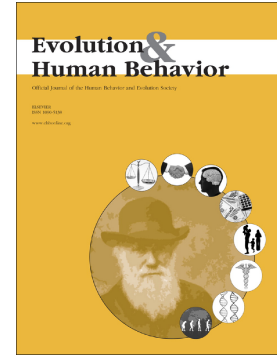


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**Impact of fresh fruit smoothie consumption on apparent health of Asian faces**

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**Impact of fresh fruit smoothie consumption on apparent health of Asian faces****Abstract**

Skin carotenoid coloration has been proposed as a valid cue to health in humans, reflecting fruit and vegetable intake, and enhancing apparent health. Supplementation with a carotenoid-rich fruit and vegetable smoothie affects skin color, but it is not known if this skin color change enhances healthy appearance. In three experiments, we examine the effects of skin color change induced by supplementation with a carotenoid-rich fruit smoothie (25mg carotenoids/d) on the apparent health of Malaysian Chinese faces. In experiment 1, observers were asked to identify the healthier looking of pairs of photographs of the same subject taken pre- and post-supplementation (or pre- and post-placebo), choosing the pre-supplementation (or pre-placebo) images. When confounding due to facial expression was eliminated in experiment 2, observers showed no preference for unmodified pre-supplementation photograph or the same image with skin color manipulated to simulate a level of smoothie-induced color change associated with 4 weeks of supplementation. In experiment 3, observers manipulated the skin color of face photographs along the smoothie-induced color change axis to optimize healthy appearance. Observers chose to induce a color change approximately equivalent to one third of the change induced by daily consumption of our carotenoid rich smoothie. This suggests that the skin color change induced by the supplementation enhanced apparent facial health, however the dose and duration of the supplementation overshot the optimal healthy-looking color of Malaysian Chinese skin. This suggests that there is an optimal carotenoid color for healthy appearance, and that this optimal level may be constrained by preferences for averageness, by the association between

very yellow skin and ill health, or by negative health impacts of very high doses of carotenoids.

## 1. Introduction

The human face conveys a range of important social and biological information about the bearer, and other humans detect and interpret such information. For example, observers make rapid, accurate judgements about age (Mouchetant-Rostaing & Giard, 2003), sex (Mouchetant-Rostaing & Giard, 2003), emotional state (De Sonneville et al., 2002), and even personality (Petrican, Todorov, & Grady, 2014) from brief exposures to faces. More recently, attention has turned to identifying cues to health in human faces, and facial features such as symmetry (Rhodes et al., 2001) or facial adiposity (Coetzee, Perrett, & Stephen, 2009) have been proposed to be valid cues to human health. These cues to health are theoretically important, as the leading adaptationist view of attractiveness posits a mechanism for identifying healthy and high quality mates (for a review, see Stephen & Tan, 2015). However, in order for a trait to be considered a valid cue to health, it must relate both to apparent health/attractiveness and to some aspect of physiological health (Stephen & Tan, 2015; Coetzee et al., 2009).

Several studies have now examined the role of skin color as a valid cue to health, with studies examining both the relationship between skin color and perceptions of health and attractiveness, and the relationship between skin color and underlying physiological health. Stephen, Law Smith, Stirrat, & Perrett (2009) allowed participants to manipulate skin color in color-calibrated photographs of Caucasian faces along CIELab L\* (luminance), a\* (red-green) and b\* (yellow-blue) axes to make the faces appear as healthy as possible. Participants chose to increase facial skin luminance (L\*), redness (a\*), yellowness (b\*) to enhance healthy appearance.

The values of these color dimensions in human skin are, of course, primarily determined by the type and density of skin pigments and the degree of blood oxygenation. Oxygenated haemoglobin in blood is bright red in color, whereas less oxygenated haemoglobin is a darker, blue-red color (Stephen, Coetzee, Law Smith, & Perrett, 2009). A higher level of oxygenated haemoglobin may reflect physical fitness and absence of respiratory and cardiac illnesses (Charkoudian, 2003; Johnson, 1998; Panza, Quyyumi, Brush, & Epstein, 1990), as well as levels of sex hormones (Jones et al., 2015). Participants choose to increase the apparent oxygenated blood content of facial skin to optimize healthy appearance, suggesting that observers may use this coloration as a cue to health (Stephen, Coetzee, et al., 2009).

The yellow component of skin color reflects the levels of melanin (which increases the  $b^*$ , and reduces the  $L^*$  components of skin color, giving it the characteristic brown appearance; (Stamatas, Zmudzka, Kollias, & Beer, 2004) and carotenoids (which primarily increase the  $b^*$  and  $a^*$  components of skin color; (Alaluf, Heinrich, Stahl, Tronnier, & Wiseman, 2002; Tan, Graf, Mitra, & Stephen, 2015) in the skin. Melanin protects the skin from sunburn and skin cancer (Branda & Eaton, 1978). Small increases in melanin coloration have been shown to enhance the apparent health of facial skin (Stephen, Coetzee, & Perrett, 2011).

Carotenoids are orange, yellow or red pigments with antioxidant properties, thought to be beneficial for human immune (Alexander, Newmark, & Miller, 1985; Seifter, Rettura, & Levenson, 1981) and reproductive (Agarwal, 2005) systems (Dowling & Simmons, 2009). It has been shown that increased skin carotenoid coloration is associated with perceived health (Stephen et al., 2011) and attractiveness (Lefevre & Perrett, 2015) in Caucasian and African (Coetzee & Perrett, 2014; Stephen et al., 2011) populations (though effects of carotenoids are more difficult to perceive in African skin (Coetzee & Perrett, 2014)). Most

studies that examined the impact of carotenoids on the appearance of human skin have used carotenoid supplements (Alaluf et al., 2002; Heinrich et al., 2003; Stephen et al., 2011) or been correlational in nature (Stephen et al., 2011; Whitehead, Re, Xiao, Ozakinci, & Perrett, 2012). We recently demonstrated in a placebo controlled trial that consumption of a carotenoid-rich fruit and vegetable smoothie increased the redness ( $a^*$ ) and yellowness ( $b^*$ ) components of human skin color (Tan et al., 2015), and correlational studies have shown that the yellowness ( $b^*$ ) of human skin is related to natural dietary intake of fruit and vegetables (Stephen et al., 2011; Whitehead et al., 2012). It is not known, however, whether experimentally-induced changes in skin color, such as those associated with supplementation with a carotenoid-rich smoothie, is perceived as healthy. Further, it is not known how quickly these effects can be seen.

