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Skin colour predicts fruit and vegetable intake in young Caucasian men: A cross-sectional study

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ABSTRACT

Aim: Current dietary assessment methods are prone to subjective bias, highlighting the demand for an objective marker of fruit and vegetable (F/V) intake. Carotenoids from F/V consumption deposit in skin and adipose tissue, contributing to changes in skin colour. Results from research in females have highlighted positive associations between skin colour assessed by reflectance spectroscopy and F/V intake. The aim of this study was to determine the relationship between (i) F/V intake, (ii) carotenoid intake and skin colour in young Caucasian men.

Methods: In this cross-sectional study reflectance spectroscopy was used to quantify skin colour in young Caucasian men. Skin colour was assessed at eight sun-exposed and unexposed body locations. A food frequency questionnaire was administered to assess F/V intake over the past month. Partial correlations were done to assess the associations between skin yellowness, F/V intake (grams) and carotenoid intake (milligrams), both with and without controlling for skin lightness.

Results: Carotenoid intake was strongly associated with F/V intake ($r = 0.8$, $p < 0.001$). Skin yellowness was found to be strongly associated with both carotenoid ($r = 0.599$, $p < 0.001$) and F/V ($r = 0.422$, $p = 0.02$) intake. When skin colour was controlled for skin lightness and measured at the forehead, biceps, palm and foot sole, a stronger association was observed (carotenoid ($r = 0.637$, $p < 0.001$); F/V ($r = 0.431$, $p = 0.02$)).

Conclusion: Skin colour is a viable biomarker of F/V intake in young Caucasian men. These findings contribute to the development of an objective marker of F/V intake, however more research is required before the method can be applied to practice.

1. Introduction

Regular consumption of fruit and vegetables (F/V) supports healthy body weight, and as epidemiological evidence suggests, is paramount in chronic disease prevention [1–4]. While healthy eating guidelines are varied between countries, the importance of adequate F/V consumption for health is consistently featured [1]. The Australian Dietary Guidelines are based on a comprehensive review of evidence that indicates F/V consumption is associated with a reduced risk of obesity, cardiovascular disease, stroke and some cancers [5]. Two thirds of Australians are either overweight or obese, while only one in 20 consume the recommended daily serves of F/V [2]. The burden of chronic disease is accumulating in Australia as the incidence of obesity among adults has rapidly increased from 18.7% to 27.9% over the past 10 years [2]. Carotenoid consumption from F/V has been linked to disease prevention in epidemiological studies [6,7]. Decreased consumption of F/V, and greater accessibility to energy-dense foods has directly contributed to chronic disease becoming the leading cause of ill health and death in Australia [8]. As the prevalence of obesity and related diseases remains high, so does the need for effective strategies to combat low F/V consumption and improve chronic disease among Australians.

Self-report dietary assessment methods are relied on to measure F/V intake in most research and clinical settings. However, self-reported data will always be limited by subjectivity. F/V intake is consistently over-reported by food frequency questionnaires (FFQ) due to issues surrounding memory distortion and social desirability [9,10]. Prospective methods such as food records tend to affect the

Abbreviations: F/V, fruit and vegetables; RS, reflectance spectroscopy; BSS, Best skin sites (averaged forehead, biceps, palm and sole b* values).

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types and amounts of foods that are consumed [11]. Current methods are also practically demanding as they require significant literacy, skill and time to complete [12]. These inherent limitations complicate the progression of nutrition research and reduce clinical accuracy, highlighting the need for an objective measure of F/V intake.

Carotenoids are red, orange and yellow pigmented fat-soluble compounds found in a wide range of F/V. As they originate in plants, almost all carotenoids in the human diet are consumed as components of F/V [13]. As they cannot be synthesised by humans these compounds represent a potential biomarker of F/V intake [14]. The most common dietary carotenoids are alpha-carotene, beta-carotene, lycopene and combined lutein-zeaxanthin [15,16]. Dietary carotenoids accumulate in skin and carotenoid status of humans has traditionally been measured by High Performance Liquid Chromatography of either excised tissue or plasma samples [17,18]. However, the invasiveness and time consuming nature of these methods do not lend them being practical measures of dietary carotenoid or F/V intake. Since they accumulate in skin (14) assessment of skin yellowness and redness can be used to estimate carotenoid status [19]. For over 50 years human skin pigments, namely blood components such as haemoglobin and melanin have been analysed using spectroscopy, however, assessment of skin carotenoid content has only become possible with improved methodology [20]. The use of spectroscopy to measure skin carotenoid status has now been validated and found to correlate with plasma carotenoid concentrations [20–23]. As spectroscopy is both objective and retrospective, methodological biases associated with traditional dietary assessment methods are eliminated [11]. These findings raise the exciting potential for F/V intake to be estimated using spectroscopic methods to assess skin colour [24].

