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Skin carotenoid status and plasma carotenoids: biomarkers of dietary carotenoids, fruits and vegetables for middle-aged and older Singaporean adults

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Abstract

Skin carotenoid status (SCS) measured by resonance Raman spectroscopy (RRS) may serve as an emerging alternative measurement for dietary carotenoid, fruit and vegetable (FV) intake although its application had not been assessed in a middle-aged and older population in Asia. This cross-sectional study aims to concurrently examine the use of SCS and plasma carotenoids to measure FV and carotenoid intake in a middle-aged and older population, taking into consideration potential socio-demographic and nutritional confounders. The study recruited 103 middle-aged and older adults (mean age: 58 years) in Singapore. Dietary carotenoids and FV, plasma carotenoid concentration and SCS were measured using 3-d food records, HPLC and a biophotonic scanner which utilised RRS, respectively. Adjusted for statistically defined socio-demographic covariates sex, age, BMI, prescription medication and cigarette smoking, plasma carotenoids and SCS showed positive associations with dietary total carotenoids (β_{plasma}: 0·020 (95 % CI 0·000, 0·040) µmol/l/mg, $P = 0·05$; β_{skin}: 265 (95 % CI 23, 506) arbitrary units/mg, $P = 0·03$) and FV (β_{plasma}: 0·076 (95 % CI 0·021, 0·132) µmol/l per FV serving, $P = 0·008$; β_{skin}: 1036 (95 % CI 363, 1708) arbitrary units/FV serving, $P = 0·003$). The associations of SCS with dietary carotenoid and FV intake were null with the inclusion of dietary PUFA, fibre and vitamin C as nutritional covariates ($P > 0·05$). This suggests a potential influence of these nutritional factors on carotenoid circulation and deposition in the skin. In conclusion, SCS, similar to plasma carotenoids, may serve as a biomarker for both dietary carotenoid and FV intake in a middle-aged and older Singaporean population.

Key words: **Fruits: Plasma carotenoids: Resonance Raman spectroscopy: Skin carotenoids: Vegetables**

A higher intake of fruits and vegetables (FV) had been consistently evidenced to be associated with a reduced risk and incidence of chronic diseases such as type 2 diabetes[1], CVD, total cancer, as well as all-cause mortality[2]. As the likelihood of developing chronic diseases increases with age, lowering this risk is especially important in middle-aged and older adults. Therefore, it is important to assess the intake of FV in this population[3–5]. Common methods used to determine dietary FV intake include FFQ and dietary recalls. While easy to administer logistically, subjective self-reported methods are prone to recollection and social desirability biases if inadequately performed[3–5]. Nevertheless, this can be complemented with the use of more objective biomarkers to improve the accuracy of dietary data and to reduce the magnitude of errors.

Carotenoids, in particular, are a group of naturally occurring organic pigments that contribute to the distinctive red, yellow and orange colours of FV. Humans and other animals, while unable to synthesise these pigments, accumulate carotenoids from the diet (e.g. in eggs from poultry and the flesh of fish such as salmon)[6,7]. As FV are the main sources of dietary carotenoids, there is potential for carotenoids to serve as an objective and reliable marker to assess dietary FV intake. From an earlier systematic review, circulating carotenoids and vitamin C had been reported to be the most commonly measured biomarkers for dietary FV intake[8].

The use of plasma carotenoids is substantially validated with numerous studies which reported significant, positive correlations between both dietary FV and carotenoids with plasma carotenoid concentrations[9–13]. However in recent years, skin carotenoid status (SCS) assessed by resonance Raman spectroscopy (RRS) had become increasingly evaluated as a potential alternative biomarker for dietary carotenoids and FV intake[3,14]. Predominantly deposited in the stratum corneum, the transfer of carotenoids to the skin is hypothesised to occur either via diffusion from blood and adipocytes or transportation through sebaceous and eccrine sweat glands[15,16]. Following oral consumption, this process was reported to take within 1–3 d[17].

Abbreviations a.u., arbitrary units; FV, fruits and vegetables; MLR, multiple linear regression; RRS, resonance Raman spectroscopy; SCS, skin carotenoid status.

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RRS is a spectroscopic technique that detects molecules based on the principle of inelastic light scattering, a frequency shift corresponding to the characteristic vibrational energy of molecules[18]. Specifically, the carbon backbone of conjugated double bonds in carotenoids yields a unique fingerprint for identification and quantification via RRS[19]. In contrast to skin biopsies and plasma carotenoids, SCS is rapid, non-invasive and without the discomfort and complications from phlebotomy and biopsy. This makes SCS particularly suited for adolescent and older populations. In addition, SCS assay may be less susceptible to fluctuations in response to recent dietary intake and potential carotenoid degradation by heat, light and oxygen during sample preparation which enhances experiment reproducibility[20–22].

