



Original Article

# Carotenoid and melanin pigment coloration affect perceived human health<sup>☆,☆☆</sup>

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## Abstract

The links between appearance and health influence human social interactions and are medically important, yet the facial cues influencing health judgments are unclear, and few studies describe connections to actual health. Increased facial skin yellowness (CIELab  $b^*$ ) and lightness ( $L^*$ ) appear healthy in Caucasian faces, but it is unclear why. Skin yellowness is primarily affected by melanin and carotenoid pigments. Melanin (dark and yellow) enhances photoprotection and may be involved in immune defense, but may contribute to vitamin D deficiency. Carotenoids (yellow) signal health in bird and fish species, and are associated with improved immune defense, photoprotection and reproductive health in humans. We present three studies investigating the contribution of carotenoid and melanin to skin color and the healthy appearance of human faces. Study 1 demonstrates similar perceptual preferences for increased skin  $L^*$  and  $b^*$  in UK-based Caucasian and black South African populations. Study 2 shows that individuals with higher dietary intakes of carotenoids and fruit and vegetables have increased skin  $b^*$  values and show skin reflectance spectra consistent with enhanced carotenoid absorption. Study 3 shows that, to maximize apparent facial health, participants choose to increase empirically derived skin carotenoid coloration more than melanin coloration in the skin portions of color-calibrated face photographs. Together our studies link skin carotenoid coloration to both perceived health and healthy diet, establishing carotenoid coloration as a valid cue to human health which is perceptible in a way that is relevant to mate choice, as it is in bird and fish species.

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## 1. Introduction

How health is manifest in physical appearance is important throughout the animal kingdom because appearance influences mate choice. In humans too, health is intimately linked with attractiveness (Jones, Little, Burt, & Perrett, 2004; Rhodes et al., 2001), yet the cues utilized in such health judgments and their validity are unclear. Most studies of how human facial characteristics are potentially linked to health focus on facial shape (Rhodes et al., 2001;

Rhodes, Chan, Zebrowitz, & Simmons, 2003), although recent studies have begun to focus on skin color and texture (Fink, Grammer, & Thornhill, 2001; Fink, Grammer, & Matts, 2006; Matts, Fink, Grammer, & Burquest, 2007; Stephen, Coetzee, Law Smith, & Perrett, 2009; Stephen, Law Smith, Stirrat, & Perrett, 2009). In three studies, we investigate the role of skin carotenoid and melanin coloration in providing a cue to a healthy diet. In Study 1, we explore cross-cultural consistency in preferences for skin lightness ( $L^*$ ) and yellowness ( $b^*$ ) by comparing the preferences of a UK-based Caucasian population and a black South African population. In Study 2, we examine the effect of carotenoids, obtained from dietary fruit and vegetable intake and from dietary supplementation, on human skin color. In Study 3, we examine the effect of skin carotenoid and melanin coloration on the perceived health of human faces.

The human face exerts a powerful influence on the impression of others and thus is a good starting point for

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studying how we judge the health of others. Yet for a health cue to be valid the trait must relate both to perceived health and to an aspect of actual health. Coetzee et al. (2009) found links from facial adiposity both to measures of actual health (respiratory illnesses and blood pressure) and to the perceived health of faces, establishing facial adiposity as a cue to health. Other robustly established facial cues to health in the literature are rare. While facial averageness (Rhodes et al., 2001, 2007), symmetry (Fink et al., 2006; Grammer & Thornhill, 1994; Jones et al., 2001; Penton-Voak et al., 2001; Rhodes et al., 2001, 2007) and female facial femininity (Law Smith et al., 2006; Rhodes et al., 2003; 2007) are perceived as healthy, attempts to link these traits to measures of actual health have produced mixed results. Thornhill and Gangestad (2006) found a relationship between female facial femininity and self-reported number and duration of respiratory infections, but Rhodes et al. (2003) found no relationship between female facial femininity and past health from medical records. Similarly, symmetry has been linked with self-reported health measures (Shackelford & Larsen, 1999; Thornhill & Gangestad, 2006), but not with past health assessed from medical records (Rhodes et al., 2001). Averageness predicts childhood health in men and adolescent and current health in women (Rhodes et al., 2001), although this relationship is driven by faces below median averageness (Zebrowitz & Rhodes, 2004).

Recent studies have investigated the contributions of aspects of skin quality, including skin color (Stephen, Law Smith, et al., 2009) and texture (Jones et al., 2004) to the healthy appearance of faces. Both *overall* skin color (Stephen, Law Smith, et al., 2009) and color *distribution* (Fink et al., 2006; Matts et al., 2007) are important to perceptions of human health. The role of skin color distribution is outside the scope of this article, however, and we focus on the effects of overall skin pigment coloration on the perceived health of human faces. Previous work has found that skin blood perfusion and oxygenation, which are relatively fast changing causes of skin color, affect the healthy appearance of human faces (Stephen, Coetzee, et al., 2009). In the current article, we concentrate on more stable causes of skin color: melanin and carotenoids.

The CIELab color space is defined by  $L^*$  (light-dark),  $a^*$  (red-green) and  $b^*$  (yellow-blue) color dimensions. It is modeled on the human visual system and designed to be perceptually uniform, a change of one unit appearing to be of approximately the same magnitude regardless of its dimension (Martinkauppi, 2002). Caucasian participants choose to increase the CIELab  $a^*$  (redness),  $b^*$  (yellowness) and  $L^*$  (lightness) values of the skin portions of Caucasian face photographs to enhance apparent health (Stephen, Law Smith, et al., 2009). Here, we evaluate cross-cultural consistency in the perception of increased skin  $L^*$  and  $b^*$  values as healthy by comparing the manipulation of Caucasian faces by UK Caucasian participants with the manipulation of black South African faces by black South African participants.

### 1.1. Carotenoids

Coloration associated with increased skin blood perfusion and oxygenation can change over a relatively short time scale. Such change primarily increases  $a^*$  values of the skin and enhances the healthy appearance of faces (Stephen, Coetzee, et al., 2009). Here, we examine the contributions of the other major skin pigments, melanin and carotenoids, to the appearance of faces. Both of these pigment types enhance skin  $b^*$  values (Alaluf, Heinrich, Stahl, Tronnier, & Wiseman, 2002; Stamatas, Zmudzka, Kollias, & Beer, 2004) and induce more long-lasting change in skin color.