Reflectance spectroscopy (RS) is an inexpensive and objective measure of skin colour that works by measuring the difference in colour and intensity between an incident beam of white light, and the reflected light [25]. Reflectance spectrophotometers typically utilise the Commission Internationale de l'Eclairage (CIE) L*a*b* colour system. L* values quantify lightness (white-black), while a* and b* values quantify colour (green-red and blue-yellow respectively) [24]. Skin carotenoid detection is primarily related to b* values, which effectively measure the extent that carotenoids have yellowed the skin. Lycopene, a carotenoid responsible for redness in F/V, can be detected in skin through analysis of a* values. However evidence supporting this is inconsistent due to the significant effect of blood perfusion on skin redness [26,27].

There is some initial evidence to support a relationship between F/V intake and skin colour. A significant correlation between skin yellowness and self-reported F/V intake has been observed in Caucasian females [16,28]. Subsequently, intervention trials explored the dose-response relationship between F/V intake and skin colour in Caucasian and Asian subjects, by providing low and high carotenoid F/V diet interventions and measuring fluctuations in skin colour [19,27]. The results from these studies demonstrated that skin yellowness increases significantly following consumption of a diet rich in high carotenoid-containing F/V. Before skin colour assessment could be routinely used to assess F/V intake, it is important to confirm whether similar associations are observed across a range of different subjects consuming a range of different types of F/V. To date these studies have been conducted predominantly in female subjects and it is therefore important to determine whether the same relationship holds in male subjects.

Adiposity is known to affect skin carotenoid status, as underweight and normal subjects are observed to have a higher skin carotenoid status than overweight/obese subjects [22]. As carotenoids are deposited in adipose tissue, a dilution effect in subjects with higher adiposity has been hypothesised [22,29]. Consequently, because of differences in adipose tissue distribution between males and females, it is unknown whether the significant associations found at skin sites measured in females would also be found among males [30]. Furthermore, Australian men typically consume less F/V than women, which may further influence readings [2]. The aim of this cross-sectional study is to examine associations between (i) F/V intake, (ii) carotenoid intake and skin colour in young Caucasian men. It is hypothesised that skin yellowness is positively correlated with F/V intake in males, and it is expected that associations between skin yellowness and carotenoid intake will be strongest.

2. Methods

2.1. Study population

This cross-sectional study was conducted at Curtin University in Perth, Western Australia (WA), from June to October 2017 during the winter season. Men aged 18–30 years (n = 41) were recruited using flyers distributed on campus, online faculty noticeboards, and personal Facebook pages. The study was approved by the Curtin University Human Research Ethics Committee (RDHS-204-15). An information statement was provided by email to those interested, and written informed consent was obtained for all participants prior to data collection.

Of the 36 participants who completed the online screening survey, 33 were eligible. Eligible participants were non-smoking men aged 18–30 years, with a BMI between 18 and 30 kg/m2. Non-Caucasian men (n = 3) were excluded to improve the homogeneity of skin lightness throughout the sample. No participants were excluded for conditions affecting skin yellowness (jaundice), using tanning products or taking carotenoid-containing supplements. Thirty-two participants completed all physical assessments and were included in the final sample. However, of the 32 participants who completed physical assessments, two were excluded from analysis for commencing high-volume plant-based diets midway through the FFQ measurement period. Their exclusion was determined based on the previously mentioned four-week period required for skin colour to reflect dietary changes.

2.2. Skin colour measurement

Skin colour was measured using a Spectro-Guide 4/50 Gloss 6801 spectrophotometer with an 11 mm diameter aperture (BYK Gardner, Maryland, USA), which was calibrated at each session according to manufacturer's instructions. Skin colour was recorded at eight body locations on the right side of the body, using the CIE L*a*b* colour space. The eight body locations were the forehead, cheek, shoulder, biceps, triceps, palm, back of the hand and sole of the foot. Body locations were chosen based on previous research and in accordance with the International Society for the Advancement of Kinanthropometry (ISAK) standards for anthropometric assessment [31]. Triplicate measurements were taken at each site, allowing time between measurements to minimise skin blanching. Overall body L*a*b* values were calculated by taking the average of all site measurements combined. The skin colour measurements were taken by a single trained investigator. The within-rater reliability of skin colour measurement was assessed on a single subject over three consecutive days using the standardised procedure and measurements were found to have a coefficient of variation of 1.8% for all body locations.