However, as biomarkers of exposure, both SCS and plasma carotenoids are vulnerable to external influences including age, race and other dietary components. At present, although SCS validation studies had been conducted in Western and younger Asian populations[3,23,24], there is a need to explore a broader spectrum of individuals including middle-aged and older adults in Asia which may have vastly different dietary preferences. Moreover, while nutrients including fat and fibre had been evidenced to affect carotenoid bioaccessibility and assimilation[25,26], limited studies evaluated the impact of these constituents on SCS. Therefore, the aim of the present study is to concurrently validate the use of SCS and plasma carotenoids to assess dietary FV and carotenoids in a middle-aged and older Singaporean population, taking into consideration potential socio-demographic and nutritional confounders for a comprehensive assessment. It is hypothesised that SCS and plasma carotenoids will be associated with both dietary FV and carotenoids.

Methods

Study design and participants

The present cross-sectional study recruited middle-aged and older adults between September 2018 and October 2019. The National Healthcare Group's Domain Specific Review Board approved the study protocol (study reference number 2018/00221) and the study was registered at clinicaltrials.gov as NCT03554954. All participants provided written informed consent and received monetary compensation for participation.

The participant inclusion criteria were as follows: (1) aged between 50 and 75 years, (2) able to give informed consent, (3) no consumption of dietary supplements such as carotenoids, multivitamins and natural extract unless willing to discontinue for a minimum of 1 month, (4) no significant changes in diet during the past year and (5) venous access sufficient for blood sampling. Following consent, participants were asked to complete a questionnaire to obtain information on their socio-demographic characteristics and medical history.

The participant flow diagram is shown in Fig. 1. From the 130 participants screened, a total of 108 participants were recruited for the study. Data from four participants who failed to provide dietary records and one participant whose plasma carotenoids and SCS were identified as an outlier via studentised residuals plots (i.e. > 3 or < –3) were excluded. Collectively, data from 103 participants were used for statistical evaluation.

Anthropometric and blood pressure measurements

Height and weight of the participants were measured using a stadiometer (Seca) recorded to the nearest 0·01 m and 0·1 kg, respectively. Waist circumference was measured with a measuring tape according to WHO standards[27]. Both resting systolic and diastolic blood pressures were measured while seated using an automated blood pressure monitor (Omron, HEM-7121). All measurements and readings were taken at least twice with the average calculated and used as the final readings.

Dietary data

Dietary data were obtained using 3-d food records collected over two weekdays and one weekend which were analysed using the Dietplan 7 software (Forestfield Software Ltd). Recording procedures were carefully instructed by trained research staff with visual aids to ensure accurate entries and portion size estimations. Clear instructions were also provided to remind participants not to deviate from their habitual diet during dietary record. Details recorded include the specific food consumed, mode of food preparation, serving size and meal timings. Nutritional information was obtained primarily from the US Department of Agriculture (USDA) database with reference to the Singapore Health Promotion Board database[28] and nutritional information panels for commercially available food products as secondary data sources. Nutritional data obtained from the secondary sources were verified and cross-referenced with close alternatives from the USDA database to ensure an accurate representation and consistency. Dietary FV intake was described according to serving number which was based on guidelines and schemes set by the WHO and National Health Service. One serving of either fruit or vegetable was defined as 80 g in its fresh or cooked form, 30 g in its dried form or 150 ml of 100 % pure juice[27,29]. This is with the exclusion of starchy vegetables (e.g. potatoes, cassava, tapioca, etc.) and pulses (e.g. beans, chickpeas, lentils, etc.).

Blood collection and blood lipid-lipoprotein and glucose measurements

Fasting-state blood was collected from the antecubital vein by a trained phlebotomist into EDTA-treated, potassium oxalate/sodium fluoride-treated and plain tubes. EDTA- and potassium oxalate/sodium fluoride-treated tubes were immediately centrifuged at 3000 ***g***, 15 min at 4°C while plain tubes were kept on ice for 15 min before centrifugation at similar settings. Aliquots (500 µl) of the plasma and serum were stored at −80°C until analysis.

Plasma glucose and the serum lipid-lipoprotein profile were measured with Siemens ADVIA Chemistry XPT and ADVIA 1800 systems (Siemens Healthcare Diagnostics) at Quest Laboratories.

Plasma carotenoid analysis by HPLC

The extraction of plasma carotenoids was adapted from Kim *et al.*[30] with minor modifications. Plasma aliquots (900 µl) were

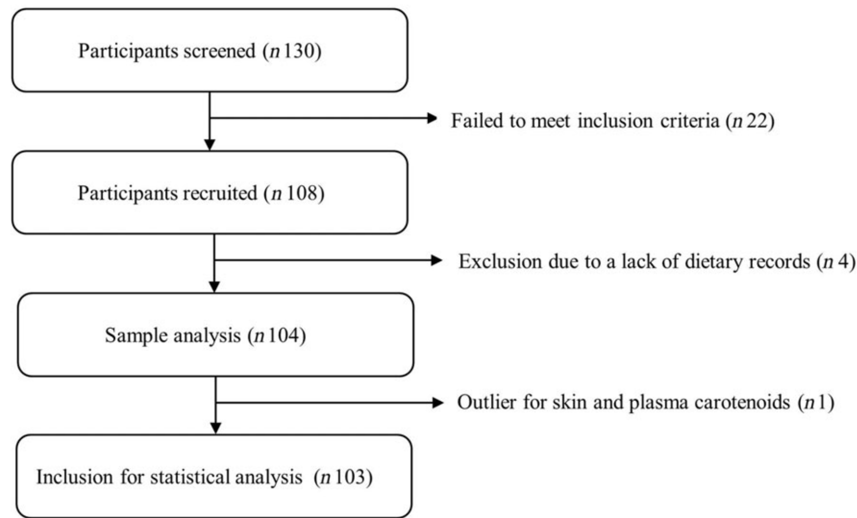

Fig. 1. Flow diagram for participants of cross-sectional study.