Carotenoids cannot be synthesized *de novo* in the body and are primarily obtained from dietary fruit and vegetables (Alaluf et al., 2002). Carotenoids contribute to human skin  $b^*$  values through deposition in the skin (Alaluf et al., 2002). Carotenoid concentrations in the skin reflect concentrations in the blood serum and are increased by dietary supplementation, an effect that can be achieved over relatively short courses (within 8 weeks) of dietary supplementation (Stahl et al., 1998), but a direct connection between carotenoid intake and skin color has not been previously reported. We predict that individuals with higher daily carotenoid intakes, from the natural diet or from dietary supplementation, will have increased skin carotenoid deposition and increased skin  $b^*$  values. We investigate this hypothesis in a dietary study.

Carotenoids are associated with immunocompetence and disease resistance in humans. Supplementation beneficially affects thymus gland growth in children (Seifter, Rettura, & Levenson, 1981) and increases T-lymphocyte number and activity in healthy adults (Alexander, Newmark, & Miller, 1985). Carotenoid levels become reduced in individuals with HIV and malaria, and in individuals with elevated levels of serum  $\alpha_1$ -antichymotrypsin (an indicator of infection; Friis et al., 2001). The mechanisms of these immune-enhancing properties are unclear, but the antioxidant properties of carotenoids may be involved in neutralizing the high levels of damaging reactive oxygen species (ROS) generated by immune function (Dowling & Simmons, 2009). The benefits from the antioxidant properties of carotenoids may be obtained through the synergistic action of other endogenous or dietary obtained antioxidants such as vitamin C and E (Vinkler & Albrecht, 2010).

Carotenoid deposition in skin may contribute to photo-protection, reducing the level of skin damage caused by both natural and artificial UV light exposure, and increasing the minimum amount of UV exposure required to cause sunburn (minimum erythmal dose; Alaluf et al., 2002; Bouilly-Gauthier et al., 2010).

Carotenoids may also be important in the human reproductive system (Agarwal, 2005; Dowling & Simmons, 2009) as they are in other mammal (Coffey & Britt, 1993) and bird species (Peters, Denk, Delhey, & Kempnaers, 2004). High levels of antioxidants including carotenoids are present in semen and are thought to protect the sperm against

ROS produced during meiosis, metabolism and immune processes (Dowling & Simmons, 2009). Seminal carotenoid levels are lower in infertile than in fertile men and carotenoid supplementation improves male fertility (Gupta & Kumar, 2002). Carotenoids may also help to protect the female reproductive system against ROS (Agarwal, 2005).

Theoretically, the use of carotenoids in ornamentation means they are unavailable for immune and detoxification responses (Dowling & Simmons, 2009). Infection with parasites reduces carotenoid levels in guppies and chickens (Olson & Owens, 1998), and nematode infection reduces the brightness of the supra-orbital comb (a carotenoid-based ornament) in red grouse (Martinez-Padilla, Mougeot, Perez-Rodrigues, & Bortolotti, 2007), suggesting that the use of carotenoids in immune defense makes them unavailable for ornamentation. Thus, carotenoid-based ornament color may be a handicapping signal demonstrating superior immunocompetence, explaining their attractiveness to the opposite sex in several bird and fish species (Lozano, 1994). Individuals with superior skills in foraging for carotenoid-rich foods or who need to use less carotenoids in immune or detoxification responses can invest more in ornamentation while still having sufficient for immune defense.

From these considerations of humans and other species, we predict that increased skin carotenoid coloration will enhance the healthy appearance of human faces.

### 1.2. Melanin

Increased melanin levels increase human skin  $b^*$  and decrease  $L^*$  values (Stamatas et al., 2004). Melanin presents health costs and benefits depending on sunlight levels. It is important in photoprotection against skin cancer and sunburn and prevents the photolysis of folate, thereby protecting against neural tube defects (Branda & Eaton, 1978). Melanocytes are involved in phagocytosis and melanosomes have lysosomal function (Burkhart & Burkhart, 2005), both roles contributing to immune defense. Conversely, melanin prevents the photoproduction of vitamin D, potentially leading to defective bone mineralization (Murray, 1934).

Melanin is associated with tanning, which is currently fashionable in Western, Caucasian populations (Melia & Bulman, 1995). Conversely, among black South Africans, the use of skin lightening products is widespread, especially for upwardly mobile, educated women. Light-skinned individuals are perceived as more desirable, affluent, attractive and trustworthy than darker skinned individuals (see Glenn, 2008). If the fashionable tanning hypothesis explains preferences for skin  $b^*$  coloration, melanin color should be increased more than carotenoid coloration in Caucasian faces. In the cross-cultural study, the fashionable tanning hypothesis predicts that Caucasian participants will increase skin  $b^*$  values and reduce skin  $L^*$  values, simulating an increase in skin melanin, while African participants are predicted to increase  $L^*$  and decrease  $b^*$  in African faces, simulating a decrease in skin melanin.

## 2. Study 1: Cross-cultural perceptions of skin color

We investigated the cross-cultural consistency of  $L^*$  and  $b^*$  preferences in a UK-based Caucasian population and a black South African population. We allowed black South African participants to manipulate black South African faces along skin lightness ( $L^*$ ) and yellowness ( $b^*$ ) axes simultaneously to enhance the apparent health of the faces. We compared the results to UK-based Caucasian participants' responses to a similar study using UK Caucasian faces (Stephen, Law Smith, et al., 2009).

### 2.1. Methods

All studies were approved by the ethics committees of the Universities of St Andrews and Pretoria as appropriate. All participants gave prior, informed consent in writing. UK data were obtained from Stephen, Law Smith, et al. (2009). Black South African participants were presented with color-calibrated black South African facial images on calibrated CRT monitors and asked to manipulate skin color along CIELab  $L^*$  and  $b^*$  axes simultaneously to “make the face as healthy as possible”.

#### 2.1.1. Photography

A Fujifilm FinePix S5Pro digital SLR camera with Nikon 60-mm fixed-length lens was used to photograph 50 black South African undergraduate students at the University of Pretoria (25 male, 25 female; aged 18–29), without skin makeup and with neutral expressions, in a booth painted Munsell N5 grey, illuminated with three Verivide F20 T12/D65 daylight simulation bulbs in high-frequency fixtures (Verivide, UK), to reduce flicker effects. The booth was located in a room with no other lighting. A Munsell N5-painted shoulder board was placed over the shoulders and a GretagMacbeth Mini ColorChecker color chart was included in the frame. We color-corrected images using a least-squares transform, from an 11-expression polynomial expansion (Hong et al., 2001) of camera RGB values for 24 ColorChecker patches to the manufacturer-specified CIELab values of the same patches. This achieved a mean color error ( $\Delta E$ ) of 2.44 between the 24 manufacturer-stated color values and the color values obtained from the corrected images ( $\Delta E$  is the Euclidean distance between two color points in CIELab space and is the standard measure of color differences).