2.3. Fruit, vegetable and carotenoid intake

Dietary intake was determined using a FFQ developed by the investigators that assesses monthly total fruit and vegetable intake

and carotenoid intake. The FFQ was conducted by interview and in conjunction with food models to assist with serve size estimation. One month of intake was assessed as previous research indicates that four weeks is an adequate time period for fluctuations in intake to confer skin colour changes [19,27]. The questionnaire contained 29 questions relating to the consumption of fruit, 36 to vegetables, one to fruit juice and one to vegetable juice. Measured fruit and vegetables are listed in appendix 1. All 100% juices were included in the analysis due to their carotenoid content and acknowledgement as a fruit or vegetable serve in the Australian Dietary Guidelines [32]. Cooking method for vegetables was also recorded (cooked/raw, with/without fat) as it is known to affect carotenoid absorption [33]. Fruit and vegetables were categorised by the types specified in the Eat for Health Educator Guide [34]. This allowed several opportunities for participants to be prompted to recall less common fruit and vegetable varieties that weren't included in the FFQ for time-purposes but would potentially be consumed (e.g. lychee, silverbeet, swede). Total weight of fruit and vegetable intake in grams was calculated by multiplying the serves of each item by the standard serve size outlined in the Australian Dietary Guidelines (150 g for fruit, 75 g for vegetables, 125 mL for juice). Total carotenoid intake was calculated from F/V intake using the carotenoids available in the US Department of Agriculture (USDA) carotenoids food composition database (α-carotene, β-carotene, lycopene and combined lutein-zeaxanthin).

2.4. Anthropometry

The subject's height, weight were measured using standard protocols by a single trained investigator. Body composition was assessed using bioelectrical impedance analysis (InBody 136, Biospace Co., Cerritos, USA). Skinfold measurements were taken at the biceps and triceps site using a skinfold calliper. These were done after skin colour had been measured to avoid the effects of skin marking and pinching on skin colour readings.

2.5. Statistical analysis

Data analysis was conducted using SPSS (Version 24; IBM corp., Armonk, USA). Significance was set at $p < 0.05$. Normally distributed variables are presented as means (±SD), while skewed variables are presented as medians with interquartile ranges (IQR). Pearson correlations were used to assess the strength of the relationship between skin yellowness (b*), F/V intake (grams per month) and carotenoid intake (milligrams per month). Partial correlations correcting for L* were also used to determine the effect of controlling for skin lightness, as melanin distribution is known to affect skin lightness and yellowness [16]. Subsequently, correlation analysis was done on the average of skin colour measurements from the most highly correlated body locations to determine the correlation strength of the most suitable skin sites for use in practice. Multiple linear regression was calculated to predict F/V intake for the month based on b* and L* values.

3. Results

Characteristics of the 30 participants included in the study are summarised in Table 1. The mean age was 21.7 ± 2.6 years; BMI 23.6 ± 3.4 kg/m2 and body fat percentage 13.8 ± 5.2%. Median (IQR) monthly F/V intake was 14158.3 (7712.6–22408.3) g or 45.34 (25.8–75.7) serves, which equates to approximately 1.5 (0.86–2.5) daily serves of fruit and vegetables. One participant consumed the daily 6 serves of vegetables recommended for men aged 19–50 years. No participants consumed the recommended daily 2 serves of fruit. Median monthly total carotenoid intake was 244.1 (138.9–505.7) mg.

There was a strong correlation ($r = 0.8$, $p < 0.001$) between reported F/V intake and calculated carotenoid intake (Fig. 1). Bivariate correlations between skin yellowness, F/V intake and carotenoid intake, as well as partial correlations controlling for skin lightness are shown in Table 2. Significant correlations were observed between skin yellowness (b*) and F/V intake at a number of sites. Correlation was strongest when examined using overall b* ($r = 0.385$, $p = 0.036$), however significant strong correlations were also observed at the individual sites of forehead ($r = 0.369$, $p = 0.045$) and biceps ($r = 0.361$, $p = 0.05$). The strength of some of these correlations improved when controlled for L*; overall b* ($r = 0.404$, $p = 0.03$) and at the biceps b* site ($r = 0.466$, $p = 0.011$), but became insignificant at the forehead b* site ($r = 0.335$, $p = 0.076$). Significant correlations between carotenoid intake and skin yellowness (b*) was found for overall b* ($r = 0.485$, $p = 0.007$), and the individual sites of forehead b* ($r = 0.549$, $p = 0.002$), right cheek b* ($r = 0.389$, $p = 0.034$), biceps b* ($r = 0.41$, $p = 0.024$), palm b* ($r = 0.446$, $p = 0.014$) and sole b* ($r = 0.555$, $p < 0.001$). Furthermore when corrected for L* these correlations either improved, or remained essentially unchanged, and correlations at two additional sites reached statistical significance: shoulder ($r = 0.450$, $p = 0.014$) and triceps ($r = 0.408$, $p = 0.028$). Across all measurement sites the correlation between carotenoid intake and skin yellowness was stronger than the correlation with F/V intake (Table 2).