Here, we report the results of three studies investigating the impact of skin colour changes associated with consumption of a carotenoid-rich fruit and vegetable smoothie on perceptions of health in Asian faces. We predict that increased smoothie-induced skin coloration will enhance the healthy appearance of Asian facial skin.

## **2. Experiment 1: Perceived health of faces before and after supplementation**

This study aimed to determine whether a 4 week supplementation with a daily carotenoid rich fruit and vegetable smoothie (containing on average 25 mg total carotenoids per day) would alter the perceived healthiness of the faces of study participants. This is a within-subjects study whereby participants were asked to compare the health status of two same-subject identity images taken at different times (before and after 4 weeks of smoothie supplementation). All experiments reported here were approved by the Ethics Committee at the University of Nottingham Malaysia Campus. All participants gave both verbal and written informed prior consent. Throughout this paper, we refer to the individuals in the

supplementation phase as “subjects”, and the individuals making perceptual judgements as “observers”.

## 2.1 Methods

**2.1.1 Stimuli.** Subjects in the supplementation group ( $n=34$ , 17 male, 17 female; mean age = 20.47, SD = 1.13) consumed 500 mL freshly prepared fruit and vegetable smoothie (containing on average 25 mg total carotenoids, for further details see Tan et al, 2015) each weekday, while subjects in the control group ( $n=34$ , 11 male, 23 female; mean age = 20.59, SD = 1.40) consumed a bottle of filtered water (500 mL; described as “purified, detoxifying water”) each weekday (Tan et al., 2015).

For each subject, one photograph was taken before the supplementation began and another one after four weeks of supplementation (Figure 1). Photographs were taken in a photo booth painted Munsell N5 grey and illuminated with nine T12/D65 fluorescent tubes (Verivide, UK) mounted in high frequency fixtures to reduce the effects of flicker. The camera was a Nikon D3100 with settings held constant at aperture f5.0, shutter speed 1/100sec, ISO 400. A GretagMacbeth mini ColorChecker was included in the frame, and images were color corrected following Stephen et al. (2009). Images were resized to 337x449 pixels, and all participants tied long hair back and wore a black hairband to remove possible confounding effects of hairstyle.

**2.1.2 Judgements of Perceived Health.** Fifty seven Malaysian Chinese (24 males, 33 females; average age = 22.35, SD= 3.17) were recruited as “observers”. All observers self-reported normal color vision and pursued undergraduate or postgraduate degrees at University Putra Malaysia (UPM) or Universiti College Sedaya International (UCSI), to reduce the likelihood of observers knowing subjects in real life.

Using the software PsychoPy (Peirce, 2009) and a 15” computer screen color-calibrated with a DataColor Spyder 4 Pro, the observers were presented with 68 pairs of

same-subject-identity facial photographs taken before and after smoothie supplementation. The first facial image was presented for 750ms, followed by a visual mask of black dots on a white background for 100ms, and then the second image of the same subject was shown for 750ms. Photographs taken at the beginning and at the end were presented in randomized order (i.e. sometimes the photograph taken before supplementation was shown as the first image and sometimes as the second image) and the order of identities was randomised. Observers were asked to decide which of the paired faces shown looked healthier, and indicate their choice using the keyboard. There was no explicit indication which of the paired photos was taken before supplementation, and which was taken after the supplementation.

## 2.2 Results

The mean number of pre-supplementation and after-supplementation images chosen as appearing healthier was calculated across participants and is presented in Table 1. Paired samples t-tests compared the frequencies of the pre-supplementation and after-supplementation images that were selected as healthier-looking, in each group. Observers deemed pre-supplementation images as healthier. However, this was observed in both the supplementation subject group,  $t(56)=2.941$ ,  $p=.005$ , (who had consumed smoothies) and the control group  $t(56)=5.121$ ,  $p<.001$  (who had consumed water). Pearson's chi-square found no difference in the strength of this effect between the supplementation and control groups of subjects  $\chi^2(1) = .001$ ,  $p = .97$ .

## 2.3 Discussion

Contrary to our hypothesis, the pre-supplementation face images were perceived as healthier than the after-supplementation images. However, the fact that this pattern was seen in the control group as well as in the smoothie group, suggests that our finding is attributable to one or more confounding factors, and not to smoothie-induced skin color change. While every



effort was taken to collect before and after photographs in identical conditions, facial characteristics can change over time due to change of season, weather, physical and psychological stress, or exercise level. It should be noted that the majority of baseline images were taken in February, while the majority of post-supplementation images were taken in March, when temperature and levels of sunlight are at their highest in Malaysia (Zain-Ahmed, Sopian, Zainol Abidin, & Othman, 2002), which could explain why pre-supplementation images were preferred for both supplementation and control groups. To exclude confounding factors, we examined in Study 2 whether the observers would detect differences in perceived health of identical facial images where only the skin tone was altered.

### **3. Experiment 2: Preferences for faces with smoothie-induced carotenoid coloration**

To eliminate potential confounding due to facial expression, posture or other variables, in experiment 2, only the skin color differed between the two presented images. Observers were asked to identify whether the unmodified pre-supplementation photograph or the same photograph in which skin color had been manipulated to replicate the measured smoothie-induced skin color change looked healthier. It was hypothesized that the faces with smoothie-induced color change would be perceived as healthier. This is a within-subjects study in which observers were asked to compare the healthy appearance of two same-subject-identity images that differ only in smoothie-induced skin colouration.