#### 2.1.2. Image manipulation

Using Matlab, we produced masks with even coloration representing the skin areas of faces (with a Gaussian blur at the edges), representing average face color  $\pm 8$  units of  $L^*$ . The masks were warped to the shape of the skin portions of each face image ( $n=50$ ) in turn. We manipulated each face by the difference in color between each of the pairs of masks (Stephen, Law Smith, et al., 2009). This produced a single manipulation composed of a series of 13 frames, in which Frame 0 had skin color reduced by 16 units of  $L^*$ , increasing incrementally so that Frame 6 was the original image and

Frame 12 was increased by 16 units of  $L^*$ . Each of these 13 frames was then transformed along the  $b^*$  axis (13 frames from  $-16$  units of  $b^*$  to  $+16$  units of  $b^*$ ), giving a set of 169 frames ( $13 \times 13$ ; Fig. 1A). This can be envisioned as a grid, 13 cells high (e.g.,  $L^*$  1 to  $L^*$  13) by 13 cells across (e.g.,  $b^*$  1 to  $b^*$  13). When the mouse pointer passes over each cell, the corresponding frame is displayed. Therefore, for each of the 50 trials, moving the mouse horizontally changes the facial skin in one color dimension (randomly assigned to either  $L^*$  or  $b^*$ ) and moving the mouse vertically changes the skin in the other color dimension, allowing both  $L^*$  and  $b^*$  to be changed simultaneously. For all transforms, hair, eyes, clothing and background remained constant.

### 2.1.3. Experimentation

Thirty-one black South African (15 male, 16 female; aged 18–24; from the University of Pretoria) participants were presented with the 50 trials, one face at a time, in random order on a CRT monitor (color-calibrated using a Color-Vision Spyder 2Pro to mean  $\Delta E$  of 2.32 for a range of skin tones reflecting faces of various ethnicity). For all trials, participants were given the instruction “make the face as healthy as possible”.

UK data represents an identical experiment run using 51 color-calibrated Caucasian faces (30 male, 21 female; ages 18–22) being manipulated by 32 UK-based Caucasian participants (12 male, 20 female, ages 18–25; from the University of St Andrews) in  $L^*$  and  $b^*$  dimensions simultaneously to “make the face as healthy as possible”.

### 2.1.4. Statistical methods

Mean color changes applied to each face were calculated. One-sample  $t$  tests ( $H_0$ : no color change) evaluated the overall color changes along the  $L^*$  and  $b^*$  axes. General linear mixed modeling (GLMM) examined the effect of test location [dependent variable=color change applied ( $L^*$  or  $b^*$ ), fixed factor=test location (Africa, UK), random factor=participant ID]. Participant ID was nested within location.

### 2.2. Results and discussion

To enhance healthy appearance, participants increased skin  $L^*$  ( $\Delta L^* = 2.64 \pm 0.10$ ;  $t_{49} = 25.98$ ;  $p < .001$ ) and  $b^*$  ( $\Delta b^* = 6.27 \pm 0.11$ ;  $t_{49} = 56.07$ ;  $p < .001$ ). No difference was found between the amount of color change applied to enhance healthy appearance by test location (Africa, UK) for

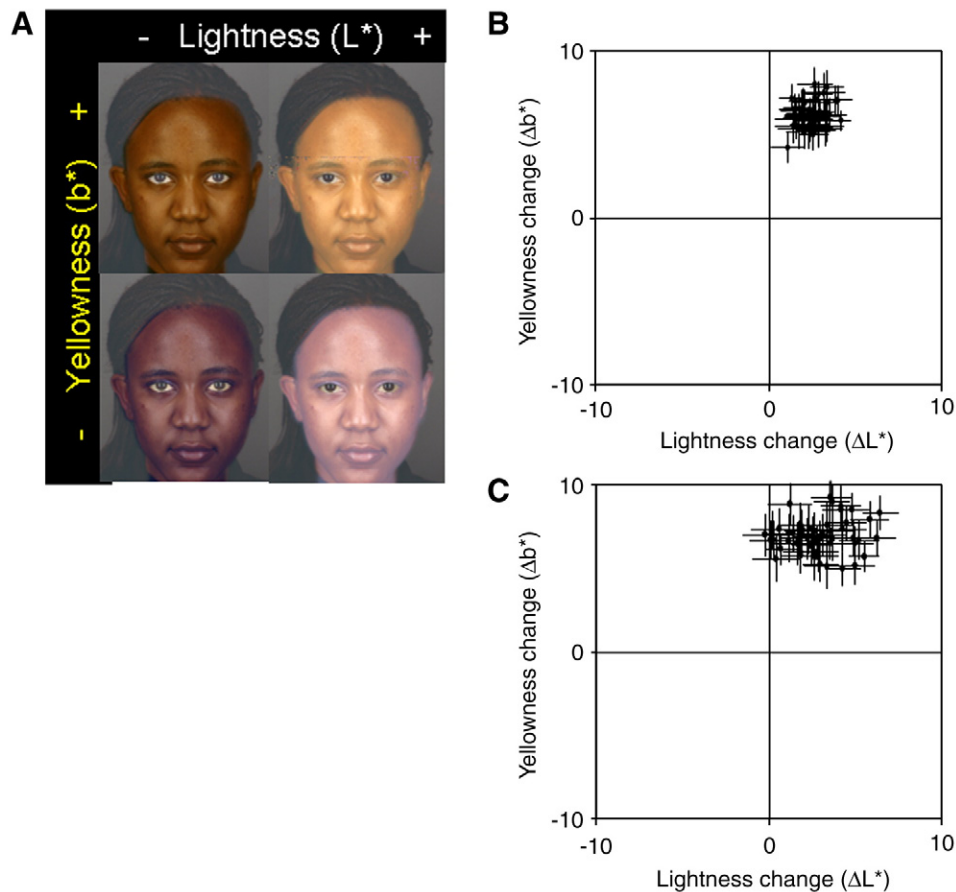


Fig. 1. (A) End points of the two-dimensional  $L^*$  vs.  $b^*$  transform in African faces. Color changes applied to enhance healthy appearance are similar in black South African (B) and Caucasian UK (C) faces, observed by their own ethnicity.

$L^*$  ( $F_{1,49.987}=0.02$ ;  $p=.889$ ) or  $b^*$  ( $F_{1,49.995}=0.785$ ;  $p=.380$ ) axis (Fig. 1B and C).