treated with methanol (1 ml) containing 0·1 % (w/v) butylated hydroxytoluene. The extraction of plasma carotenoids was carried out with acetone–petroleum ether (1:2, v/v) (4 ml) and petroleum ether (2 ml twice). The extracts were evaporated to dryness under N_2 gas and reconstituted in methanol–methyl tert-butyl ether (1:1, v/v) with 0·1 % butylated hydroxytoluene.

Six plasma carotenoids, namely α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein and zeaxanthin, were quantified by HPLC (Waters Alliance e2695 Separation Module; Waters) with photodiode array at 450 nm, with the exception of lycopene (470 nm). Separation of carotenoids was conducted using a C_{30}, 250 × 4·6-mm, bonded silica reversed-phase column (YMC). A pair of mobile phases (methanol–methyl tert-butyl ether–water (81:15:4, by vol.) and methanol–methyl tert-butyl ether–water (6:90:4, by vol.)) were used for gradient elution at 1·0 ml/min, 25°C. The standard curves and retention times of pure carotenoid standards were used for the identification and quantification of plasma carotenoid concentrations. Acetone, methanol and petroleum ether were purchased from VWR International while butylated hydroxytoluene, methyl tert-butyl ether and the carotenoid standards were purchased from Sigma-Aldrich.

Skin carotenoid analysis by resonance Raman spectroscopy

Utilising RRS, SCS was measured with a Pharmanex S3 biophotonic scanner (NuSkin). Participants were guided to place their palm on the sensor for an analysis which lasted around 30 s. The score obtained had a range from 10 000 to 89 000 arbitrary units (a.u.), where a high score would indicate higher carotenoids concentration in the skin. A minimum of two readings was obtained, and the average SCS was calculated.

Power calculation and statistical analysis

SCS was not a primary outcome of interest for the present cross-sectional study and was not used for the derivation of the original sample size estimates. Retrospectively, *post hoc* power analysis was conducted with G * Power 3.1 (Heinrich-Heine-Universität)[31] based on the associations between SCS and dietary FV at $\alpha = 0·05$ (coefficient of determination = 0·0825; two-tailed). Based on the population size of 103, the present study would yield a sufficiently high power of 86 %.

An evaluation of potential confounding factors was performed using multiple linear regressions (MLR) and ANCOVA for continuous and categorical variables, respectively. Dietary total carotenoids were controlled to identify population characteristics which influenced SCS and plasma carotenoids independent of the dietary source. SCS and plasma carotenoids were compared against socio-demographic characteristics (age, sex, race, BMI, waist circumference, cigarette smoking and use of prescription medication (for blood glucose, lipid and blood pressure control)), as well as with a thorough coverage of nutritional data which included energy, macronutrients (carbohydrate, sugar, fibre, protein, total fat, saturated fat, monounsaturated fat, polyunsaturated fat) and micronutrients (Na, K, Ca, vitamin A, vitamin B_9, vitamin B_{12}, vitamin C, vitamin D and vitamin E).

The associations between dietary FV and carotenoids with both SCS and plasma carotenoids were first examined with simple linear regression. Pearson's correlation was further used for pairwise comparisons between skin, plasma and dietary carotenoids. Following which, adjusted for defined socio-demographic covariates, a MLR model was used to assess the associations between dietary FV and carotenoids with both SCS and plasma carotenoids. Nutritional covariates were also incorporated into the statistical models if they were either highly established confounders to carotenoid bioavailability (i.e. fat and fibre)[25,26] or were likewise identified to impact plasma carotenoids or SCS as independent covariates.

Data analyses were conducted using STATA/MP 13 (STATACORP LP). All data are presented as means with their standard errors or as β-coefficients with 95 % CI, with $P < 0·05$ considered for statistical significance.

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Table 1. Baseline population characteristics
(Mean values with their standard errors; ranges; numbers)