#### **3.1 Methods**

**3.1.1 Stimuli preparation: Skin colour measurement.** Facial skin color from three different regions (left cheek, right cheek, and forehead) were measured before and after smoothie supplementation using a Konica Minolta CM2600D spectrophotometer, and  $L^*$ ,  $a^*$  and  $b^*$  values were averaged across the three regions. Four weeks of smoothie supplementation decreased skin luminance by  $\Delta L^* = -.913$ , increased skin redness by  $\Delta a^* =$

1.077 and increased skin yellowness by  $\Delta b^* = 3.382$  (Tan et al., 2015). Thirty face photographs from subjects in the smoothie group were randomly selected for experiment 2, with an equal number from each gender. Matlab was used to produce masks with even coloration representing the skin areas of faces, with a Gaussian blur at the edges. One mask was created to represent average face color adjusted by the amount of color change associated with 4 weeks of smoothie supplementation ( $\Delta L^* = -.913$ ,  $\Delta a^* = 1.077$ ,  $\Delta b^* = 3.382$ ), and one with average face color with this color change reversed ( $\Delta L^* = .913$ ,  $\Delta a^* = -1.077$ ,  $\Delta b^* = -3.382$ ). Psychomorph (Tiddeman, Burt, & Perrett, 2001) was used to manipulate the skin portions of the face photographs by 50% of the difference between the two masks to simulate the color change associated with 4 weeks of smoothie supplementation. All non-skin portions of the images remained unchanged. All the facial images (manipulated and unmanipulated) were standardized in terms of size and eye level (Stephen, Law Smith, et al., 2009). An example of the images used is presented in Figure 2.

**3.1.2 Judgements of perceived health.** An identical procedure was used as for experiment 1, except for the stimuli, as described above. Forty-two different Malaysian Chinese (20 males, 22 females) were recruited as “observers” (average age 22.29; SD= 3.42). All observers self-reported normal color vision and pursued undergraduate or postgraduate degrees at University Putra Malaysia (UPM) or Universiti College Sedaya International (UCSI).

### 3.2 Results

The mean number of original and color-manipulated images chosen as appearing healthier was calculated across participants and is presented in Table 2. A paired samples t-test found no significant difference in observers’ preference for original images or images manipulated to simulate smoothie-induced skin colour change,  $t(41)=1.07$ ,  $p=.291$ .

### 3.3 Discussion

Contrary to our initial hypothesis, participants showed no significant preference for original or colour-manipulated images. It may be that the magnitude of change in skin colour induced by the smoothie supplementation was not appropriate to optimize the apparent health of Malaysian Chinese faces. Although faces with increased carotenoid coloration were perceived as healthy (Stephen et al., 2011) and attractive (Lefevre, Ewbank, Calder, von dem Hagen, & Perrett, 2013; Lefevre & Perrett, 2015) in previous studies, overly yellow and red faces may not be perceived as healthy, and may even resemble the symptoms of certain physical illnesses such as jaundice and rosacea, and during climateceterium (Cohen, Wong, & Stevenson, 2010; Izikson, English, & Zirwas, 2006; Mansor et al., 2012). In guppies (small fish that display sexually-selected colorful carotenoid-based ornaments), bigger, brighter carotenoid-based ornaments are preferred by the opposite sex, however, overly yellow colouration is not preferred (Houde, 1987). In humans, perceptual studies have tended to use a method of adjustment task to identify the healthiest level of carotenoid coloration. Ceiling effects have not been observed in these previous studies in Caucasian and African faces (Stephen et al., 2011; Stephen, Law Smith, et al., 2009), suggesting that increasing skin yellowness does not improve healthy appearance indefinitely. Our findings may suggest that extreme values of skin yellowness may not be preferred. It may be suggested that, conversely, the amount of change in coloration induced by smoothie supplementation is too small to be detectable. However, it has previously been found that much smaller color changes can be detected in human faces (Tan & Stephen, 2013), making this explanation appear unlikely.

#### 4. Experiment 3: What is the optimal amount of carotenoid induced skin colouration?

It was hypothesized that observers prefer enhanced carotenoid-induced skin colouration, but not to the extent seen in experiment 1 and 2.

##### 4.1 Materials and Methods

**4.1.1 Stimuli preparation.** To create stimuli for Experiment 3, Matlab was used to produce masks with even coloration representing the skin areas of faces, with a Gaussian blur at the edges. One mask was created to represent average face color with 4 times the change observed in the smoothie supplementation study (henceforth referred to as intervention units; decreased luminance by 3.652 units, increased redness by 4.308 units and increased yellowness by 13.528 units) and another one with average face color -4 intervention units of color change (increased luminance by 3.652 units, decreased redness by 4.308 units and decreased yellowness by 13.528 units). Images were transformed into a series of 41 frames such that image 0 had facial color reduced by 4 supplementation units, increasing incrementally so that image 20 was the original image and image 40 had color increased by 4 supplementation units. For all the transformations, participants' hair, eyes and clothing and the background remained unchanged (Figure 3).

**4.1.2 Observers.** Fifty seven different Malaysian Chinese (24 males, 33 females; mean age = 22.35, SD= 3.17) observers were recruited for this experiment. All observers were students from University Putra Malaysia (UPM) and Universiti College Sedaya International (UCSI) and self-reported normal color vision.

**4.1.3 Procedure.** Stimuli were presented using computers which had been color calibrated using a DataColor Spyder 4 Pro. Participants were presented with 30 facial images (15 males, 15 females, mean age = 20.37, SD = 1.19), one image at a time, and were asked to

adjust the color of skin portions of the facial images presented. By moving the computer mouse horizontally, the app cycled through the 41 frames of the color change for a single face identity to give the appearance that the participant was manipulating the color of the face along the smoothie-induced color axis in real time. The sequence of the individual faces presented and the starting frame in the color transform sequence were randomized.

Participants were asked to “make the face as healthy as possible”.

## 4.2 Results

One supplementation unit in this experiment is equivalent to an increment in skin yellowness of 3.382 units, an increment in skin redness of 1.077 units, and a decrement in skin luminance by -.913 units (the amount of skin color change seen in the supplementation study).

