Dietary Carotenoids Contribute to Normal Human Skin Color and UV Photosensitivity

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ABSTRACT The aim of the current study was to determine whether dietary carotenoids influence skin pigmentation and UV photosensitivity in a healthy unsupplemented panel (n = 22) of Caucasian (skin Type II) subjects. Skin spectrophotometric and tristimulus (L*a*b*) CR200 chromameter readings were made at various body sites to objectively measure skin carotenoid levels and skin color, respectively. The minimal erythemal dose (MED) was also measured to determine the intrinsic UV photosensitivity of the skin. We found that tristimulus b* values (but not L* and a* values) were consistently and closely correlated with skin carotenoid levels at a number of body sites including the back (r = 0.85, P < 0.00001), forehead (r = 0.85, P < 0.00001), inner forearm (r = 0.75, P < 0.00001) and palm of the hand (r = 0.78, P < 0.00001). Skin carotenoid levels and MED were also correlated in these subjects (r = 0.66, P < 0.0001), as were tristimulus b* values and MED (r = 0.71, P < 0.0002). From these observations, we conclude that carotenoids from a normal, unsupplemented diet accumulate in the skin and confer a measurable photoprotective benefit (at least in lightly pigmented Caucasian skin), that is directly linked to their concentration in the tissue. Carotenoids also appear to contribute measurably and significantly to normal human skin color, in particular the appearance of “yellowness” as defined objectively by CR200 tristimulus b* values. On the basis of these findings we believe that objective measurements of skin color, in particular tristimulus b* values, may be a potentially useful means of monitoring dietary carotenoid status and assessing UV photosensitivity in Caucasian populations.

KEY WORDS: pigmentation, chromameter, antioxidants, melanin, photoprotection, humans

The color of human skin is dominated by two major biological pigments: hemoglobin, which provides red coloration via the vascular network of microcapillaries in the skin, and melanin, which provides varying degrees of brown coloration at the skin surface. Accurate and objective measurements of human skin color can be made using a tristimulus chromameter (1–6). This instrument utilizes the Commission Internationale de l’Eclairage (CIE)2 L*a*b* color system to determine skin color objectively. Color is quantified using the 3-digit output L* a*b*: L* measures skin reflectance or lightness—this is a gray scale with values from 0 to 100 where 0 is black and 100 is white; a* measures the color saturation from red to green—the scale is from -60 to +60, where positive values indicate varying intensities of red; b* measures the color saturation from yellow to blue—the scale is also from +60 to -60, where positive values indicate varying intensities of yellow.

A number of studies have been performed (mostly with Caucasian subjects) which report close associations between chromomter a* values (redness) and erythema or blood flow in the skin (7–10). Similarly, it has been suggested that both L* values (reflectance) and b* values (yellowness) are closely associated with melanin content and tanning (7–10; our unpublished data).

Although these studies indicate that much of the variation in tristimulus L*a*b* values is under the influence of hemoglobin and melanin in the skin, there is also considerable evidence to suggest that a third class of pigments, carotenoids, which are ingested through the diet, also have a significant influence on human skin color. Carotenoids such as β-carotene, lycopene and lutein are highly colored (yellow-red), fat-soluble antioxidants that are found in a wide variety of fruit and vegetables. Consumed as part of the human diet, they influence on human skin color. Carotenoids such as β-carotene, lycopene and lutein are highly colored (yellow-red), fat-soluble antioxidants that are found in a wide variety of fruit and vegetables. Consumed as part of the human diet, they

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2 Abbreviations used: CIE, Commission Internationale de L’Eclairage; IL, interleukin; MED, minimal erythemal dose.
known as carotenodermia), which is caused by the accumulation of carotenoid pigments in the skin (17–19,21). However, although it is clear that ingestion of very large quantities of carotenoids (particularly β-carotene) can alter skin color in the short term, much less is known about the effect of normal levels of dietary carotenoids on human pigmentation and UV photosensitivity.

The aim of the current study was therefore to determine whether carotenoids play a significant role in regulating skin pigmentation and UV photosensitivity in an unsupplemented human population, and further, to investigate whether tristimulus (L*a*b*) color measurements can be used to accurately and noninvasively predict carotenoid status and UV photosensitivity in human skin.