Characteristics	Mean/*n*	SEM	Range
Socio-demographic characteristics			
Age (years)	59	1	50–75
Sex			
Male	45	–	–
Female	58	–	–
Race			
Chinese	90	–	–
Indian	6	–	–
Malay	2	–	–
Caucasian	5	–	–
Cigarette smoking			
Never	89	–	–
Past	14	–	–
Use of prescription medication			
No	84	–	–
Yes	19	–	–
Health characteristics			
BMI (kg/m^2)	23·8	0·4	17·4–42·5
Waist circumference (cm)	83·0	1·2	63·0–136·5
Systolic blood pressure (mmHg)	117	2	79–178
Diastolic blood pressure (mmHg)	75	1	50–107
Glucose (mg/dl)*	98·3	190·9	75·7–210·8
Total cholesterol (mg/dl)*	206·3	405·0	112·1–321·0
HDL (mg/dl)*	61·6	184·0	27·1–119·9
LDL (mg/dl)*	123·6	353·4	54·1–232·0
TAG (mg/dl)*	107·1	502·4	35·4–265·7
Dietary biomarkers and characteristics			
Plasma total carotenoids (μmol/l)	2·07	0·09	0·50–4·65
α-Carotene (μmol/l)	0·27	0·01	0·06–0·90
β-Carotene (μmol/l)	0·47	0·03	0·07–1·85
β-Cryptoxanthin (μmol/l)	0·48	0·03	0·10–1·96
Lycopene (μmol/l)	0·36	0·03	0·06–1·69
Lutein (μmol/l)	0·35	0·02	0·08–1·00
Zeaxanthin (μmol/l)	0·15	0·00	0·07–0·32
Skin carotenoid status (a.u.)	33 277	1102	12 000–64 500
Dietary fruit and vegetable intake (servings/d)	4·7	0·3	0·0–11·7
Fruit intake (servings/d)	2·3	0·2	0·0–8·6
Vegetable intake (servings/d)	2·4	0·2	0·0–7·4
Dietary total carotenoids (mg/d)	10·52	0·80	0·64–35·88
α-Carotene (mg/d)	0·79	0·10	0·00–3·81
β-Carotene (mg/d)	4·66	0·46	0·08–28·85
β-Cryptoxanthin (mg/d)	0·15	0·02	0·00–1·11
Lycopene (mg/d)	1·89	0·27	0·00–11·95
Lutein and zeaxanthin (mg/d)	3·02	0·28	0·21–16·96
Dietary total fat (g/d)	67·1	3·0	23·1–210·2
Saturated fat (g/d)	23·7	1·0	7·7–66·8
Monounsaturated fat (g/d)	20·3	1·2	4·4–71·4
Polyunsaturated fat (g/d)	12·8	0·8	3·5–62·5
Fibre (g/d)	19·9	0·9	4·4–56·8
Vitamin A (μg/d)	245	36	14–3375
Vitamin C (mg/d)	103	8	10–482

a.u., Arbitrary units.
* To convert glucose in mg/dl to mmol/l, multiply by 0·0555. To convert cholesterol in mg/dl to mmol/l, multiply by 0·0259. To convert TAG in mg/dl to mmol/l, multiply by 0·0113.

Results

Socio-demographic characteristics

The characteristics of 103 middle-aged and older participants (age: 59 (SEM 1) years; BMI: 23·8 (SEM 0·4) kg/m^2) are shown in Table 1. Both females (*n* 58) and males (*n* 45) were recruited, with racial Chinese making up the majority (*n* 90). The population was generally healthy based on their BMI, blood pressure, plasma glucose and serum lipid-lipoprotein concentrations as well as their cigarette smoking history and use of prescription medication. The mean plasma carotenoid concentration and SCS were 2·07 (SEM 0·09) μmol/l and 33 277 (SEM 1102) a.u., respectively.

Dietary characteristics

The population dietary characteristics are tabulated in Table 1. The intake of dietary FV was 2·3 (SEM 0·2) and 2·4 (SEM 0·2) servings/d, respectively. Dietary total carotenoid intake was 10·52 (SEM 0·80) mg/d with individual carotenoids β-carotene, lutein and

Table 2. Regression analyses of plasma carotenoids with daily fruit, vegetable and carotenoid intake
(Regression coefficients and 95 % confidence intervals)

	Total plasma carotenoids (μmol/l)								
				Multiple linear regression					
	Simple linear regression			Model 1*			Model 2†		
Dietary factors	β	95 % CI	P	β	95 % CI	P	β	95 % CI	P
Fruits and vegetables (servings/d)	0·086	0·023, 0·149	0·008	0·076	0·021, 0·132	0·008	0·097	0·039, 0·156	0·001
Total carotenoids (mg/d)	0·021	–0·002, 0·043	0·07	0·020	0·000, 0·040	0·05	0·023	0·003, 0·043	0·03
	Corresponding individual plasma carotenoids (μmol/l)								
				Multiple linear regression					
	Simple linear regression			Model 1*			Model 2†		
Individual dietary carotenoids	β	95 % CI	P	β	95 % CI	P	β	95 % CI	P
α-Carotene (mg/d)	0·039	0·014, 0·063	0·002	0·032	0·009, 0·055	0·008	0·038	0·015, 0·061	0·001
β-Carotene (mg/d)	0·008	–0·006, 0·022	0·25	0·005	–0·007, 0·018	0·40	0·006	–0·007, 0·019	0·34
β-Cryptoxanthin (mg/d)	0·432	0·195, 0·670	< 0·001	0·395	0·174, 0·616	< 0·001	0·407	0·179, 0·635	0·001
Lycopene (mg/d)	0·044	0·027, 0·061	< 0·001	0·048	0·031, 0·064	< 0·001	0·053	0·036, 0·070	< 0·001
Lutein and zeaxanthin (mg/d)	0·010	–0·004, 0·024	0·15	0·009	–0·004, 0·021	0·16	0·009	–0·003, 0·022	0·15

* Model 1 was adjusted for age, BMI and prescription medication.
† Model 2 was adjusted for age, BMI, prescription medication and daily total fat intake.

zeaxanthin, lycopene, α-carotene and β-cryptoxanthin intake ranked in descending order.