Observers significantly increased the amount of smoothie-induced color in the facial skin to enhance healthy appearance,  $t(29) = 24.65$ ,  $p < .001$ , on average adding .34 supplementation units of smoothie-induced colour change ( $SD = .074$ ). This amount of change is equivalent to a decrement in skin luminance of .31 units of  $L^*$ , an increment in skin redness of .37 units of  $a^*$ , and an increment in skin yellowness of 1.15 units of  $b^*$ . The amount chosen was only one third of the change in facial color that was experienced by the subjects in the supplementation study (Tan et al., 2015; Table 3).

**4.2.1 Relationship between colouration adjustment and initial facial colour.** To examine whether the chosen preferred skin colour was influenced by the initial skin colour, Pearson’s correlation was performed. All variables were normally distributed, and results showed a significant correlation between the chosen amount of colour adjustment and the initial colour of the face (Figure 4). This means faces which were initially lighter received more increment ( $r(29) = .542$ ,  $p = .002$ ), and faces which were initially redder ( $r(29) = -.387$ ,  $p = .038$ ) or yellower ( $r(29) = -.708$ ,  $p < .001$ ) received less increment to optimise healthy appearance.

**4.2.2 Gender difference in the initial face colouration and effects on the adjustment.** Independent samples t-tests showed that unmanipulated male faces were significantly darker,  $t(28)=4.30$ ,  $p<.001$  and yellower  $t(28)=2.28$ ,  $p = .031$  and marginally significantly redder  $t(28)=1.98$ ,  $p = .058$  than female faces (Table 4), in line with previous studies.

An independent samples t-test was conducted to examine the relationship between gender and degree of colouration adjustment. The result was significant,  $t(28)=2.194$ ,  $p=.037$ , where male faces received less colouration increase (mean=.31, SD=.065) than female faces (mean=.36, SD=.074).

An ANCOVA was conducted (with amount of color change chosen as the dependent variable, gender as fixed factor, and initial skin colour [ $L^*$ ,  $a^*$  and  $b^*$ ] as covariates) to examine if the gender difference still exists after controlling for the initial facial colours. No significant gender difference was found  $F(1,25) = .014$ ,  $p = .908$ . This suggests that the gender difference in amount of color change applied was driven by the initial skin colour between men and women.

### 4.3 Discussion

In line with our hypothesis, observers chose to increase smoothie-induced facial skin color to optimize the healthy appearance of faces. However, the chosen color change was of a smaller magnitude (approximately 34%) than the color change induced by our smoothie supplementation. This suggests that the carotenoid dose in the smoothie (Tan et al., 2015) was higher than the dose that would best enhance the apparent health of Asian faces. To deliver the optimal carotenoid dose within a smoothie, carotenoid concentration in the smoothie, volume consumed or the frequency of consumption could be reduced. Faces that were initially redder, yellower and darker (i.e. showed color consistent with higher skin carotenoid content) received less colour adjustment. This provides additional support for the

hypothesis that there is an optimal level of skin carotenoid coloration that best enhances the healthy appearance of Asian faces.

## 5. General Discussion

In experiment 1, observers preferred the pre-supplementation facial images over the post-supplementation images, regardless of whether the images were from the experimental group or the control group. This clearly indicates the presence of one or more confounding factors, such as facial expression, facial shape and skin texture which were beyond of the control of the experimenters. Therefore, experiment 2 was designed to examine the effect of smoothie-induced skin color change on apparent facial health, while controlling for possible confounding factors. Observers chose between same-subject-identity pairs of facial images that differed only in skin coloration. However, no significant preference was found. We therefore hypothesized that the change in facial coloration induced by the smoothie supplementation might have overshot the optimum color change and therefore be too great to be perceived as healthy. The third perceptual experiment was designed to identify the preferred amount of skin colour change. Observers chose a carotenoid-induced colouration equivalent to only one third of the amount of colouration change that was induced by smoothie consumption. The results suggest that the carotenoid level of the prescribed smoothies may have exceed the optimum dose.

### 5.1 Constrained directional selection

We know from previous studies (Lefevre, Ewbank, Calder, Hagen, & Perrett, 2013; Stephen et al., 2011) that increments in facial yellowness and redness are perceived as healthy.

However, results presented here suggest that there may be a ceiling to this preference, and faces which are overly red or yellow will no longer be seen as healthy and attractive. While it may be tempting to attribute this phenomenon to the preference for averageness seen in facial

attractiveness studies (Apicella, Little, & Marlowe, 2007; Rhodes et al., 2001), our data suggest that constrained directional selection may better explain the pattern of results. The averageness hypothesis predicts that skin coloration close to the average in the sample would be perceived as most healthy. We found instead that participants choose to increase the amount of smoothie-induced coloration in all of the faces in our sample to enhance healthy appearance, but that the amount of increment applied was not the maximum amount available to participants. Further, the amount of increment applied was positively correlated with the initial luminance ( $L^*$ ) and negatively correlated with the initial redness ( $a^*$ ) and yellowness ( $b^*$ ) of the faces. This suggests that, rather than more smoothie-induced color change indefinitely enhancing healthy facial appearance, there may be an optimal amount of smoothie-induced color change to enhance healthy facial appearance that is higher than the average but not infinitely high. This suggests that skin coloration may be under constrained directional selection. This finding is in line with previous studies in humans, where skin color preferences have been found to be negatively related with initial skin color of the faces (Stephen, Coetzee, et al., 2009; Stephen et al., 2011; Stephen, Law Smith, et al., 2009).

We find here that the preferred amount of smoothie-induced color change to optimize healthy appearance of Asian faces is approximately 34% of the change induced by supplementation with a daily smoothie containing 25mg of carotenoids for 4 weeks (which was long enough to see a plateau in skin color change, Tan et al. (2015)), suggesting that an optimal dose of supplemental carotenoids for enhancing healthy appearance may be around 8.5mg per day. This is similar to Stephen et al. (2011), who found that the preferred amount of carotenoid color change to optimize healthy appearance of Caucasian facial photographs was approximately 56% of the color change induced by 6 weeks of daily supplementation with 15mg of beta-carotene in capsule form, which suggests an optimal dose for enhancing healthy appearance may be around 8.4mg per day. While the primary issuers of nutritional



Recommended Daily Allowances (RDAs), the European Food Safety Authority (2006) and the U.S. Food and Nutrition Board (2000), do not issue RDAs for carotenoids due to a lack of large scale studies, the U.S Food and Nutrition Board (2001) notes that to achieve the RDA for vitamin A from carotenoid intake, an adult would need to consume between 2.8 and 21.6 mg per day, depending on the source, bioavailability and type of the carotenoids, and the sex of the individual. The amount associated with optimized healthy appearance in Asian and Caucasian young adult faces is therefore within this range.