SUBJECTS AND METHODS

Study panelists. A healthy group of Caucasian volunteers (skin phototype II) comprising 8 men and 14 women aged 26–67 y was recruited at the Institute of Experimental Dermatology (Universität Witten-Herdecke, Germany). Subjects smoking >3 cigarettes per day or with a history of malabsorption diseases, liver diseases, diseases of lipid metabolism or photosensitivity disorders were excluded from the study. All procedures were subjected to internal ethical approval before commencement of the study, and all subjects were required to give witnessed, written informed consent before participating.

Determination of the minimal erythemal dose (MED). Dorsal skin was irradiated at multiple sites with varying doses of UV radiation using a solar simulator (SOL3 Hńle, Munich, Germany). Each site was clinically graded by an expert assessor and the MED determined 24 h after irradiation.

Skin carotenoid measurements. Carotenoid levels in the skin were determined noninvasively by reflection spectrophotometry using the method described by Stahl et al. (14). Briefly, the tissue was illuminated with a 5-W, 1-cm diameter halogen lamp (MBR GmbH, Herdecke, Germany) with a penetration depth into the skin of 0.1–0.85 mm. The reflection spectrum of skin was collected between 350 and 850 nm with a Multiscan OS 20 spectrophotometer (MBR GmbH) coupled to an all-silica fiber optic reflectance bundle (Top Sensor Systems, Eerbeek, Netherlands). Analysis of the spectrum allowed the carotenoid component to be quantified. Because of the low penetration of the incident light, carotenoids (largely β-carotene and lycopene) were analyzed only within the dermis and epidermis, and carotenoids in the adipose tissue were not detected. Measurements were made on the back, forehead, inner forearm and hand (back and palm). All measurements were performed at the Universität Witten-Herdecke.

Skin color measurements. Skin color measurements were made using a Minolta CR200 chromameter (Ahrensburg, Germany) on the back, forehead, inner forearm and hand (back and palm).

Statistical analysis. All statistical analyses were performed on raw (untransformed) data using the computer software package SigmaStat Version 2.03 (SPSS UK, Surrey, UK). Correlation analysis was performed by Student’s paired t test for normally distributed data (normality test passed, significant difference P < 0.05) or by Mann-Whitney Rank Sum test for nonnormally distributed data (normality test failed, significant difference P < 0.05).

RESULTS

Relationship between skin color and MED. Pearson product moment correlation analysis was performed on measurements of skin color and MED as described in Subjects and Methods. There was an inverse relationship between L* values and MED in these subjects, suggesting that the darker the skin (i.e., the more melanin it contains) the higher the level of endogenous sun protection (Fig. 1). However, the correlation coefficient for MED and L* values was not particularly high (r = −0.47) (Fig. 1). In addition, no correlation was observed between a* values and MED, whereas a strong and significant correlation (r = 0.71, P < 0.0002) was observed between b* values and MED (Fig. 1). The strength of this association was greater than the strength of the correlation between MED and L* values (Fig. 1). This was surprising because melanin has a well-documented role in UV photoprotection (22,23). The implication of this result was that human skin contains pigments that not only contribute selectively to b* values (yellowness) but, in addition to melanin, also provide the skin with a large measure of UV-photoprotection.

Relationship between skin carotenoids and MED. Carotenoids are plausible candidates for these pigments because they are both highly pigmented (yellow-red) and possess potent antioxidant properties (24–28). To determine whether an association might exist between carotenoid levels in skin and sensitivity to UV-induced erythema, Pearson product moment correlation analysis was performed on dorsal skin carotenoid levels and MED values determined on the same site. This analysis revealed a significant positive association (r = 0.66, P < 0.001) between the carotenoid levels in skin determined by reflectance spectroscopy and the level of endogenous UV photoprotection (Fig. 2).