Thus, black South Africans show similar preferences for a combined increase in  $L^*$  and  $b^*$  coloration in faces to Caucasian UK-based participants (Stephen, Law Smith, et al., 2009) and therefore are likely to show similar preferences for carotenoid and melanin skin coloration. Neither the Caucasian nor the African  $L^*$  and  $b^*$  preferences support the fashionable tanning hypothesis which predicts that health perceptions are driven by fashions for increased melanin in Caucasian populations and decreased melanin in African populations. Both the Caucasian and African results are instead consistent with a preference for increased carotenoid coloration of the skin.

### 3. Study 2: skin color and carotenoids in natural and supplemented diets

Study 1 showed that judgments of a healthy skin color were consistent with raised levels of carotenoid pigments. Indeed, variation in skin color within a population could reflect individual differences in intake of carotenoids from diet. We sought to establish this carotenoid–skin color relationship by (a) comparing skin color to natural dietary consumption of carotenoids, (b) using a spectral analysis to determine the specific skin pigments associated with any relationship between skin color and dietary carotenoid intake, and (c) measuring skin color change in response to dietary supplementation with carotenoids.

We assessed the relationship of skin  $b^*$  (yellowness) values (measured using a spectrophotometer) to daily intakes of both fruit and vegetables and dietary carotenoids. It is plausible that individuals who eat a healthy diet rich in fruit and vegetables also follow other aspects of a healthy lifestyle. If so, they may be more exposed to the sun (and consequently acquire higher skin melanin levels) while engaging in additional exercise. To control for any potential effects of exercise frequency on skin  $b^*$  values, we statistically controlled for participants' exercise levels.

Relationships between skin reflectance spectra (measured by spectrophotometry) and natural daily intakes of fruit and vegetables ( $\rho_{F+V}$ ) and  $\beta$ -carotene ( $\rho_c$ ) were calculated and compared to spectral signatures of skin pigments (carotenoids, melanin, hemoglobin) to identify the pigments responsible for any relationship between skin color and dietary fruit and vegetable and carotenoid intake.

We further assessed the impact of dietary  $\beta$ -carotene supplementation on skin  $b^*$  values by measuring skin color before and after participants completed an 8-week course of oral  $\beta$ -carotene supplementation.

#### 3.1. Methods

Estimates of daily fruit and vegetable intake for each of 82 Caucasian participants (aged 18–26, 34 male, 48 female) were made by using a food item intake (obtained

by a food frequency questionnaire; Margetts, Cade, & Osmond, 1989) and summing across items. Consumption was scaled by the mean portion size of fruit and vegetable items typically eaten per sitting (obtained from 22 further participants; aged 19–28; 11 male, 11 female). All 82 participants in the diet survey indicated in a questionnaire that they had not recently used any artificial tanning product.

Estimated  $\beta$ -carotene intake was calculated by multiplying individual food item intake by their  $\beta$ -carotene content (Canadian Nutrient File v2007b) and summing across items.

Weekly Leisure Activity Score (WLAS), a validated measure of exercise frequency (Godin & Shephard, 1985), was calculated for 76 (33 male, 43 female) of the 82 participants.

Skin color measurements (CIELab) were made from the left outer shoulder, outer and inner upper arm, and ventral interosseous region of the palm of the hand, using the 8-mm aperture on a Konica Minolta CM-2600d — an accurate and affordable portable spectrophotometer. For 37 of the participants (29 female, 8 male), reflectance spectra were also obtained at 10-nm intervals from 360 to 740 nm. Reflectance at each wavelength was divided by total reflectance for each individual to control for overall skin lightness. Standard spectral absorption coefficient values were obtained for melanin (Sarna & Swartz, 1988), hemoglobin (Prahl, 1998) and three common carotenoids ( $\alpha$ -carotene,  $\beta$ -carotene and lycopene; Miller 1937). A mean carotenoid absorption spectrum was computed. For values, see Table S1.

For the supplementation aspect of the study, 10 participants (two male, eight female; aged 19–22) completed 8 weeks of  $\beta$ -carotene supplementation (15 mg/day; Holland and Barrett, Ltd). Spectrophotometer measurements of skin color (CIELab) were taken from the left outer shoulder, inner upper arm, the ventral interosseous region of the palm of the hand, the forehead, and left and right cheeks, before and after the course of supplementation.

#### 3.1.1. Statistical methods

Some variables were non-normally distributed and so Spearman's rank correlations were used to relate daily intake of fruit and vegetables and  $\beta$ -carotene to skin  $b^*$ . Non-parametric partial correlations (Spearman's  $\rho$ ) were used to test relationships controlling for WLAS.

Spearman's  $\rho$  was calculated for the relationship between daily  $\beta$ -carotene and skin reflectance values at the wavelengths at which carotenoids absorb light (400–540 nm) and defined as  $\rho_c$ . This was done similarly for fruit and vegetable intake, defining  $\rho_{F+V}$ . Non-parametric correlations compared  $\rho_{F+V}$  and  $\rho_c$  to carotenoid absorption spectra.

Paired-samples  $t$  tests were used to test whether a significant change in skin  $b^*$  values occurred between the before- and after-supplementation conditions.

Table 1  
Relationship between skin  $b^*$  values and diet

	Daily intake							
	Fruit+vegetables ( $n=82$ )		$\beta$ -Carotene ( $n=82$ )		Fruit+vegetables (controlling exercise, $df=73$ )		$\beta$ -Carotene (controlling exercise, $df=73$ )	
	$\rho$	$p$	$\rho$	$p$	$\rho$	$p$	$\rho$	$p$
Shoulder	0.21	.057	0.23	.039*	0.23	.062	0.23	.045*
Upper arm	0.17	.124	0.19	.082	0.17	.135	0.19	.096
Under arm	0.20	.079	0.27	.015*	0.20	.080	0.28	.017*
Palm	0.45	<.001*	0.47	<.001*	0.43	<.001*	0.45	<.001*
Mean	0.25	.026*	0.27	.009*	0.25	.034*	0.29	.013*

For different body locations, the correlation coefficient ( $\rho$ ) and probability ( $p$ ) are given for daily fruit and vegetable consumption and  $\beta$ -carotene intake, with and without controlling for exercise.

\* Indicates significant relationships.