The sites that had the highest correlation with skin colour and carotenoid intakes were the forehead, palm, biceps, and sole. The average skin colour of these sites was determined and is referred to as the best skin sites (BSS) in Table 2. This combined skin colour measure was more strongly correlated with carotenoid intake than was any of the individual sites ($r = 0.599$, $p < 0.001$); a finding that also held true when correlated with F/V intake ($r = 0.422$, $p = 0.02$). The BSS measure was also more strongly correlated with both carotenoid and F/V intake than overall b* was. When controlled for L*, the BSS b* correlations were further improved for both F/V intake ($r = 0.431$, $p = 0.02$) and carotenoid intake ($r = 0.637$, $p < 0.001$).

Given the observation that average skin colour at the BSS was more strongly correlated with both carotenoid and F/V intakes multiple linear regression analysis was performed using BSS b* and L* values to predict monthly F/V intake (in kilograms). Results of this analysis are summarised in Table 3. The multiple linear regression analysis revealed that predicted F/V intake is equal to −56.62 + 2.66 (BSS b*) + 0.53 (BSS L*). F/V intake increased 2.66 kg for each increment increase in BSS b* and 0.53 kg for each increment increase in BSS L*. Significance of b* as a predictor of F/V intake improved using the BSS b* values ($p = 0.02$) compared to overall b* ($p = 0.03$). L* was not a significant predictor of F/V intake ($p = 0.56$). Individually, b* was a significant predictor of F/V intake at the bicep only ($p = 0.011$). Whereas L* was not a significant predictor of F/V intake at any skin site.

There were significant negative correlations between b* and skinfold thickness, controlling for carotenoid intake at the biceps ($r = -0.371$, $p = 0.05$) and triceps ($r = -0.549$, $p = 0.002$). Controlling for L* improved significance at the biceps ($r = -0.413$, $p = 0.03$), but not at the triceps ($r = -0.479$, $p = 0.01$).

4. Discussion

This cross-sectional study examined associations between skin yellowness and self-reported F/V intake in 30 Caucasian men aged 18–30 years. When skin yellowness was averaged across all measurement sites, a significant positive correlation was observed with both F/V ($r = 0.404$, $p = 0.03$) and carotenoid ($r = 0.591$, $p = 0.001$)

Table 1
Characteristics of young Caucasian men (n = 30) participating in a cross-sectional evaluation of fruit, vegetable and carotenoid intake and skin colour.

Characteristic	Result
Age (years) mean ± SD	21.7 ± 2.6
Height (cm) mean ± SD	181.7 ± 6.5
Weight (kg) mean ± SD	78.2 ± 13.7
BMI (kg/m2) mean ± SD	23.6 ± 3.4
Body fat percentage mean ± SD	13.8 ± 5.2
Biceps skinfold (cm) mean ± SD	4.4 ± 2.2
Triceps skinfold (cm) mean ± SD	10.0 ± 4.5
Fruit and vegetable intake (g/month) median (IQR)	14158.3 (7712.6–22408.3)
Fruit and vegetable intake (serves/month) median (IQR)	45.34 (25.8–75.7)
Fruit intake (serves/month) median (IQR)	13.1 (5.8–18.7)
Vegetable intake (serves/month) median (IQR)	33.5 (15.8–56.1)
Total carotenoid intake (mg/month) median (IQR)	244.1 (138.9–505.7)
Overall L* mean ± SD	63.2 ± 2.1
Overall a* mean ± SD	10.6 ± 2.0
Overall b* mean ± SD	14.9 ± 1.7
BSS L* mean ± SD	64.4 ± 1.9
BSS a* mean ± SD	9.9 ± 2.8
BSS b* mean ± SD	14.5 ± 1.6

BSS = Best skin sites; averaged forehead, biceps, palm and sole b* values.