Identification of covariates

Socio-demographic covariates for plasma carotenoids were identified to be: age, BMI and prescription medication, and for SCS: sex, BMI, cigarette smoking and prescription medication (online Supplementary Table S1). Waist circumference, while identified as a covariate, was not adjusted for as it exhibited strong collinearity with BMI. Among the nutritional data, dietary vitamin C was identified as a covariate for both plasma carotenoids and SCS while vitamin A was identified as a covariate for SCS only (online Supplementary Table S2). Coupled with well-established confounders of fat and fibre, nutritional covariates were singularly added to and adjusted for in separate MLR models.

Regression analyses

Simple linear regression, as tabulated in Tables 2 and 3, yielded positive associations between dietary FV with plasma carotenoids and SCS (β_{plasma} (regression coefficient): 0·086 (95 % CI 0·023, 0·149) μmol/l per FV serving, $P = 0·008$; β_{skin}: 1133 (95 % CI 387, 1878) a.u./FV serving, $P = 0·003$). For total dietary carotenoids, only SCS showed a significant correlation (β_{skin}: 274 (95 % CI 9, 540) a.u./mg, $P = 0·04$). According to Fig. 2 however, no linearity was observed between dietary carotenoids and both plasma carotenoids and SCS. With individual dietary carotenoids, positive associations were observed with corresponding plasma carotenoids for α-carotene (β_{plasma}: 0·039 (95 % CI 0·014, 0·063) μmol/l per mg, $P = 0·002$), β-cryptoxanthin (β_{plasma}: 0·432 (95 % CI 0·195, 0·670) μmol/l per mg, $P < 0·001$) and lycopene (β_{plasma}: 0·044 (95 % CI 0·027, 0·061) μmol/l per mg, $P < 0·001$) while SCS depicted positive associations with α-carotene (β_{skin}: 2095 (95 % CI 51, 4139) a.u./mg, $P = 0·04$) and lutein and zeaxanthin (β_{skin}: 804 (95 % CI 52, 1556) a.u./mg, $P = 0·04$). Between plasma carotenoids and SCS, a significant positive association was observed (ρ (correlation coefficient) = 0·626; $P < 0·001$) (Fig. 2).

Upon the adjustment of socio-demographic covariates (model 1; Tables 2 and 3), dietary FV continued to show positive associations with plasma carotenoids (β_{plasma}: 0·076 (95 % CI 0·021, 0·132) μmol/l per FV serving, $P = 0·008$) and SCS (β_{skin}: 1036 (95 % CI 363, 1708) a.u./FV serving, $P = 0·003$). Likewise, significant associations were observed with dietary carotenoids (β_{plasma}: 0·020 (95 % CI 0·000, 0·040) μmol/l per mg, $P = 0·05$; β_{skin}: 265 (95 % CI 23, 506) a.u./mg, $P = 0·03$). Similar to the simple linear regression, model 1 continued to depict positive associations between individual carotenoids with the corresponding plasma carotenoids (α-carotene ($P = 0·008$), β-cryptoxanthin ($P < 0·001$) and lycopene ($P < 0·001$)). SCS, on the other hand, depicted associations with both lutein and zeaxanthin ($P = 0·04$)) and lycopene ($P = 0·04$).

With the inclusion of total fat intake as a covariate (model 2; Tables 2 and 3), the significant associations between both SCS and plasma carotenoids with dietary FV as well as carotenoids (total and individual) were maintained with reference to model 1 (without dietary covariates). This remained largely true with the inclusion of SFA (model 3), MUFA (model 4) and PUFA (model 5) (Table 3 and online Supplementary Table S3) as covariates, with the exception of the association between SCS and dietary carotenoids after PUFA was adjusted ((β_{skin}: 247 (95 % CI –13, 508) a.u./mg, $P = 0·06$); model 5). Correspondingly, a similar change was detected for individual dietary carotenoids lutein and zeaxanthin (β_{skin}: 690 (95 % CI –2, 1383) a.u./mg, $P = 0·05$) and lycopene (β_{skin}: 730 (95 % CI –58, 1518) a.u./mg, $P = 0·07$) (Table 3).