### 3.2. Results and discussion

Individuals with higher daily intakes of fruit and vegetables and  $\beta$ -carotene had yellower skin (higher  $b^*$  values) at multiple body sites (Table 1). This relationship was maintained when exercise was controlled (Table 1; Fig. 2). Significant relationships were found between  $\rho_{F+V}$ ,  $\rho_c$  and carotenoid absorption spectra (Tables 2 and 3, Fig. 3). Thus the relationship between skin reflectance and dietary intake is consistent with the pattern of carotenoid light absorption. Supplementary Figures 1 and 2 show that  $\rho_{F+V}$  and  $\rho_c$  are unrelated to light absorption by melanin and hemoglobin. This provides strong evidence that individuals with higher dietary fruit and vegetable and carotenoid intakes have higher skin carotenoid levels and yellower skin, and that this relationship cannot be attributed to differences in melanin and hemoglobin pigmentation that may be associated with other aspects of a healthy lifestyle such as increased levels of exercise.

Increased skin  $b^*$  values were also seen over the course of oral  $\beta$ -carotenoid supplementation in five of the six skin regions tested (Table 4).

### 4. Study 3: Perception of carotenoid and melanin skin pigmentation

We examined the effects of skin carotenoid and melanin (the main pigments that contribute to skin  $b^*$  values; Aluluf et al., 2002; Stamatas et al., 2004) coloration on perceived health. Caucasian participants were asked to manipulate the skin color of color-calibrated Caucasian facial images along empirically measured carotenoid (Fig. 4A) and melanin (Fig. 4C) color axes to “make the face as healthy as possible”. Trials were performed that allowed participants to adjust each pigment color axis (melanin or carotenoid) separately (single-axis trials). Additional trials were performed in which participants could adjust melanin and carotenoid pigment coloration levels simultaneously (two-dimensional trials; Fig. 4E).

#### 4.1. Methods

##### 4.1.1. Photography

We took and color-calibrated (Stephen, Coetzee, et al., 2009; Stephen, Law Smith, et al., 2009) photographs of 51

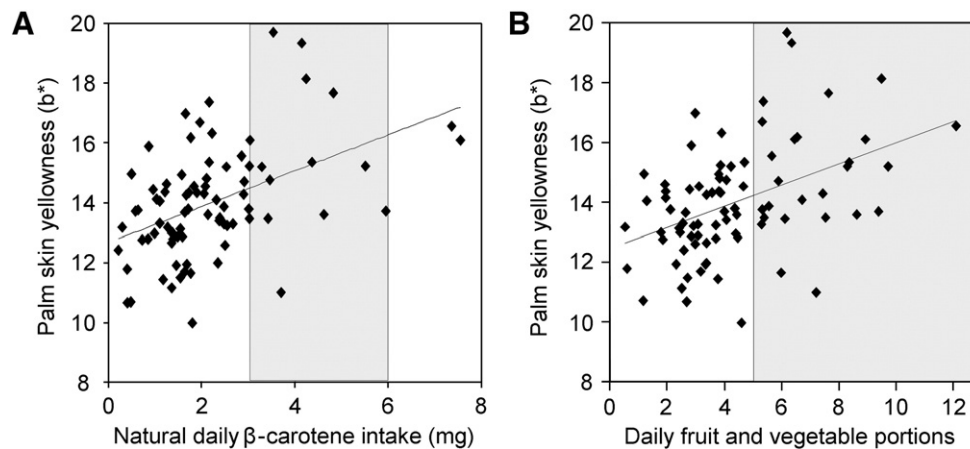


Fig. 2. (A) Individuals with higher daily  $\beta$ -carotene intake have higher skin  $b^*$  values. Shaded zone indicated recommended daily dietary intake of  $\beta$ -carotene (Institute of Medicine, 2000). (B) Individuals with higher daily fruit and vegetable intake have higher skin  $b^*$  values. Shaded zone indicates recommended daily fruit and vegetable intake (Institute of Medicine, 2000).

Table 2

Relationship between correlation coefficients ( $\rho_c$ ) calculated between carotenoid intake and skin reflectance at 10-nm intervals between 400 and 540 nm and absorption spectra of common carotenoids

	Inner arm	Right cheek	Left cheek	Forehead	Upper arm	Shoulder	Palm
$\beta$ -Carotene	$\rho=-0.68$ $p=.006^*$	$\rho=-0.72$ $p=.003^*$	$\rho=-0.61$ $p=.015^*$	$\rho=-0.88$ $p<.001^*$	$\rho=-0.58$ $p=.024^*$	$\rho=-0.40$ $p=.137$	$\rho=-0.61$ $p=.017^*$
$\alpha$ -Carotene	$\rho=-0.70$ $p=.004^*$	$\rho=-0.58$ $p=.023^*$	$\rho=-0.42$ $p=.117$	$\rho=-0.74$ $p=.002^*$	$\rho=-0.39$ $p=.148$	$\rho=-0.18$ $p=.529$	$\rho=-0.39$ $p=.154$
Lycopene	$\rho=-0.31$ $p=.265$	$\rho=-0.76$ $p=.001^*$	$\rho=-0.82$ $p<.001^*$	$\rho=-0.73$ $p=.002^*$	$\rho=-0.86$ $p<.001^*$	$\rho=-0.82$ $p<.001$	$\rho=-0.85$ $p<.001^*$
Mean	$\rho=-0.63$ $p=.013^*$	$\rho=-0.74$ $p=.002^*$	$\rho=-0.64$ $p=.010^*$	$\rho=-0.85$ $p<.001^*$	$\rho=-0.63$ $p=.012^*$	$\rho=-0.49$ $p=.066$	$\rho=-0.65$ $p=.008^*$

\* Indicates significant relationships.

Caucasian participants (21 male, 30 female; aged 18–22), without skin makeup and with neutral expressions, as described in Section 2.1.1. We calculated mean CIELab values across skin pixels for each image to define initial face color.

#### 4.1.2. Empirical pigment color measurement

**4.1.2.1. Carotenoid.** Masks were produced with even coloration representing the skin areas of faces with low and high carotenoid coloration from the mean pre-supplementation and post-supplementation skin CIELab values from Study 2. To produce the masks, the color change from the non-face skin regions was used since they are less photoexposed and hence less subject to seasonal tanning, giving a less noisy measure of carotenoid-based skin color change than could be obtained from more photoexposed areas such as the face (Table S2).

**4.1.2.2. Melanin.** Spectrophotometer measurements were taken on the dorsal side of the upper arm, 2 cm proximal to the elbow (the more melanized ‘upper arm’) and 2 cm distal to the shoulder (the less melanized ‘shoulder’). Nine (six male, three female; aged 20–23) of 19 participants were more tanned at the elbow than at the shoulder. From these nine participants, mean CIELab values for the upper arm and shoulder values were used for the low- and high-melanin color masks, respectively (Table S2).