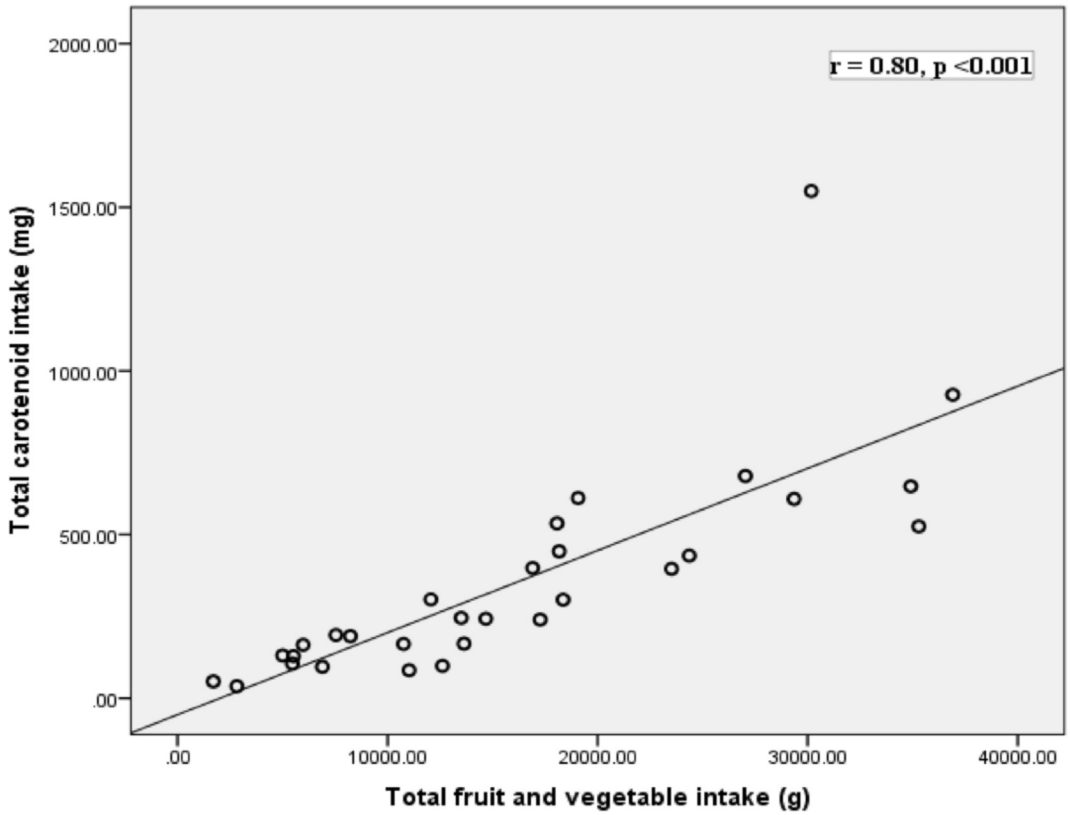

Fig. 1. Correlation between estimated total fruit and vegetable intake and total carotenoid intake in a sample of young Caucasian men.

intake, with the latter being a stronger correlation (Table 2). The stronger correlations with carotenoid intake and skin yellowness is likely due to the variability of carotenoid content in commonly consumed F/V. The F/V intake data includes foods containing a range of low and high carotenoid contents. Nonetheless a strong positive correlation ($r = 0.8$, $p < 0.001$) between total carotenoid intake and F/V intake was observed (Fig. 1). After controlling for skin lightness, the yellowness of the forehead, right cheek, shoulder, biceps, triceps, palm and sole of the foot sites were significantly correlated with carotenoid intake. Whereas the correlation between F/V intake and skin yellowness occurred with the average of all of the sites, biceps and the four best skin sites (Table 2). As the extent of correlation of skin colour with both F/V and carotenoid intake varied across the range of different measurement sites we also examined these correlations using a combined measure of the 4 sites that were most strongly correlated (best skin sites: forehead, biceps, palm, and sole). This combined measure was more strongly correlated with both F/V ($r = 0.431$, $p < 0.05$) and carotenoid ($r = 0.637$, $p < 0.001$) intakes when controlled for L*. Taken together these findings suggest that the previously reported ability of skin

Table 2
Correlations between skin yellowness, fruit and vegetable intake and carotenoid intake in a sample of young Caucasian men, uncontrolled and controlled for skin lightness.

	Uncontrolled		Controlled for L*	
	r	p value	r	p value
Overall b*				
Fruit and vegetable intake (kg/month)	0.385	0.036*	0.404	0.030*
Carotenoid intake (mg/month)	0.485	0.007**	0.591	0.001**
BSS b*				
Fruit and vegetable intake (kg/month)	0.422	0.020*	0.431	0.020*
Carotenoid intake (mg/month)	0.599	<0.001***	0.637	<0.001***
Forehead b*				
Fruit and vegetable intake (kg/month)	0.369	0.045*	0.335	0.076
Carotenoid intake (mg/month)	0.549	0.002**	0.580	0.001**
Right cheek b*				
Fruit and vegetable intake (kg/month)	0.334	0.072	0.354	0.060
Carotenoid intake (mg/month)	0.389	0.034*	0.379	0.043*
Shoulder b*				
Fruit and vegetable intake (kg/month)	0.257	0.171	0.339	0.072
Carotenoid intake (mg/month)	0.268	0.153	0.450	0.014*
Biceps b*				
Fruit and vegetable intake (kg/month)	0.361	0.05*	0.466	0.011*
Carotenoid intake (mg/month)	0.410	0.024*	0.590	0.001**
Triceps b*				
Fruit and vegetable intake (kg/month)	0.263	0.160	0.344	0.068
Carotenoid intake (mg/month)	0.306	0.100	0.408	0.028*
Palm b*				
Fruit and vegetable intake (kg/month)	0.301	0.106	0.313	0.099
Carotenoid intake (mg/month)	0.446	0.014*	0.448	0.015*
Back of hand b*				
Fruit and vegetable intake (kg/month)	0.221	0.241	0.061	0.75
Carotenoid intake (mg/month)	0.189	0.317	0.143	0.46
Sole of foot b*				
Fruit and vegetable intake (kg/month)	0.344	0.063	0.309	0.10
Carotenoid intake (mg/month)	0.555	<0.001***	0.531	0.003**