Increased carotenoid intake is thought to be beneficial for humans' physical health, such as augmentation of immunity (Hughes, 2001), visual acuity and photoprotection of the skin (Fraser & Bramley, 2004; Krinsky & Johnson, 2005). However, an extremely high level of skin yellowness might be an indication of certain diseases and ailments, such as jaundice (Cohen et al., 2010; Mansor et al., 2012).

Dietary carotenoids can act as pro or as antioxidant, depending on the cellular environment (Lowe, Vlismas, & Young, 2003; Palozza, Serini, Di Nicuolo, Piccioni, & Calviello, 2003; Palozza, 1998). Specifically, the pro-oxidant character of carotenoids is enhanced if their consumption exceeds the normal dietary level (Palozza et al., 2003; Palozza, 1998). Lowe and colleagues (2003) suggest that there is an optimal dose of carotenoids which results in maximum antioxidant effectiveness in human cells, and these optimal levels differ for different types of carotenoids, and for different organs and cells. Therefore, excessive carotenoid intake, associated with large increases in skin carotenoid color, may not be optimal for health outcomes.

Houde (1987) found that female guppies preferred males with more-than-average amounts of orange in their color patterns. However, this increment in female responsiveness levelled off with increasing amounts of orange, suggesting that there may not be an advantage for carotenoid coloration above a certain level in a non-human species.

Skin which is overly red is not always perceived as healthy either. Even though increment in skin redness is associated with aerobic fitness and changes in fertility (Armstrong & Welsman, 2001; Jones et al., 2015), sudden and temporary flushing of the skin, especially in an exaggerated manner, can also be the symptoms of fever, hyperthermia and neurological diseases (Izickson et al., 2006).

Previous research has established the impact of carotenoids on skin yellowness (Alaluf et al., 2002; Coetzee & Perrett, 2014; Stephen et al., 2011) which was perceived as healthy in Caucasian and African samples (Coetzee & Perrett, 2014; Stephen et al., 2011). The results of the present study however suggests that there may be a ceiling to this preference for carotenoid colouration in humans. Faces with excessive levels of carotenoid colouration, after a supplementation of approximately 25mg carotenoids for 4 weeks, were not perceived as healthier than the initial face colouration.

## **5.2 Gender difference in the initial face colouration and effects on the adjustment**

Results showed a negative correlation between the level of adjustment and initial colour of the faces. Participants added more carotenoid-induced colouration to those faces that were initially lower in redness, yellowness and lightness, suggesting that there may be an optimal level of carotenoid coloration for enhancing the apparent health of faces. There was a gender difference in the amount of adjustment being applied to the faces, which may be driven by gender differences in initial face colour. It should also be noted that the preferred amount of carotenoid-induced coloration was lower in the current study compared with preferred carotenoid-induced coloration in a Caucasian population (Lefevre et al., 2013; Lefevre & Perrett, 2015; Stephen et al., 2011). This may be attributable to the higher initial yellowness and darkness of Asian, compared to Caucasian faces (Wee, Chong, & Quee, 1997).

## **6. Conclusion**

In conclusion, in line with results from a Caucasian population (Lefevre et al., 2013; Lefevre & Perrett, 2015; Stephen et al., 2011), observers felt that elevated carotenoid-induced skin color in an Asian population looked also healthy. However, the degree of preferred carotenoid coloration in the Asian population was lower than previously seen in Caucasians, possibly because of the yellower initial skin color of Asian faces, and lower than the color change induced by consuming a carotenoid-rich fruit and vegetable smoothie daily for 4 weeks. This suggests that preferences for skin carotenoid color may be constrained by associations between very yellow skin and ill health, negative health impacts of very high doses of carotenoids, or an overall preference for averageness in faces.

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#### **Data Availability**

The data associated with this research are available at [link].

## References

- Agarwal, A. (2005). Role of oxidative stress in male infertility and antioxidant supplementation. *Business Briefing: US Kidney and Urological Disease*, 122–.
- Alaluf, S., Heinrich, U., Stahl, W., Tronnier, H., & Wiseman, S. (2002). Human nutrition and metabolism: Dietary carotenoids contribute to normal human skin color and UV photosensitivity. *Journal of Nutrition*, 132, 399–403.
- Alexander, M., Newmark, H., & Miller, R. G. (1985). Oral beta-carotene can increase the number of OKT4+ cells in human blood. *Immunology Letters*, 9, 221–224.
- Apicella, C. L., Little, A. C., & Marlowe, F. W. (2007). Facial averageness and attractiveness in an isolated population of hunter-gatherers. *Perception*, 36(12), 1813–1820. <http://doi.org/10.1068/p5601>
- Armstrong, N., & Welsman, J. (2001). Peak oxygen uptake in relation to growth and maturation in 11- to 17-year-old humans. *European Journal of Applied Physiology*, 85(6), 546–551. <http://doi.org/10.1007/s004210100485>
- Branda, R. F., & Eaton, J. W. (1978). Skin color and nutrient photolysis: an evolutionary hypothesis. *Science*, 201(4356), 625–6.
- Charkoudian, N. (2003). Skin blood flow in adult human thermoregulation: how it works, when it does not, and why. *Mayo Clinic Proceedings. Mayo Clinic*, 78(5), 603–12. <http://doi.org/10.4065/78.5.603>
- Coetzee, V., & Perrett, D. I. (2014). Effect of beta-carotene supplementation on African skin. *Journal of Biomedical Optics*, 19(2), 25004. <http://doi.org/10.1117/1.JBO.19.2.025004>
- Coetzee, V., Perrett, D. I., & Stephen, I. D. (2009). Facial adiposity: A cue to health? *Perception*, 38(11), 1700–1711. <http://doi.org/10.1068/p6423>
- Cohen, R. S., Wong, R. J., & Stevenson, D. K. (2010). Understanding Neonatal Jaundice: A Perspective on Causation. *Pediatrics & Neonatology*, 51(3), 143–148. [http://doi.org/10.1016/S1875-9572\(10\)60027-7](http://doi.org/10.1016/S1875-9572(10)60027-7)
- De Sonnevile, L. M. J., Verschoor, C., Njiokiktjien, C., Op het Veld, V., Toorenaar, N., & Vranken, M. (2002). Facial identity and facial emotions: speed, accuracy, and processing strategies in children and adults. *Journal of Clinical and Experimental Neuropsychology*, 24(2), 200–213. <http://doi.org/10.1076/jcen.24.2.200.989>
- Dowling, D. K., & Simmons, L. W. (2009). Reactive oxygen species as universal constraints in life-history evolution. *Proceedings. Biological Sciences*, 276(1663), 1737–45. <http://doi.org/10.1098/rspb.2008.1791>
- European Food Safety Authority Scientific Committee on Food. (2006). *Tolerable Upper Intake Levels for Vitamins and Minerals*.
- Fraser, P. D., & Bramley, P. M. (2004). The biosynthesis and nutritional uses of carotenoids. *Progress in Lipid Research*, 43(3), 228–65. <http://doi.org/10.1016/j.plipres.2003.10.002>
- Heinrich, U., Ga, C., Wiebusch, M., Eichler, O., Sies, H., Tronnier, H., & Stahl, W. (2003). Human Nutrition and Metabolism: Supplementation with beta-carotene or a similar amount of mixed carotenoids protects humans from UV-induced erythema. *Human Nutrition and Metabolism*, 133, 98–101.