For plasma carotenoids, the adjustment of fibre contributed to no changes in regression analyses. However, significant associations with both FV and dietary total carotenoids were null

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Table 3. Regression analyses of skin carotenoids with daily fruit, vegetable and carotenoid intake
(Regression coefficients and 95 % confidence intervals)

	Skin carotenoids (a.u.)								
	Simple linear regression			Multiple linear regression: Model 1*			Multiple linear regression: Model 2†		
Dietary factors	β	95 % CI	*P*	β	95 % CI	*P*	β	95 % CI	*P*
Fruits and vegetables (servings/d)	1133	387, 1878	0·003	1036	363, 1708	0·003	1142	423, 1861	0·002
Total carotenoids (mg/d)	274	9, 540	0·04	265	23, 506	0·03	269	21, 516	0·03
α-Carotene (mg/d)	2095	51, 4139	0·04	1522	–350, 3394	0·11	1527	–390, 3445	0·12
β-Carotene (mg/d)	282	–186, 750	0·23	172	–258, 603	0·43	168	–267, 603	0·45
β-Cryptoxanthin (mg/d)	6461	–2414, 15 335	0·15	4302	–3988, 12 593	0·31	4267	–4313, 1417	0·33
Lycopene (mg/d)	342	–448, 1132	0·39	780	58, 1502	0·04	820	62, 1579	0·03
Lutein and zeaxanthin (mg/d)	804	52, 1556	0·04	732	47, 1416	0·04	729	41, 1417	0·04

	Multiple linear regression								
	Model 3‡			Model 4§			Model 5‖		
Dietary factors	β	95 % CI	*P*	β	95 % CI	*P*	β	95 % CI	*P*
Fruits and vegetables (servings/d)	1129	444, 1814	0·001	1132	391, 1873	0·003	1066	316, 1817	0·006
Total carotenoids (mg/d)	271	29, 514	0·03	259	10, 509	0·04	247	–13, 508	0·06
α-Carotene (mg/d)	1548	–332, 3427	0·11	1454	–489, 3398	0·14	1310	–709, 3329	0·20
β-Carotene (mg/d)	177	–255, 609	0·42	158	–277, 593	0·47	112	–321, 565	0·59
β-Cryptoxanthin (mg/d)	4739	–3654, 13 132	0·27	3794	–5026, 12 615	0·40	3542	–4909, 11 993	0·41
Lycopene (mg/d)	831	100, 1562	0·03	785	15, 1556	0·05	730	–58, 1518	0·07
Lutein and zeaxanthin (mg/d)	728	41, 1415	0·04	724	37, 1411	0·04	690	–2, 1383	0·05

a.u., Arbitrary units.
* Model 1 was adjusted for sex, BMI, prescription medication and cigarette smoking.
† Model 2 was adjusted for sex, BMI, prescription medication, cigarette smoking and daily total fat intake.
‡ Model 3 was adjusted for sex, BMI, prescription medication, cigarette smoking and daily saturated fat intake.
§ Model 4 was adjusted for sex, BMI, prescription medication, cigarette smoking and daily monounsaturated fat intake.
‖ Model 5 was adjusted for sex, BMI, prescription medication, cigarette smoking and daily polyunsaturated fat intake.

