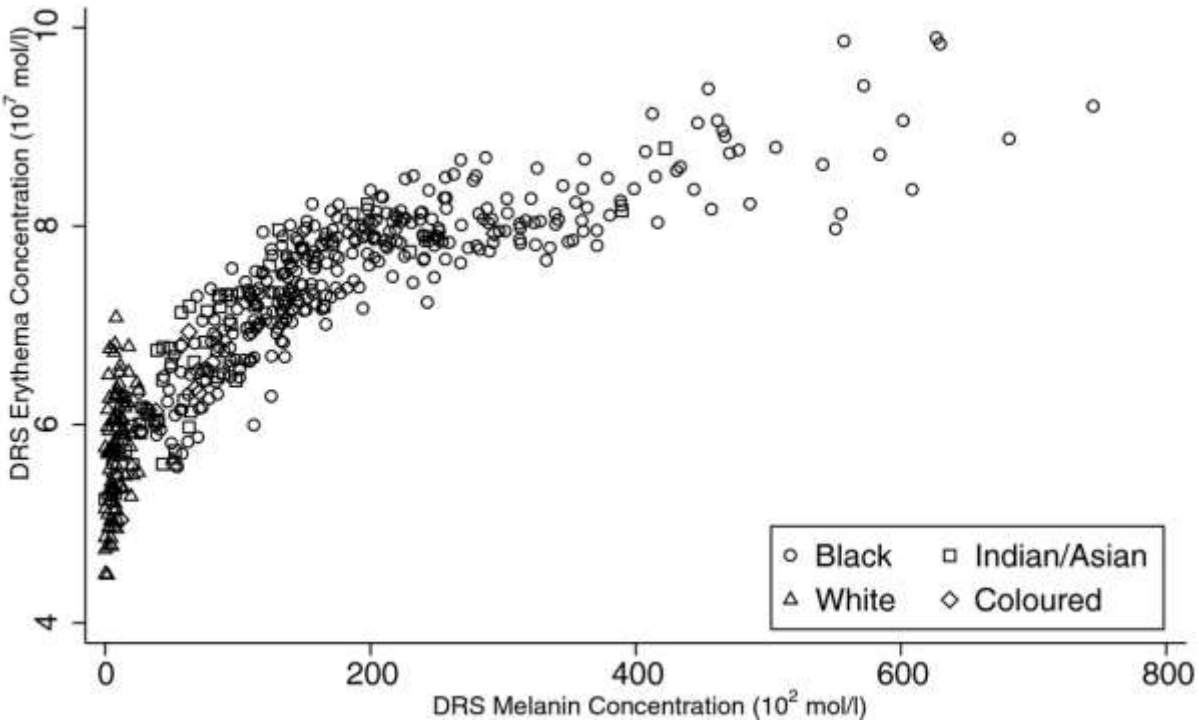

Figure 3. DRS melanin concentrations (mol/l) versus DRS erythema concentrations (mol/l) demonstrate a positive correlation (n=503). DRS melanin and erythema concentrations were multiplied by 10^2 and 10^7 for illustrative purposes, respectively.

DISCUSSION

While DRS has been used with reasonable confidence to estimate erythema in fair skin, our findings suggest that it is unlikely to be a reliable technique for use among individuals with dark skin. One reason for this finding may be that the wavelength at which melanin is measured is too similar to the wavelength at which haemoglobin, as a proxy for erythema, is measured. As a consequence, the instrument cannot differentiate between melanin and erythema, particularly in deeply pigmented skin. We found a positive correlation between melanin and erythema measurements using both the Mexameter® MX18 and the DRS probe. We consider this to be a spurious result. Despite having sophisticated algorithms meant to separate melanin and erythema values, the correlation results in darker skin always having high erythema readings, despite there

being no biological plausibility for this finding. The correlation is likely due to the difficulty in measuring red (vascular) and brown (pigment) colour without interference (brown comprises primarily red with some green pigment) (12). We suggest that the Mexameter® MX18 EI and the DRS are inappropriate methods to use to evaluate erythema among individuals with dark skin. Other handheld reflectometers, such as the Photovolt ColorWalk colorimeter (Photovolt Instruments, 6323 Cambridge St, Minneapolis, MN, 55416, USA) and the DermaSpectrometer (Cortex Technology, Plastvaenget 9, 9560 Hadsun, Denmark), have been used to measure skin and hair colour among European Americans, African Americans, South Asians and East Asians (9) and could perhaps also be tested among Black Africans living in Africa too.

Future studies using spectrophotometry to measure melanin content should include a questionnaire item on whether participants were using hormonal treatments, known to affect melanin and pigmentation, at the time of the study. The majority of this sample were Black African and the three other population group sample sizes were relatively small in comparison, thereby reducing the statistical power for correlation in the analyses. Future research could include larger samples of these populations groups.

Finding reliable, valid, affordable and non-invasive ways to measure melanin in the field is important for determining skin phototype (7), predicting sensitivity to solar UVR (13) and skin cancer risk (14). Few studies have assessed melanin concentration in African participants (6, 8). Here, good agreement was found between DRS, considered to be a more sophisticated technique for measuring melanin content, and Mexameter melanin assessments, including among self-reported Black African participants with a wide range in self-reported natural skin colours. There is evidence that Black Africans suffer adverse health effects of excess sun exposure (3). In addition, sub-Saharan Africa has a high prevalence of HIV infection, currently estimated as 10%

of the Black population. This may lead to a higher-than-expected incidence of keratinocyte cancers in Black Africans, probably due to the lack of an effective immune response to combat the initial or a progressive stage in tumour development (15,16). Further research and analysis is needed to fully investigate optimal and valid techniques for assessing melanin content, erythema concentrations, and skin phototype among individuals with dark skin to inform skin cancer prevention and safe sun awareness information and interventions.

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