#### 4.1.3. Image manipulation

Following Stephen, Law Smith, et al. (2009) and Stephen, Coetzee, et al. (2009), the skin portion of each of the 51 face

photographs was manipulated by a multiple of the difference between the pairs of color masks, to avoid ceiling effects (Table S2). Thirteen images were generated in equal steps from high to low color, for each pigment (Fig. 4A and C), simulating the addition or subtraction of carotenoid or melanin pigmentation. Single-axis trials allowed a participant to cycle through the 13 frames by moving the mouse horizontally to select the frame that appeared the healthiest, for each face.

Two-dimensional transforms were produced, as described in Section 2.1.1, by transforming each of the 13 single-axis melanin frames along the carotenoid axis. These allowed participants to simultaneously adjust skin melanin and carotenoid coloration by horizontal and vertical movements of the mouse, to select any of the 169 (13 melanin by 13 carotenoid) frames. Hair, eyes, clothing and background remained constant.

#### 4.1.4. Experimentation

We recruited 22 Caucasian participants (10 male, 12 female, aged 20–26) for single-axis carotenoid manipulations, 26 participants (12 male, 14 female, aged 18–24) for melanin color manipulations and 22 participants (12 male, 10 female, aged 18–25) for two-dimensional manipulations.

Participants were presented with 51 trials (melanin, carotenoid or two-dimensional), one face at a time, in random order on a color-calibrated (Stephen, Coetzee, et al., 2009; Stephen, Law Smith, et al., 2009) CRT monitor and instructed to “make the face as healthy as possible”.

Table 3

Relationship between correlation coefficients ( $\rho_{F+V}$ ) calculated between fruit and vegetable intake and skin reflectance at 10nm intervals between 400 and 540nm and absorption spectra of common carotenoids

	Inner arm	Right cheek	Left cheek	Forehead	Upper arm	Shoulder	Palm
$\beta$ -Carotene	$r=-0.61$ $p=.016^*$	$r=-0.69$ $p=.005^*$	$r=-0.63$ $p=.012^*$	$r=-0.76$ $p=.001^*$	$r=-0.59$ $p=.020^*$	$r=-0.34$ $p=.219$	$r=-0.51$ $p=.052$
$\alpha$ -Carotene	$r=-0.66$ $p=.008^*$	$r=-0.53$ $p=.041^*$	$r=-0.44$ $p=.101$	$r=-0.74$ $p=.002^*$	$r=-0.40$ $p=.136$	$r=-0.12$ $p=.668$	$r=-0.28$ $p=.313$
Lycopene	$r=-0.30$ $p=.277$	$r=-0.76$ $p=.001^*$	$r=-0.85$ $p<.001^*$	$r=-0.34$ $p=.221$	$r=-0.88$ $p<.001^*$	$r=-0.81$ $p<.001^*$	$r=-0.80$ $p<.001^*$
Mean	$r=-0.59$ $p=.022^*$	$r=-0.72$ $p=.003^*$	$r=-0.67$ $p=.007^*$	$r=-0.69$ $p=.004^*$	$r=-0.65$ $p=.009^*$	$r=-0.43$ $p=.111$	$r=-0.55$ $p=.035^*$

\* Indicates significant relationships.

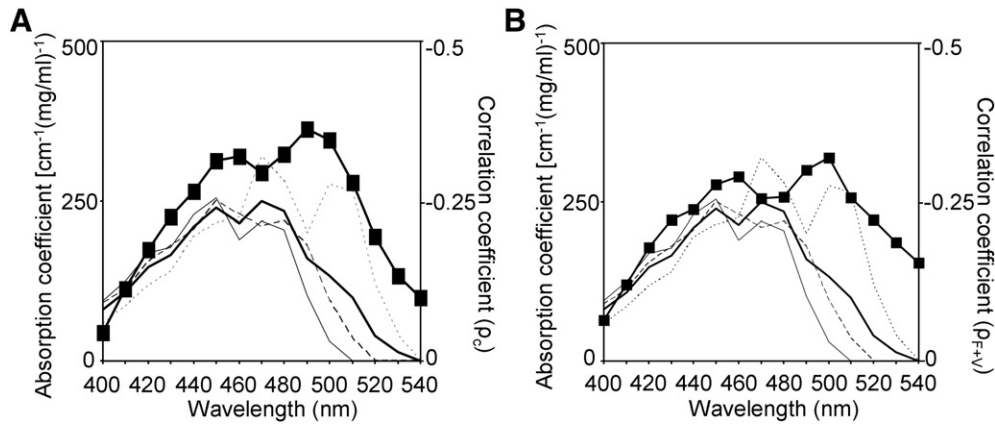


Fig. 3. (A) Correlation coefficients ( $\rho_c$ ) between skin reflectance values and daily carotenoid intake (bold line with squares) plotted with absorption spectra of common carotenoids: lycopene (dotted line),  $\beta$ -carotene (dashed line),  $\alpha$ -carotene (thin solid line) and mean carotenoid absorption (bold line, no squares). (B) Black squares represent correlation coefficients ( $\rho_{F+V}$ ) between skin reflectance values and daily fruit and vegetable intake. The absorption parallels the correlation coefficient in both plots. See supplementary information for similar plots with melanin and blood pigments.

4.1.5. Statistical methods

Mean color changes applied to each face were calculated. One-sample *t* tests ( $H_0$ : no color change) evaluated overall color changes. A general linear mixed model tested for the effects of face sex, participant sex and the interaction between the two on color change applied (dependent variable=color change; fixed factors=face sex, participant sex; random factor=participant ID; covariates=initial face color:  $L^*$ ,  $a^*$  and  $b^*$ ). A GLMM performed on the data from both melanin and carotenoid single-axis trials tested whether participants changed carotenoid and melanin color by different amounts (dependent variable=color change; fixed factor=pigment type; random factor=participant ID).

4.2. Results and discussion

In the single-pigment transforms, all faces were increased in carotenoid (Fig. 4B; color change  $\Delta E=6.39\pm 0.15$ ;  $t_{50}=43.208$ ,  $p<.001$ ) and melanin (Fig. 4D;  $\Delta E=3.51\pm 0.12$ ;  $t_{50}=29.584$ ,  $p<.001$ ) color to improve healthy appearance.

No effects of face sex or participant sex, or their interaction were found. Participants increased melanin and carotenoid color more in faces that were initially low in  $b^*$ . Initial  $L^*$  and  $a^*$  values had smaller effects (Table 5). Participants increased carotenoid more than melanin coloration ( $F_{1,46}=21.12$ ;  $p<.001$ ).