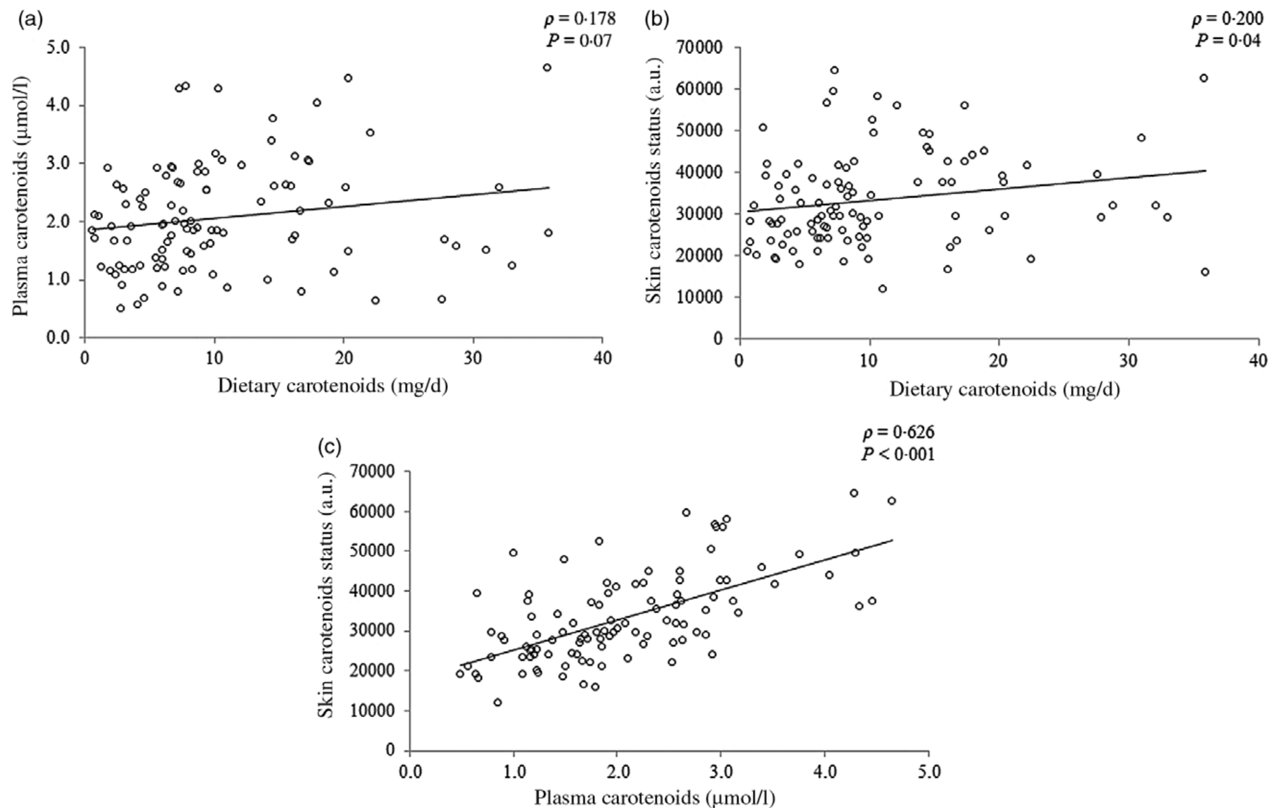

Fig. 2. Scatterplots depicting correlations between (a) plasma *v.* dietary carotenoids, (b) skin *v.* dietary carotenoids and (c) skin *v.* plasma carotenoids. ρ, Correlation coefficient; a.u., arbitrary units.

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($P > 0·05$) when controlled for daily vitamin C intake (online Supplementary Table S4). On the other hand for SCS, adjustment of vitamin A contributed to no effects although adjustments of both vitamin C and fibre resulted in no associations with dietary FV and carotenoids ($P > 0·05$; online Supplementary Table S5).

Discussion

FV are the main sources of dietary carotenoids. Apart from the blood, carotenoids can accumulate in the skin which serves as a site for deposition[19]. SCS measured by RRS had received emerging interest due to its ease of measurement and accurate representation of FV and carotenoid intake. Previous cross-sectional studies reported positive associations between plasma carotenoids as well as SCS with both dietary FV and carotenoids[3,4,14,23,32,33]. In the present study, the authors also observed positive correlations which support the use of both plasma carotenoids and SCS as biomarkers for dietary FV and carotenoid intake in a middle-aged and older Singaporean population.

The mean SCS reported in the present study was similar to participants from the Strong Hearts, Healthy Communities study (mean = 34 100 a.u.) which recruited predominantly Caucasian, obese women (n 157) and used an identical device for SCS analysis[3]. This was coupled with similar dietary total carotenoids (mean = 10·0 mg/d) compared with the present population (mean = 10·5 mg/d). However, there was discrepancy in the intake pattern of dietary carotenoids compared with Western populations whose carotenoid intakes consisted mostly of lycopene[34]. The present results bore closer resemblance to a previous assessment on middle-aged and older Asian adults which likewise consumed predominantly β-carotene, followed by lutein and zeaxanthin[35].

In alignment with the hypothesis, both dietary carotenoids and FV showed positive associations with plasma carotenoids and SCS after the socio-demographic covariates were controlled. Plasma carotenoids in particular had been extensively validated as a biomarker for dietary carotenoids and FV intake. This included a systematic review and meta-analysis conducted by Burrows *et al.*[36] which observed a positive relationship between dietary and plasma carotenoids. Additionally, the European Prospective Investigation into Cancer and Nutrition (EPIC), a large-scale multicentre prospective cohort study, also demonstrated its usefulness as a biomarker for dietary FV[9]. Examining individual carotenoid species, the results indicated that while a majority of carotenoids depicted a similar trend, there was an absence of associations between dietary and plasma β-carotene, lutein and zeaxanthin.

Among the carotenoids, β-carotene in particular displays the highest substrate affinity to β-carotene dioxygenase-1, making it most susceptible to vitamin A conversion[37]. Compared with other provitamin A carotenoids, β-carotene can be enzymatically cleaved to yield 2 molecules of retinal via a single-step process in enterocytes. This may have increased plasma β-carotene depletion and hence, the absence of significant associations with dietary β-carotene. For the most abundantly consumed xanthophylls lutein and zeaxanthin, this lack of association may be attributed to its markedly lower half-life and more rapid clearance from the circulation[38]. Conjugation with oxygen increases hydrophilicity which influences the orientation of xanthophylls in circulating lipoproteins[39]. In contrast to the hydrophobic carotenes packaged in the lipoprotein core, xanthophylls were postulated to be metabolised and deposited more efficiently[40].

Carotenoids in the skin were reported to reflect dietary intake and its bioavailability from food sources[41]. This is supported by the present positive associations between SCS with dietary carotenoids and FV. The uptake and depletion kinetics was described by Jahns *et al.*[42] who reported that SCS mirrored the changes in plasma carotenoids. In comparison however, the skin, which serves as a site for deposition, was regarded to be less responsive than fluctuations in blood, which functioned as a transport medium[14,43]. This makes SCS a promising indicator of long-term carotenoid intake.

Moreover, in contrast to plasma carotenoids, SCS reflects total carotenoids. This includes, for instance, fucoxanthin and capsanthin which may be present in lower quantities in FV, with reference to the six main plasma carotenoids detected. Therefore, caution ought to be exercised when interpreting associations with single dietary carotenoids and SCS since carotenoids more abundantly present may reflect stronger associations. This was evidenced by the marked correlations with lycopene, lutein and zeaxanthin which are the most consumed carotenoids in this population after β-carotene.

