

## Letter to the Editor

### **Towards a reliable, non-invasive melanin assessment for pigmented skin**

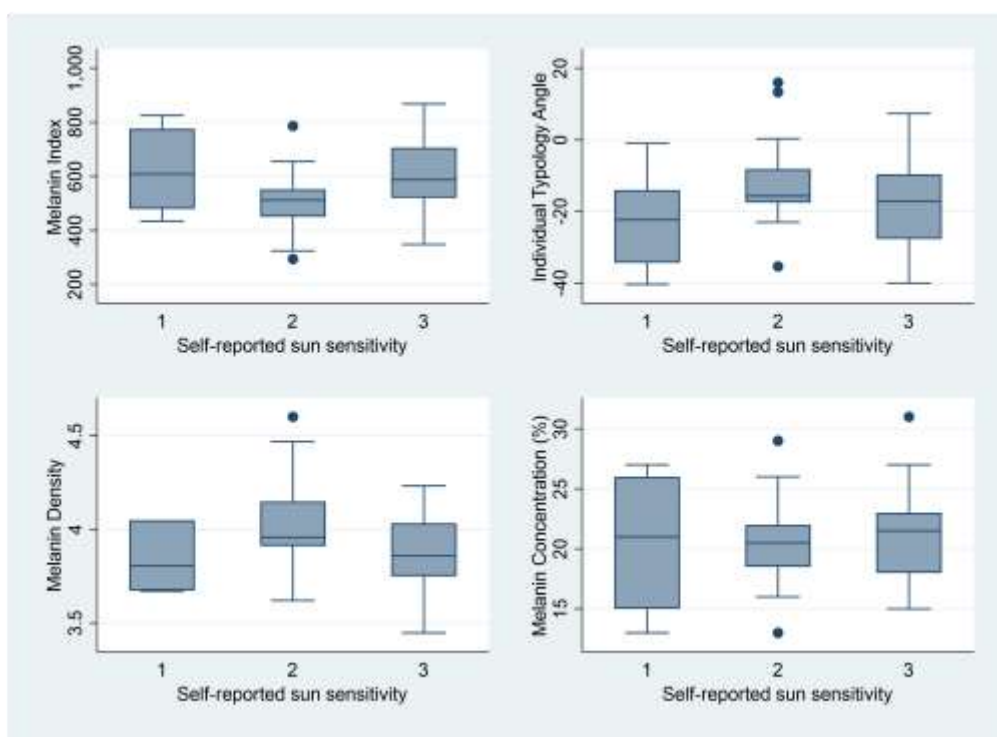
Spectrophotometry is used to estimate melanin density (MD) in Caucasians<sup>1</sup> but applicability of the calculation to people with pigmented skin, who also experience sun sensitivity, is not known. Here, we directly compared biopsy melanin concentration (MC) with Melanin Index (MI), Individual Typology Angle (ITA) values,<sup>2</sup> calculated Melanin Density (MD) and Self-Reported Sun Sensitivity (SRSS), with an aim to identify a non-invasive, reliable melanin assessment technique for deeply-pigmented skin.

Participants (n=50) were drawn from employees of the Council for Scientific and Industrial Research (CSIR) in Pretoria, South Africa from May 9-17, 2016. The CSIR Research Ethics Committee approved the study protocol (Certificate number 79/2013). Healthy study participants gave written informed consent, spoke English, cleaned their non-dominant arm with a sanitary wipe, and answered a short questionnaire to self-identify sex, SRSS (just burn and not tan; burn first then tan afterwards; not burn at all, just tan) and population group (Black African; Indian/Asian; Coloured (which is defined in South Africa as mixed population group); or White).<sup>3</sup>

We determined MI and ITA using a Mexameter MX 18 and Skin Colorimeter CL 400, respectively [both Courage+Khazaka Electronic] and MD<sup>1</sup> using a portable Spectrophotometer CM-2600d [Konica Minolta], taking an average of three measurements of skin on the inner surface of the upper, non-dominant arm. After a half-hour rest, a trained nurse took a 2 mm punch biopsy of the measurement area. Biopsies

were fixed, stained and analysed according to a standard protocol<sup>4</sup> to determine MC. We used Stata (v.14.0) for statistical analysis, with Pearson's correlation coefficient ( $r$ ) used to assess the correlation between MC and all other variables,<sup>1</sup> and linear regression to test predictive models of MC using likelihood ratios.