Table 4  
Yellowness ( $b^*$ ) change over  $\beta$ -carotene supplementation period

	$b^*$ before supplementation	$b^*$ after supplementation	$b^*$ change
Shoulder	16.39	17.65	$t_9=2.61$ ; $p=.028^*$
Inner arm	15.48	16.20	$t_9=1.43$ ; $p=.186$
Palm	13.22	14.44	$t_9=5.36$ ; $p<.001^*$
Forehead	15.46	16.54	$t_9=2.51$ ; $p=.033^*$
Left cheek	14.47	15.88	$t_9=3.30$ ; $p=.009^*$
Right cheek	14.18	15.79	$t_9=5.10$ ; $p=.001^*$

\* Indicates significant relationships.

In two-dimensional trials, participants induced an overall color change (Fig. 4F;  $\Delta E=5.07\pm 0.08$ ;  $t_{50}=61.417$ ;  $p<.001$ ) composed of a small change in melanin color ( $\Delta E=0.45\pm 0.16$ ;  $t_{50}=2.875$ ;  $p=.006$ ) and a significantly larger ( $t_{50}=22.803$ ;  $p<.001$ ) change in carotenoid color ( $\Delta E=5.88\pm 0.12$ ;  $t_{50}=49.264$ ;  $p<.001$ ). The combined color change represented an overall increase in skin  $L^*$  ( $\Delta L^*=0.23\pm 0.09$ ;  $t_{50}=2.520$ ;  $p=.015$ ) and  $b^*$  ( $\Delta b^*=5.66\pm 0.12$ ;  $t_{50}=48.864$ ;  $p<.001$ ) values. This is consistent with the results of the cross-cultural study, where increased  $L^*$  and  $b^*$  values were also chosen to enhance healthy appearance. Whilst 68% of faces were increased in melanin, all were increased in carotenoid color.

These results suggest that carotenoid coloration impacts more strongly on apparent health of human faces than does melanin coloration.

5. General discussion

Study 1 established a similar influence of increased skin  $L^*$  and  $b^*$  on perceived health in black South African and Caucasian UK-based populations, suggesting that similar preferences for skin melanin and carotenoid coloration may be predicted in both populations. Study 2 established a link between carotenoid intake (both naturally in the diet and supplemented) and skin yellowness ( $b^*$ ) in a Caucasian population. Study 3 established that Caucasian participants choose to increase empirically measured skin carotenoid more than melanin color to enhance healthy appearance of Caucasian faces.

In line with the UK-based Caucasian participants, black South African participants chose to apply a large increase in skin yellowness ( $b^*$ ) and a smaller increase in skin lightness ( $L^*$ ) to enhance healthy appearance. This is consistent with the hypothesis that carotenoid (which increases skin  $b^*$  values), rather than melanin (which increases skin  $b^*$  values and decreases skin  $L^*$  values),



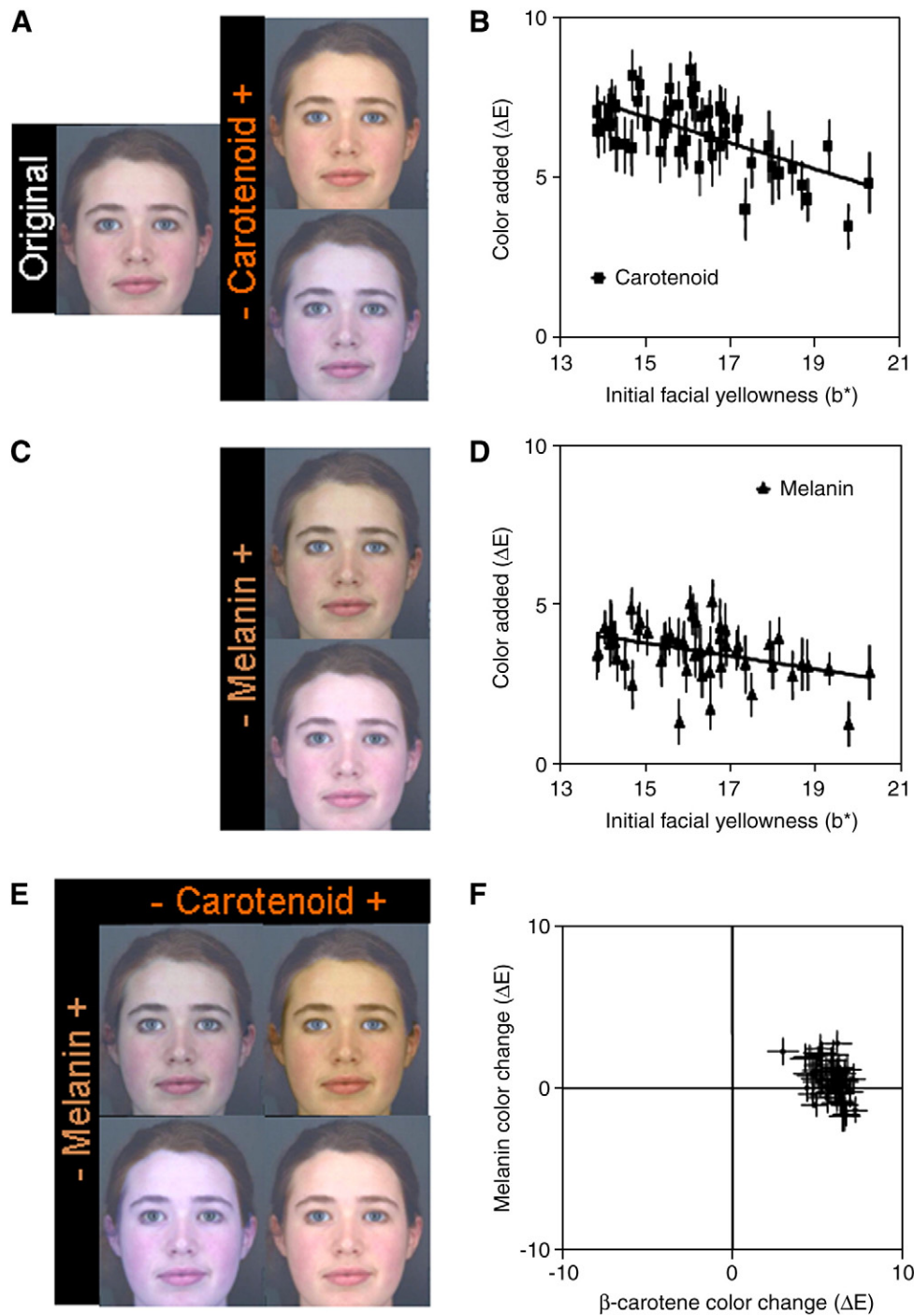


Fig. 4. (A) Original image (left) and image with increased (top) and decreased (bottom) carotenoid coloration. (B) Initial facial  $b^*$  correlates with the carotenoid ( $R^2=0.38$ ) color change to maximize healthy appearance for 51 Caucasian faces (mean $\pm$ S.E.). (C) Image with increased (top) and decreased (bottom) melanin coloration. (D) Initial facial  $b^*$  correlates with the melanin color change ( $R^2=0.15$ ) applied to maximize healthy appearance. (E) End points of the two-dimensional carotenoid vs. melanin transform. (F) Participants increase carotenoid more than melanin color to maximize healthy appearance.