In addition to established socio-demographic confounders such as sex, BMI and smoking history which aligned with the covariates identified from previous clinical studies[22,44], dietary factors including fibre and fat intake also play an important role in the bioaccessibility and bioavailability of carotenoids. However, limited studies considered the influence of dietary confounders on plasma carotenoids and SCS[25,45]. With the adjustment of total fat as well as SFA, MUFA and PUFA individually, the present study largely showed no marked change in association, in contrast to MLR model 1 (controlled for socio-demographic covariates only). While the role of dietary fat in increasing carotenoid absorption had been well studied in post-prandial experiments, patterning of food intake needs to be taken into consideration[30,46,47]. Specifically, carotenoid absorption may only be enhanced when carotenoid-rich foods are co-consumed with dietary fat[46]. However, as this is a cross-sectional study, it is not optimised to take into consideration the variable of time. This could possibly explain the absence of significant associations.

Nevertheless, it is noteworthy to highlight that the association between dietary carotenoids and SCS was null when adjusted for PUFA. This suggests that among the dietary fats, PUFA intake in particular may confound the observed associations between dietary carotenoids and SCS. Previous studies on genetically obese rats have shown that PUFA administration raised the mRNA and levels of hepatic scavenger receptor class B1 type 1 (SR-B1) compared with controls fed with SFA-rich groundnut oil[48] while fish oil intake was also observed to increase CD36 expression in abdominal adipocytes of spontaneously hypertensive rats[49]. Similarly expressed in epidermal keratinocytes, lipid membrane transporters SR-B1 and CD36 had been thought to facilitate carotenoids absorption into the skin[50–52]. Therefore,

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its raised expression may explain the influence of PUFA on SCS although this hypothesis will need to be validated. Thus, future studies could investigate the effects of co-consuming dietary fat at different levels of saturation on the eventual deposition of carotenoids in the skin.

Corresponding to the adjustments with dietary fat, controlling for vitamin A did not exert any considerable influence on the associations between SCS with dietary FV and carotenoids. However, correlations for both SCS and plasma carotenoids were mostly non-significant following the inclusion of fibre and vitamin C into the MLR models. This indicates a possible influence of vitamin C and fibre on the status of plasma and skin carotenoids. Notably, dietary fibre had been established to lower carotenoid bioavailability as a result of matrix entrapment during digestion[53,54] while vitamin C, which may exhibit antioxidant synergy with carotenoids, could aid in the extension of its half-life[55,56]. Nonetheless, in contrast to dietary fat, vitamin C and fibre are synonymous with and strongly correlated with FV intake (data not shown). This results in multicollinearity which can weaken the regression model's precision and statistical power.

The strengths of the present study include the use of a rapid, non-invasive method to analyse carotenoids in the skin, where skin biopsies would otherwise be challenging in this population due to the discomfort from excision[15]. Another strength lies in the rigorous collection of dietary information. This was challenging to achieve, particularly in the present, predominantly Asian population whose cuisines are often composite, with a variety of ingredients. To maintain dietary data precision, each food item was deconstructed according to its ingredients with careful consideration of the cooking method, portion size and product details. This yields a thorough depiction of the nutritional data which subsequently allow for a more meticulous identification of potential dietary confounders to be applied in the present study. Furthermore, quantification of FV as servings in accordance to WHO and National Health Service guidelines also provides a more characteristic description, in contrast to earlier studies which measured FV intake by weight[27,29]. This quantification method standardises the dietary data of FV intake according to typical consumption quantities *v.* the absolute weights which improve research translatability and representation from a nutritional perspective. For instance, a serving of dried fruit (30 g) deviates substantially in absolute weight in contrast to fresh alternatives (80 g) although the weight of the fruit pre-processing may be similar.

Nevertheless, a weakness which needs to be highlighted is the discrepancy in optical properties of carotenoids despite the similar conjugated carbon molecular structure. In particular, this may be a limitation for specific colourless carotenoids including phytoene and phytofluene which can be further investigated in future studies. The representativeness of SCS for FV intake may also be challenged since some FV contains little to no carotenoids while other foods which are not FV (e.g. eggs) may contain carotenoids. Nevertheless, carotenoid status can serve as a reflective marker of nutritional factors which are abundant in FV beyond carotenoids alone. As deduced in the present study, antioxidants such as vitamin C were also strongly correlated with SCS. While these do not serve as a direct carotenoid source, synergism between antioxidants, as described earlier, may extend carotenoid half-life. This indirectly raises plasma and skin concentrations and hence, supports its use as FV biomarkers. However, it should be noted that the large lipid to protein ratio of the skin may inherently favour the deposition of the more hydrophobic carotenes, as deduced by an earlier trial which examined the correlations using skin biopsy samples[57]. Lastly, it is worth noting that while the use of 3-d food records at present yields greater accuracy, specifically in the context of dietary carotenoids data, it may lack representability for long-term dietary patterns.

In conclusion, skin carotenoids, similar to plasma carotenoids, may serve as a biomarker for both dietary FV and carotenoid intake in the middle-aged and older Singaporean population. The application of carotenoid biomarkers, however, may be susceptible to confounding from socio-demographic and other nutritional influences which ought to be considered for a more comprehensive assessment.

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The authors report no conflicts of interest.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114521000143

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