- Houde, A. (1987). Mate Choice Based Upon Naturally Occurring Color-Pattern Variation in a Guppy Population. *Evolution*, 41(1), 1–10.
- Hughes, D. a. (2001). Dietary carotenoids and human immune function. *Nutrition (Burbank, Los Angeles County, Calif.)*, 17(10), 823–7. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/11684388>
- Institute of Medicine Food and Nutrition Board. (2000). Beta-carotene and other carotenoids. In *Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids*. (pp. 325–400). Washington, D.C.: National Academy Press.
- Institute of Medicine Food and Nutrition Board. (2001). *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. Washington, D.C.: National Academy Press.
- Izikson, L., English, J. C., & Zirwas, M. J. (2006). The flushing patient: differential diagnosis, workup, and treatment. *Journal of the American Academy of Dermatology*, 55(2), 193–208. <http://doi.org/10.1016/j.jaad.2005.07.057>
- Johnson, J. M. (1998). Physical training and the control of skin blood flow. *Medicine and Science in Sports and Exercise*, 30, 382–386.
- Jones, B., Hahn, A. C., Fisher, C. I., Wincenciak, J., Kandrik, M., Roberts, S. C., ... DeBruine, L. M. (2015). Facial coloration tracks changes in women's estradiol. *Psychoneuroendocrinology*, 56, 29–34. <http://doi.org/10.1016/j.psychneuen.2015.02.021>
- Krinsky, N. I., & Johnson, E. J. (2005). Carotenoid actions and their relation to health and disease. *Molecular Aspects of Medicine*, 26(6), 459–516. <http://doi.org/10.1016/j.mam.2005.10.001>
- Lefevre, C. E., Ewbank, M. P., Calder, a J., von dem Hagen, E., & Perrett, D. I. (2013). It is all in the face: carotenoid skin coloration loses attractiveness outside the face. *Biology Letters*, 9(6), 20130633. <http://doi.org/10.1098/rsbl.2013.0633>
- Lefevre, C. E., & Perrett, D. I. DI. (2015). Fruit over sunbed: Carotenoid skin colouration is found more attractive than melanin colouration. *The Quarterly Journal of Experimental Psychology*, 68(2), 284–293. <http://doi.org/10.1080/17470218.2014.944194>
- Lowe, G. M., Vlismas, K., & Young, A. J. (2003). Carotenoids as prooxidants? *Molecular Aspects of Medicine*, 24(6), 363–369. [http://doi.org/10.1016/S0098-2997\(03\)00032-3](http://doi.org/10.1016/S0098-2997(03)00032-3)
- Mansor, M. N., Yaacob, S., Hariharan, M., Basah, S. N., Jamil, S. H. F. S. A., Khidir, M. L. M., ... Saad, S. a. (2012). Jaundice in Newborn Monitoring using Color Detection Method. *Procedia Engineering*, 29, 1631–1635. <http://doi.org/10.1016/j.proeng.2012.01.185>
- Mouchetant-Rostaing, Y., & Giard, M. H. (2003). Electrophysiological correlates of age and gender perception on human faces. *Journal of Cognitive Neuroscience*, 15(6), 900–10. <http://doi.org/10.1162/089892903322370816>
- Palozza, P. (1998). Prooxidant actions of carotenoids in biologic systems. *Nutrition Reviews*, 56(1), 257–265. <http://doi.org/10.1111/j.1753-4887.1998.tb01762.x>
- Palozza, P., Serini, S., Di Nicuolo, F., Piccioni, E., & Calviello, G. (2003). Prooxidant effects of  $\beta$ -carotene in cultured cells. *Molecular Aspects of Medicine*, 24(6), 353–362.