Overall, MC was available for 49 participants. Of these, 65% were women (with missing sex data for one participant), and all but one person (who self-reported as Coloured) self-identified as Black African. Participants did not relate to SRSS terms such as 'sunburn' and 'tan', evident in the lack of meaningful comparisons with objective measures of skin sensitivity to the sun (Figure 1).



**FIGURE 1** Distribution of participants' (a) MI values (n=47), (b) ITA values (n=49), (c) spectrophotometric MD (n=49), and (d) MC (n=49), vs. SRSS (1=just burn and not tan; 2=burn first then tan afterwards; 3=not burn at all, just tan). The upper whisker is the 95th percentile, the upper box line is the 75th percentile, the middle line in the box is the median, the lower line of box is the 25th percentile and the lower whisker is the 5th percentile.

There were statistically significant inverse correlations between MC and ITA, and MC and MD (Table 1) and there was a significant positive correlation between MC and MI but no significant correlation between MC and SRSS. Correlations between the other measures of skin type were as we have previously reported.<sup>5,6</sup>

**TABLE 1** Correlations of estimates of MC with MI, ITA, MD and SRSS. Mean and range are given in brackets for ITA, MI, MD and MC, with n per SRSS category provided for SRSS.

	<b>MC</b> (n=49) (21; 13 – 31)	<b>MI</b> (n=47) (580; 294 – 868)	<b>ITA</b> (n=49) (-17; -40 – 16)	<b>MD</b> (n=49) (4; 3 – 5)	<b>SRSS</b> (n=49) (1:7; 2:16; 3:26)
<b>MC</b>	1	0.331* (p=0.02)	-0.276 (p=0.05)	-0.280 (p=0.05)	0.129 (p=0.38)
<b>MI</b>		1	-0.864 (p<0.001)	-0.783 (p<0.001)	0.070 (p=0.64)
<b>ITA</b>			1	0.921 (p<0.001)	0.014 (p=0.92)
<b>MD</b>				1	-0.059 (p=0.69)

For the sample with full data, the best fitting multiple linear regression model for MC (explaining 26% of the variance) included only sex ( $\beta=-3.31$ ,  $p=0.004$ ) and MI ( $\beta=0.005$ ,  $p=0.23$ ). We tested different ways of combining the MD input data (reflectance at wavelengths of 400 and 420 nm), and adding phenotypic characteristics, but were unable to find any algorithm that explained more than 29% of the variance in biopsy MC ( $p=0.02$ ), which is much less than that previously shown for Caucasian skin (i.e. 68%).<sup>1</sup>

Here, the best skin type predictor of MC was MI; however, MI alone explained only 11% of the variance in MC, with a greater contribution when sex was also included in the model. The spectrophotometric MD, previously shown to predict MC well in Caucasians,<sup>1</sup> was only a weak (and non-significant) predictor of MC in people with deeply-pigmented skin. In studies of the effect of sun exposure on human health, for example, for production of vitamin D or for sun sensitivity to sunburn or immune suppression, an accurate measure for 'skin type' is essential.

Although calculated MD is commonly used for this purpose, we show here that the algorithm developed for Caucasian populations is not suitable for use in people with deeply-pigmented skin. Developing an accurate, non-invasive estimate of MC in people with deeply-pigmented skin – or that is generalizable to the range of skin types - will prove useful in epidemiology studies, in clinical dermatology and application of laser therapy treatments.

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## **LIST OF SUPPORTING INFORMATION**

## **ACKNOWLEDGEMENTS**

We thank Dr Kevin Sevier for training our research nurse, Louise Renton for performing the laboratory work, Patricia Albers and Mirriam Mogotsi for assisting with data collection and the participants in the study.

## **FUNDING INFORMATION**

This study was supported in part by funding from the South African Medical Research Council and the National Research Foundation of South Africa.

## **CONFLICT OF INTEREST**

The authors state no conflict of interest.