BSS = Best skin sites; averaged forehead, biceps, palm and sole b* values, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

colour to predict F/V intake in young Caucasian females (16) also holds true in young Caucasian males.

The significant correlation observed between F/V intake and carotenoid intake (Fig. 1) supports use of the RS method to predict fruit and vegetable intake. Optical quantification of skin carotenoid status has been validated as a measure of plasma carotenoid concentrations [20–23]. The results of the present study support this validation via the strong correlations observed between carotenoid intake and skin yellowness. Hence the ability to demonstrate that carotenoid intake is also strongly associated with F/V intake provides further evidence to support the use of skin colour as a biomarker of F/V intake. The majority of the participants show very strong correlations between F/V intake and carotenoid intake. Fig. 1 shows that one participant had a particularly high carotenoid intake compared to the reported F/V intake. This value was attributed to the consumption pattern of large volumes of high carotenoid containing F/V (pumpkin, carrot) during the FFQ period. Removing this participant's data did not alter the correlations seen and future research conducted among a large sample size will account for a broader range of intake patterns.

Across the range of F/V measured in the FFQ, there are extremes of carotenoid content. Low carotenoid-containing fruits and vegetables include mushrooms and beans and those that are characteristically carotenoid-rich include pumpkin and carrot. Although we showed that F/V and carotenoid intake are strongly correlated, they cannot be perfectly correlated if subjects consume either predominantly low or high carotenoid-containing varieties. This means that skin colour may not always predict total F/V intake, but instead may reflect a consumption pattern of extreme-carotenoid-containing F/V. The Australian Dietary Guidelines promotes consumption of a wide variety of F/V, and vegetables of different types and colours in order to maintain nutritional adequacy (32). Lower skin yellowness results may indicate participants who are consuming insufficient variety within these food groups and this may still because for nutritional concern. However, given the strong and significant correlation between F/V and carotenoid intake, the RS method for the most part appears, to be a valid measure of F/V intake.

The significant associations found between skin colour and F/V intake are consistent with results from studies using the RS method in predominantly female populations, which have also found positive correlations between skin yellowness and F/V intake overall, and at the forehead, palm and sole. These skin sites are known to contain some of the highest concentrations of total carotenoids in the body (22), which may explain why they are consistently found to be the areas that exhibit the strongest associations with carotenoid and F/V intake. These sites are also unaffected by increased adiposity in females, making them suitable sites for measurement for both genders [35].

The significant negative correlations found between skinfold thickness and skin yellowness strengthen the hypothesis that skin carotenoids are diluted with increases in adiposity [22,29]. Our results indicate that for the same carotenoid intake, subjects with a higher fat mass at the biceps and triceps display lower skin yellowness at these areas. Research has suggested other explanations for this phenomenon. It is possible that obesity-mediated inflammation causes increased clearance of carotenoids from adipose tissue [22,29]. This is plausible as plasma carotenoid concentrations are inversely correlated with inflammatory biomarkers such as IL-6 and C-reactive protein [22]. However, in our population, this explanation is unlikely as all participants were of a healthy weight, non-smoking and free of chronic disease. This finding is topical as it highlights that lower skin carotenoid status shown in obese subjects (22) may not necessarily reflect poor dietary choices or inflammation. As the dilution effect impacts the ability of the RS method to predict F/V intake at skin sites where fat mass is variable, suitability of the palm and sole sites is further supported.

Sun exposure increases the effects of melanin interference on skin colour measurements. Melanin contributes to skin yellowness and lightness, and hinders the accuracy of the RS method to assess carotenoid status [16,24]. Additionally, UV irradiation is known to decrease carotenoid levels in the skin. This may explain why weaker correlations were observed at the right cheek, shoulder and back of the hand, as they are areas of higher sun exposure. Although the forehead is also sun-exposed, it has a thick stratum corneum, which is the outermost layer of skin where carotenoids are predominantly found [36]. This gives the forehead a greater carotenoid content than other sun-exposed locations. The significant correlations observed at the biceps in this sample could be attributed to men typically having higher upper body muscle mass and lower adipose tissue in that area than women [37], lessening the carotenoid dilution effect. The mean biceps skinfold thickness in this sample was low (4.4 mm ± 2.2), but also varied as some participants exhibited a thickness as high as 11.5 mm. Sun exposure at the biceps is also varied, and can depend on the season as well as individual lifestyle factors. The palm and sole are generally limited in their exposure to the sun, which increases their viability as a universal measurement site.