[http://doi.org/10.1016/S0098-2997\(03\)00031-1](http://doi.org/10.1016/S0098-2997(03)00031-1)

- Panza, J., Quyyumi, A., Brush, J., & Epstein, S. (1990). Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *New England Journal of Medicine*, 323(1), 22–27.
- Petrican, R., Todorov, A., & Grady, C. (2014). Personality at Face Value: Facial Appearance Predicts Self and Other Personality Judgments among Strangers and Spouses. *Journal of Nonverbal Behavior*, 38(2), 259–277. <http://doi.org/10.1007/s10919-014-0175-3>
- Rhodes, G., Zebrowitz, L. a., Clark, A., Kalick, S. M., Hightower, A., & McKay, R. (2001). Do facial averageness and symmetry signal health? *Evolution and Human Behavior* : *Official Journal of the Human Behavior and Evolution Society*, 22(1), 31–46. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/11182573>
- Seifter, E., Rettura, G., & Levenson, S. M. (1981). Carotenoids and cell mediated immune responses. In G. Charamblouis & G. Inglett (Eds.), *The Quality of Foods and Beverages: Chemistry and Technology*. New York: Academic Press.
- Stamatas, G. N., Zmudzka, B. Z., Kollias, N., & Beer, J. Z. (2004). Non-invasive measurements of skin pigmentation in situ. *Pigment Cell Research*, 17, 618–626.
- Stephen, I. D., Coetzee, V., Law Smith, M., & Perrett, D. I. (2009). Skin blood perfusion and oxygenation colour affect perceived human health. *PLoS One*, 4(4), e5083. <http://doi.org/10.1371/journal.pone.0005083>
- Stephen, I. D., Coetzee, V., & Perrett, D. I. (2011). Carotenoid and melanin pigment coloration affect perceived human health. *Evolution and Human Behavior*, 32(3), 216–227. <http://doi.org/10.1016/j.evolhumbehav.2010.09.003>
- Stephen, I. D., Law Smith, M. J., Stirrat, M. R., & Perrett, D. I. (2009). Facial Skin Coloration Affects Perceived Health of Human Faces. *International Journal of Primatology*, 30(6), 845–857. <http://doi.org/10.1007/s10764-009-9380-z>
- Stephen, I. D., & Tan, K. W. (2015). Healthy body, healthy face? Evolutionary approaches to attractiveness perception. In *Culture and Cognition: A Collection of Critical Essays*. Peter Lang International Publishers.
- Tan, K. W., Graf, B. A. A., Mitra, S. R. R., & Stephen, I. D. D. (2015). Daily Consumption of a Fruit and Vegetable Smoothie Alters Facial Skin Color. *PLoS ONE*, 10(7), e0133445. <http://doi.org/10.1371/journal.pone.0133445>
- Tan, K. W., & Stephen, I. D. (2013). Colour detection thresholds in faces and colour patches. *Perception*, 42(7), 733–741. <http://doi.org/10.1068/p7499>
- Tiddeman, B., Burt, D. M., & Perrett, D. I. (2001). Prototyping and transforming facial textures for perception research. *Computer Graphics and Applications*, 21(5), 42–50.
- Wee, L. K. S., Chong, T. K., & Quee, D. K. S. (1997). Assessment of skin types, skin colours and cutaneous responses to ultraviolet radiation in an Asian population. *Photodermatology Photoimmunology and Photomedicine*, 13, 169–172.
- Whitehead, R. D., Re, D., Xiao, D., Ozakinci, G., & Perrett, D. I. (2012). You are what you eat: within-subject increases in fruit and vegetable consumption confer beneficial skin-color changes. *PLoS ONE*, 7(3), e32988. <http://doi.org/10.1371/journal.pone.0032988>

Zain-Ahmed, A., Sopian, K., Zainol Abidin, Z., & Othman, M. Y. H. (2002). The availability of daylight from tropical skies—a case study of Malaysia. *Renewable Energy*, 25(1), 21–30. [http://doi.org/10.1016/S0960-1481\(00\)00209-3](http://doi.org/10.1016/S0960-1481(00)00209-3)

Table 1.

*Frequency with which pre- and after-supplementation images were chosen as healthier.*

*Means and standard deviations calculated across participants separately for images of subjects in the supplementation and control groups.*

Group / Preference	Pre Supplementation		After Supplementation	
	Mean	SD	Mean	SD
Supplementation (N=34)	19.25 (56.6%)	5.76 (16.9%)	14.75 (43.3%)	5.76 (16.9%)
Control (N=34)	19.07 (56.1%)	3.05 (9.0%)	14.92 (43.9%)	3.05 (9.0%)

Table 2.

*Frequency with which original and color-manipulated images were chosen as healthier.*

*Means and standard deviations calculated across participants.*

	Original images		Colour-manipulated images	
	Mean	SD	Mean	SD
Preference	16.05	6.347	13.95	6.347
	(53.5%)	(21.1%)	(46.5%)	(21.1%)

Table 3.

*Color change chosen to optimize healthy appearance (left) and color change observed in the supplementation study (right).*

	Color change chosen	Supplementation color change
Luminance	-.31	-.91
Redness	.37	1.08
Yellowness	1.15	3.38

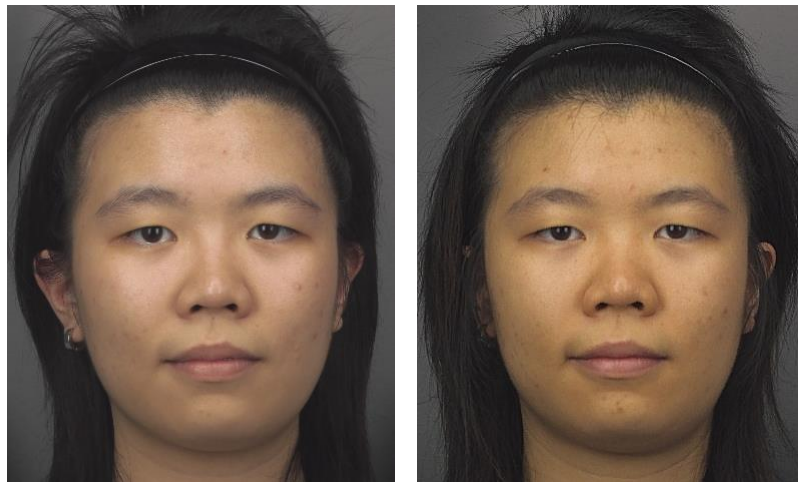


Table 4.