Relationship between carotenoids and skin color. To investigate whether an association might also exist between carotenoids and skin color, we next compared skin carotenoid levels determined at various body sites by reflectance spectroscopy with corresponding tristimulus L*a*b* values also obtained from those sites. Pearson product moment correlation analysis revealed strong and significant (r = 0.747–0.854, P < 0.0001) positive linear associations between these carotenoid values and skin tristimulus b* values (yellow) on the back, forehead, inner forearm and palm of the hand together with a weaker association (r = 0.475, P < 0.03) on the back of the hand (Fig. 3). This association was very selective for b* values because similar associations with L* and a* values generally were not observed (Fig. 3).

In spite of the apparent relationship between carotenoids and b* values in human skin, it was also clear from a comparison of these parameters across different body sites that not all of the variation in b* values in human skin could be accounted for by variation in carotenoid levels alone (Table 1). This was exemplified by the fact that some subjects had almost undetectable levels of skin carotenoids (by reflectance spectroscopy), but an appreciable b* value (see Fig. 3). This is perhaps
not too surprising because melanin has a significant effect on b* values in human skin (7; our unpublished data).

The most obvious discrepancy was observed between the palm of the hand where a high carotenoid concentration was detected (in spite of a relatively low b* value) and the dorsal (or back) skin which appeared to have a lower carotenoid content but a higher b* value (Table 1). Carotenoid pigments in the skin are excreted through the sebaceous glands and partly reabsorbed by the stratum corneum (29). Hence, sites like the palm of the hand (which has a very thick stratum corneum), tend to retain high concentrations of carotenoids relative to other body sites (14,16). In contrast, the palm of the hand has very few melanocytes compared with the back, which has a relatively high concentration of these cells (~1.6 fold greater) (30,31). The skin of the back therefore contains much more melanin than the palm of the hand and thus had a correspondingly higher b* value (Table 1).

DISCUSSION

Reactive oxygen species are induced in the skin by solar UVA and UVB radiation and have long been suspected of contributing to the deleterious effects of cutaneous photodamage (32,33). Species such as singlet oxygen and superoxide, as well as hydroxyl and peroxyl radicals are believed to promote lipid peroxidation, protein oxidation and cross-linking, enzyme inactivation and DNA damage (32,33). In addition, it has been suggested that singlet oxygen mediates UVA-induced upregulation of interstitial collagenase in the skin, through induction of the inflammatory cytokine interleukin (IL)-1 and IL-6 (34,35). These properties of reactive oxygen species can result in compromised cell viability and biological function as well as increased degradation of the dermal extracellular matrix, all of which may mediate the appearance of two key phenotypes associated with cutaneous photodamage, i.e., photocarcinogenesis and photoaging (36,37).

Dietary carotenoids such as β-carotene, lycopene, zeaxanthin and lutein have potent antioxidant functionality and are among the most effective naturally occurring scavengers of singlet oxygen and peroxy radicals (24–28). Reflectance spectrophotometry has previously been used to detect the rise in skin levels of such carotenoids after elevated dietary intake of carotenoid supplements or carotenoid rich foods (14,15,19,20). This effect is preceded by ~2 wk with a significant increase in the serum levels of these antioxidants (12,14,15,19,20). In the current study, we used the same spectrophotometric methods to quantify the levels of carotenoids in the skin of unsupplemented Caucasian men and women and compared these values with objective measurements of skin color and UV photosensitivity. Our findings suggest that there is a significant relationship between normal, unsupplemented levels of dietary carotenoids in the dermis and epidermis of Caucasian skin and endogenous UV photosensitivity as determined by MED (see Fig. 2). This supports the view that carotenoid antioxidants from a normal, unsupplemented diet accumulate in the skin and confer a measurable photoprotective benefit that is directly linked to their concentration in the tissue. Our data also suggests that the yellow component of human skin color, which is quantified by the tristimulus b* value, is closely associated with carotenoid levels in the skin of the back, forehead, inner forearm and palm of the hand (see Fig. 3). These observations are linked by a third finding, which suggests a selective and positive association between skin MED...
measurements and b* values. These data suggest that in Caucasian skin, carotenoids (and by implication, other skin antioxidant defenses, both endogenous and exogenous) may play an important role in mediating UV-photoprotection.