coloration is important in enhancing the apparent health of human faces. Also consistent with this hypothesis were the results of Study 3, where participants chose to increase carotenoid coloration more than melanin coloration when given the option of changing both pigments (Fig. 4). When only change in melanin pigmentation was

permitted (single-axis trials), participants did raise melanin coloration to maximize healthy appearance, possibly because this was the only method available to increase skin yellowness.

If the preference for increased skin yellowness ( $b^*$ ) could primarily be explained by a socially driven

Table 5  
Summarized statistics for factors affecting amount of pigment color to optimize healthy appearance

	Dimension manipulated	
	Melanin	Carotenoid
Initial face $L^*$	$F_{1,1295}=30.10; p<.001$	$F_{1,1095}=5.21; p<.023$
Initial face $a^*$	$F_{1,1295}=7.54; p<.001$	$F_{2,1095}=2.27; p=.132$
Initial face $b^*$	$F_{1,1295}=41.82; p<.001$	$F_{1,1095}=41.77; p<.001$
Participant sex	$F_{1,24,057}=0.21; p=.653$	$F_{1,20,052}=1.50; p=.234$
Face sex	$F_{1,1295}=3.03; p=.082$	$F_{1,1095}=0.25; p=.621$
Participant sex×Face sex	$F_{1,1295}=0.03; p=.861$	$F_{1,1095}=0.40; p=.525$
Participant sex (participant ID)	$F_{24,1295}=27.09; p<.001$	$F_{20,1095}=24.88; p<.001$

Columns give GLMM factors.

preference for tanned skin, we would expect a significantly greater addition of melanin than carotenoid coloration in both single-axis and two-dimensional transforms. The opposite was seen. Further, white European and black African participants chose similar increases in lightness ( $L^*$ ) and yellowness ( $b^*$ ) to maximize apparent health of own-ethnicity faces (Study 1), suggesting cross-cultural consistency in the role of carotenoids in skin appearance.

While cross-cultural similarity in  $L^*$  and  $b^*$  preferences may be interpreted as supporting an innate, domain-specific role for skin color in the apparent health of human faces, further study is necessary before this conclusion can be made. For example, the skin color of black South Africans and UK-based Caucasians is very different. So, while both ethnicities have similar preferences for increased yellowness ( $b^*$ ) in own-ethnicity faces, it remains to be shown whether this will be the case with other-ethnicity faces. It may be hypothesized therefore that learning mechanisms are involved in calibrating people's perceptions of normal and healthy skin colors. Further study should address this issue.

We have established links from skin  $b^*$  to dietary fruit and vegetables and carotenoid intake and also from empirically measured skin carotenoid coloration to apparent health of human faces. Together, these lines of evidence suggest that, in humans, the enhanced skin  $b^*$  values associated with increased carotenoid intake represent a valid cue to a healthy diet. Whereas previous work found that increased skin  $b^*$  enhances apparent health of human faces (Stephen, Law Smith, et al., 2009), the current study provides an explanation for the preference in terms of health-relevant pigmentation. Of course, skin carotenoid levels and  $b^*$  coloration may also be affected by factors such as immune system status and the amount of carotenoids used by the body in neutralizing ROS (Dowling & Simmons, 2009; Vinkler & Albrecht 2010). Future studies addressing these factors would be valuable.

Young women's decisions to eat fruit and vegetables are significantly motivated by a belief that it will improve their appearance (Chung, Hoerr, Levine, & Coleman, 2006). Our

study linking skin carotenoid coloration to healthy appearance may therefore provide a powerful message for promoting healthy eating, increasing fruit and vegetable intake towards widely missed government recommendations (5–13 portions; Fig. 2; Institute of Medicine, 2000).

We have demonstrated that human skin coloration represents a valid cue to dietary carotenoid and fruit and vegetable intake. Carotenoid coloration may also indicate several health attributes including enhanced immune function and fertility (Dowling & Simmons, 2009; Vinkler & Albrecht 2010), as it does in many bird and fish species (see Lozano, 1994). Since skin color contributes to apparent health, and apparent health is a major component of attractiveness (Jones et al., 2004), skin color may affect mate choice in humans, similarly to other species. Past or present mate choice for carotenoid coloration may represent selection for individuals who are most capable of acquiring healthy food resources and/or are most able to resist disease (and can therefore expend carotenoid resources on skin coloration), particularly in view of the cross-cultural agreement in skin color preferences seen in Study 1. Work investigating a possible link between skin carotenoid coloration, mate choice and specific health outcomes in humans would be a valuable next step.

The moderate impact on apparent health from an increase in melanin reflects a balance between costs and benefits. The enhanced healthy appearance caused by increased melanization might be attributed to increased skin yellowness, which resembles carotenoid coloration. Costs follow a decrease in skin lightness which can affect apparent socioeconomic status (Glenn, 2008) and an impairment of vitamin D synthesis (Murray, 1934).

While the current study focuses on overall skin pigment coloration, the distribution of pigments across the face also affects the apparent health of human faces (Fink et al., 2006; Matts et al., 2007). The spectrophotometer (used in Studies 2 and 3 to measure skin color) does not measure color distribution, instead it takes a single color reading from a small area of skin, 8 mm in diameter. Since skin carotenoid deposition may also help to protect against UV-induced photodamage (Alaluf et al., 2002; Bouilly-Gauthier et al., 2010), it is likely that carotenoids may also play a role in helping to reduce the loss of skin color homogeneity (evenness) across the face, further enhancing healthy appearance. Further study to examine potential relationships between pigmentation levels, pigment distribution and healthy appearance would be valuable.

In summary, for the first time in a mammalian species, we have shown that skin color reflects healthiness of diet. For humans, skin color within a population represents a cue to health condition, which is perceptible in a way that is relevant to mate choice and may be subject to sexual selection (Jones et al., 2004).

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.evolhumbehav.2010.09.003.

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