*Initial face color for male and female stimuli. Men's faces were significantly redder, yellower and darker than women's.*

	Male		Female	
	Mean	SD	Mean	SD
Luminance (L*)	59.64	2.01	62.94	2.17
Redness (a*)	14.20	1.17	13.27	1.42
Yellowness (b*)	19.18	1.81	17.86	1.33

*Figure 1: An example of the paired-images used in Study 1. The image on the left was taken before supplementation, while the image on right was taken after 4 weeks of supplementation.*



*Figure 2: An example of the paired-images used in experiment 2. The image on the left is the original image, while the image on right has been manipulated to simulate skin color change induced by 4 weeks of supplementation with the carotenoid-rich smoothie.*

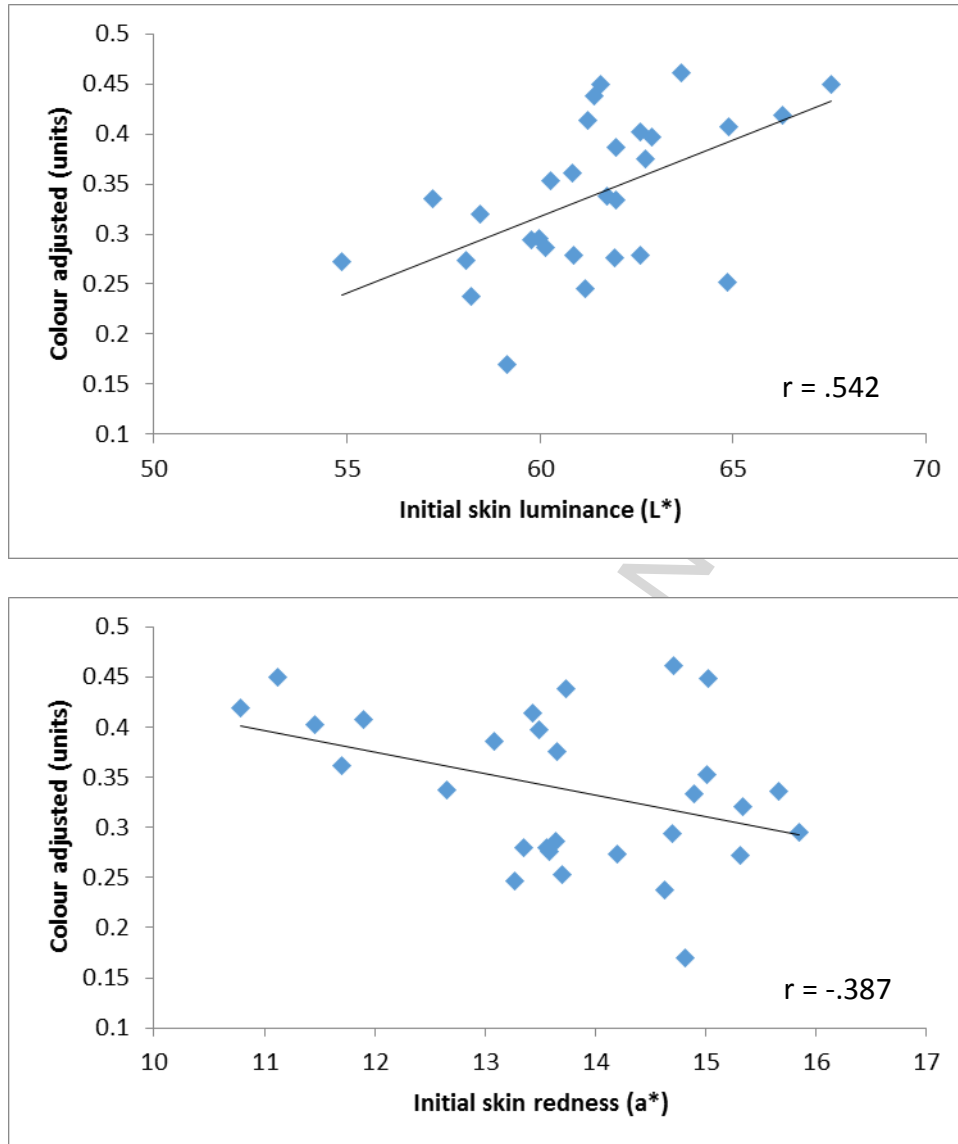


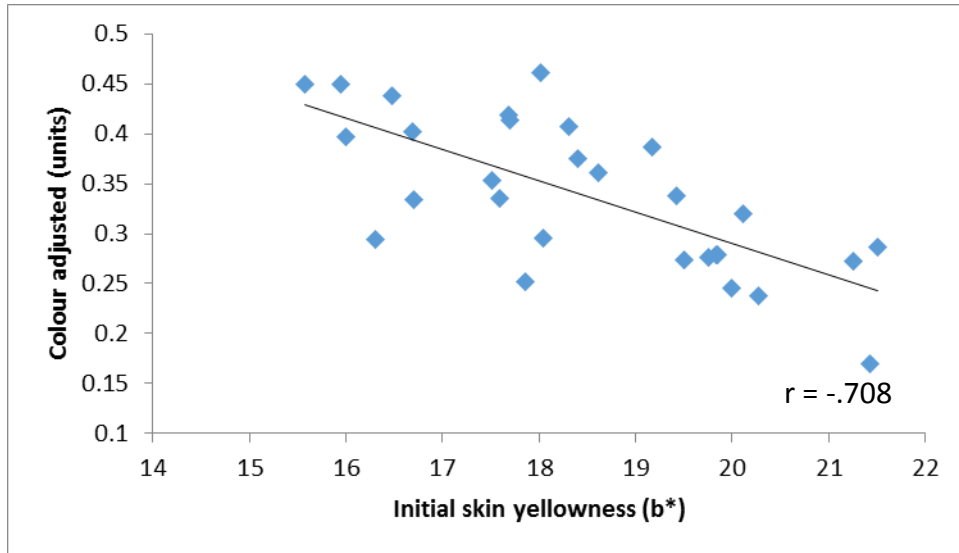
*Figure 3: Examples of facial images used in experiment 3. The image on the left shows 4 intervention units of decrement (frame 0); the image in the middle is the original image (frame 20); the image on the right shows 4 intervention units of increment (image 40).*



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Figure 4. Relationship between coloration adjustment and initial coloration. Change applied by observers to optimise healthy appearance (y axis) and initial skin luminance (top), redness (middle) and yellowness (bottom) of the faces (x axis).





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