These findings should be supported by additional studies employing a larger number of subjects and more empirical determinations of skin carotenoids, e.g., by HPLC analysis. If confirmed, these findings suggest that hand-held chromameters could be used as noninvasive tools to monitor the levels of dietary carotenoids in human skin. Moreover, because of the potential link among carotenoids, b* values and UV-photosensitivity, it may also be possible to use these noninvasive measurements to provide further information about the antioxidant status of the skin. This may have useful long-term applications, such as assessing the susceptibility of skin to UV-induced photodamage, or even predicting the risk of developing certain forms of skin cancer. This latter proposal follows the recent suggestion that low levels of skin carotenoids may be associated with an increased predisposition to develop actinic keratosis and basal cell carcinoma (16).

In recent years, a number of studies have suggested a close association between diets rich in carotenoids, and a reduced incidence of cancer, cardiovascular disease and macular degeneration in the human population (38–41). As well as being informative about the skin’s antioxidant status, dietary intervention studies have suggested that skin carotenoid concentrations are also quite closely associated with circulating serum carotenoids (12,14,19). Therefore it is possible that simple measurements of carotenoid concentrations in skin could also be informative about long-term circulating levels of carotenoids and thereby overall carotenoid status in the body. It is then conceivable that simple skin color or spectrophotometric measurements could be used in conjunction with dietary carotenoid intake data in prospective epidemiologic studies to determine the effect of tissue carotenoid status on the risk of developing cancer and cardiovascular disease.

With respect to pigmentation, it seems likely that in Caucasian subjects, even unsupplemented carotenoid levels in the skin may contribute measurably and significantly to normal human skin color, in particular the appearance of “yellowness” as defined objectively by tristimulus b* values (see Fig. 3). However, epidermal melanin also contributes significantly to b* values (7, our unpublished data). It is therefore possible that the relative influence of carotenoids on human pigmentation may diminish in darker skin types which contain more melanin. However, unlike melanin, skin carotenoids determined by reflectance spectroscopy appear to affect only b* values selectively (see Fig. 3). Therefore it is possible that within an ethnic group, tristimulus b* values may still be predictive of intersubject variability in skin carotenoid levels (see Fig. 1). Similarly, sites like the palm of the hand which are rich in carotenoids, have a very low concentration of melanin regardless of ethnicity, and therefore appear to be an ideal site for making noninvasive measurements of skin carotenoids.

In the current study we have studied the carotenoid content of Caucasian skin types only. Therefore to determine whether tristimulus b* values are associated with carotenoid status in other skin types, further studies with a wider range of ethnic groups will have to be conducted.

**LITERATURE CITED**


**TABLE 1**

Significant variation at certain human body sites in skin carotenoid levels and tristimulus b* values

<table>
<thead>
<tr>
<th>Site</th>
<th>Carotenoids (nmol/g wet weight)</th>
<th>b* Values (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Back</td>
<td>0.23 ± 0.18</td>
<td>16.1 ± 3.4</td>
</tr>
<tr>
<td>Forehead</td>
<td>0.26 ± 0.26</td>
<td>15.7 ± 2.3</td>
</tr>
<tr>
<td>Inner arm</td>
<td>0.21 ± 0.22</td>
<td>13.5 ± 2.8</td>
</tr>
<tr>
<td>Palm of hand</td>
<td>0.32 ± 0.18</td>
<td>12.9 ± 2.2</td>
</tr>
<tr>
<td>Back of hand</td>
<td>0.29 ± 0.14</td>
<td>15.9 ± 1.8</td>
</tr>
</tbody>
</table>

1 Skin carotenoid levels and tristimulus b* values were measured in a group of Caucasian men and women as described in Subjects and Methods. Measurements were taken across a range of body sites including the back, forehead, inner forearm, palm of the hand and back of the hand.

2 Carotenoid levels were significantly different between the palm of the hand and the inner forearm (Mann-Whitney Rank Sum Test, P < 0.03, n = 22). No significant difference in carotenoid levels was observed between any other body sites.

3 Tristimulus b* values for the palm of the hand and the inner forearm were found to be significantly lower than for all other body sites (Students t test, P < 0.01, n = 22). No significant difference in b* values was observed between any other body sites.