After the biceps, taking the average of the most highly correlated sites (BSS) provided the strongest correlation between skin colour and F/V intake. Because of the variability discussed with regards to

Table 3
Summary of multiple linear regression to predict monthly fruit and vegetable intake in a sample of young Caucasian men.

	Predictor variable	β -Coefficient ± SE	95% Confidence Interval	p value
Overall b*				
Fruit and vegetable intake (kg/month)	Constant	−67.6 ± 69.1	−209.4, 74.1	0.34
	b*	2.70 ± 1.17	0.29, 5.10	0.030*
	L*	0.69 ± 0.94	−1.25, 2.62	0.47
BSS b*				
Fruit and vegetable intake (kg/month)	Constant	−56.6 ± 61.6	−183.1, 69.8	0.37
	b*	2.66 ± 1.07	0.46, 4.86	0.020*
	L*	0.53 ± 0.90	−1.32, 2.37	0.56
Forehead b*				
Fruit and vegetable intake (kg/month)	Constant	23.4 ± 70.0	−101.7, 148.5	0.70
	b*	2.05 ± 1.11	−0.23, 4.33	0.076
	L*	−0.62 ± 0.87	−2.41, 1.17	0.48
Right cheek b*				
Fruit and vegetable intake (kg/month)	Constant	23.0 ± 51.5	−82.6, 128.6	0.66
	b*	2.49 ± 1.27	−0.11, 5.10	0.060
	L*	−0.66 ± 0.82	−2.34, 1.02	0.43
Shoulder b*				
Fruit and vegetable intake (kg/month)	Constant	−70.4 ± 58.8	−191.1, 50.4	0.24
	b*	1.77 ± 0.95	−0.17, 3.72	0.072
	L*	0.86 ± 0.69	−0.55, 2.27	0.22
Biceps b*				
Fruit and vegetable intake (kg/month)	Constant	−95.8 ± 51.2	−200.9, 9.4	0.073
	b*	2.69 ± 0.98	0.68, 4.71	0.011*
	L*	1.090 ± 0.62	−0.20, 2.37	0.092
Triceps b*				
Fruit and vegetable intake (kg/month)	Constant	−58.9 ± 40.1	−141.2, 23.4	0.15
	b*	1.57 ± 0.83	−0.12, 3.26	0.068
	L*	0.79 ± 0.52	−0.28, 1.87	0.14
Palm b*				
Fruit and vegetable intake (kg/month)	Constant	29.1 ± 44.9	−63.1, 121.3	0.52
	b*	1.42 ± 0.83	−0.28, 3.11	0.099
	L*	−0.54 ± 0.72	−2.02, 0.94	0.46
Back of hand b*				
Fruit and vegetable intake (kg/month)	Constant	67.5 ± 50.0	−28.9, 163.9	0.16
	b*	0.34 ± 1.052	−1.82, 2.50	0.75
	L*	−0.94 ± 0.61	−2.20, 0.32	0.14
Sole of foot b*				
Fruit and vegetable intake (kg/month)	Constant	−35.7 ± 29.8	−96.9, 25.5	0.24
	b*	1.47 ± 0.87	−0.32, 3.26	0.10
	L*	0.47 ± 0.45	−0.45, 1.39	0.31

BSS = Best skin sites; averaged forehead, biceps, palm and sole b* values, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

the biceps, and the body of evidence supporting measurement at the forehead, palm and sole, taking the combined average of these four sites in a practical setting may allow for more consistent results. The forehead, biceps, palm and sole are all easily accessible sites, which is likely to decrease subject discomfort in comparison to previously studied sites where subjects are required to expose their torso [16,19].

The impact of melanin interference on skin yellowness measurement is illustrated by the improved correlations observed when skin lightness is controlled for. However, skin lightness was not found to be a significant predictor of F/V intake in the regression analysis. This indicates that skin lightness was not a significant predictor of F/V intake in this Caucasian male sample and strengthens the applicability of skin yellowness as a biomarker of F/V intake for the Caucasian population. However, as skin lightness is a significant predictor at the biceps and is a known confounder of the relationship between skin yellowness and F/V intake, L* values are an important input in the regression equation. The lack of significance for L* may be explained as the subjects were of primarily Anglo-Celtic origin. It is likely L* would be a greater determining factor for b* measurements among more olive-toned complexions (e.g. Mediterranean, Arabic ethnic groups). Ethnicity is also an important consideration in future research due to the implications of cultural and regional F/V consumption patterns and to increase the applicability of this potential biomarker to ethnically diverse populations.

The limitations of the present study should be recognised in order to guide future research directions. A relatively small sample size limits the applicability of this study. It would be beneficial to analyse a greater range of BMI, ages and intakes to achieve a more accurate representation of the Western Australian Caucasian male population. No participants in the study sample consumed the recommended daily serves of F/V, compared to 1.75% of Australian 18–34 year old males [2]. These results highlight the pattern of very low fruit and vegetable consumption that currently exists among young Australian men. Many volunteers had a personal interest in the research due to their own health and fitness goals but were unable to meet current dietary recommendations. In future, more adequate-level intakes should be analysed to determine the suitability of this method to predict an adequate level of F/V consumption. This study is also limited by its external validity, which is held for the Western Australian winter season only. Performing this research in winter was beneficial, as decreased sun exposure limits the effect of UV irradiation and melanin interference on skin colour measurements. Controlling for these effects was a strength of this study, however, further research is required to determine whether the observed associations hold true at various times of year and particularly in summer. Additionally, calculation of carotenoid intake was limited using the USDA carotenoid database, which is specific to the US food supply. Australia does not have a

comprehensive carotenoid database, as the Food Standards Australia New Zealand (FSANZ) NUTTAB 2010 database only provides information on α-carotene, β-carotene and cryptoxanthin for an incomplete number of foods. As such, the accuracy of the measured total carotenoid intake is limited. This highlights the requirement for an updated Australian carotenoid database, for application to this topic and across the nutrition field. Fortunately, the strong correlation between carotenoid intake and F/V intake indicates that the USDA database is somewhat suitable for the Australian population. Future research should include measurement of plasma carotenoid status to further examine the relationship between carotenoid intake and F/V intake.

This study adds to the body of research conducted in Caucasian populations using the RS method. Caucasian subjects have been used primarily to facilitate determination of the best skin sites for measurement, as measuring the reflectance properties of skin in racial groups with darker skin tones is challenging due to high melanin interference. There is potential for the palm and sole to be used across skin tones because melanin does not deposit in these areas [24]. The present study adds to the growing evidence that supports measurement at the palm and the sole for various reasons, and further research is required to explore the use of these skin sites among non-Caucasian racial groups.

5. Conclusions

The present study provides evidence that skin yellowness is positively correlated with F/V intake and carotenoid intake. We show proof of concept that skin yellowness is viable as a biomarker to predict F/V intake in young Caucasian men. This evidence supports findings from studies conducted in female populations and provides insight into the skin sites that are most likely to be viable in a practical setting. As research in this area is still limited, it is important to examine this relationship in a wider range of subjects consuming a variety of F/V. This additional data will increase the applicability of an equation to potentially predict F/V intake from BSS skin colour.

Authorship declaration

Georgia S Bixley: Acquisition of data; statistical analysis; primarily responsible for writing the manuscript.

Karin M Clark: Conception and design of the study; writing the manuscript.

Anthony P James: Conception and design of the study; writing the manuscript.

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Conflicts of interest

The authors declare no conflict of interest.

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Appendix I. Fruit and vegetables listed in the food frequency questionnaire

Fruit	Vegetables	Juice
Citrus	Dark green	Fruit juice
Grapefruit	Broccoli	Vegetable juice
Lemon	Broccolini	
Mandarin	Brussels sprout	
Orange	Asian greens	
Other	Cabbage	
	Cauliflower	
Stone	Lettuce	
Avocado	Kale	
Apricot	Spinach	
Peach	Other	
Plum		
Nectarine	Root	
Other	Potato	
	Sweet potato	
Tropical	Onion	
Banana	Beetroot	
Pineapple	Carrot	
Mango	Leek	
Papaya	Artichoke	
Other	Spring onion	
	Other	
Berries		
Blueberry	Legumes/beans	
Raspberry	Canned beans	
Strawberry	Lentils	
Cranberry	Split peas	
Other	Green peas	
	Green beans	
Pome	Snow/snap peas	
Apple	Other	
Pear		
Asian pear	Gourd	
Other	Pumpkin	
	Zucchini	
Melons	Cucumber	
Rockmelon	Other	
Watermelon		
Other	Other	
	Capsicum	
Other	Chilli	
Grape	Corn	
Fig	Eggplant	
Kiwifruit	Tomato	
Date	Mushroom	
Pomegranate	Celery	
Raisin	Bean sprout	
Passionfruit	Olive	

Appendix J. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jnim.2018.06.001.

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