

## Short Communication

# A non-invasive assessment of skin carotenoid status through reflection spectroscopy is a feasible, reliable and potentially valid measure of fruit and vegetable consumption in a diverse community sample

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### Abstract

**Objective:** To assess the feasibility, reliability and validity of reflection spectroscopy (RS) to assess skin carotenoids in a racially diverse sample.

**Design:** Study 1 was a cross-sectional study of corner store customers ( $n$  479) who completed the National Cancer Institute Fruit and Vegetable Screener as well as RS measures. Feasibility was assessed by examining the time it took to complete three RS measures, reliability was assessed by examining the variation between three RS measures, and validity was examined by correlation with self-reported fruit and vegetable consumption. In Study 2, validity was assessed in a smaller sample ( $n$  30) by examining associations between RS measures and dietary carotenoids, fruits and vegetables as calculated from a validated FFQ and plasma carotenoids.

**Setting:** Eastern North Carolina, USA.

**Results:** It took on average 94.0 s to complete three RS readings per person. The average variation between three readings for each participant was 6.8%. In Study 2, in models adjusted for age, race and sex, there were statistically significant associations between RS measures and (i) FFQ-estimated carotenoid intake ( $P < 0.0001$ ); (ii) FFQ-estimated fruit and vegetable consumption ( $P < 0.010$ ); and (iii) plasma carotenoids ( $P < 0.0001$ ).

**Conclusions:** RS is a potentially improved method to approximate fruit and vegetable consumption among diverse participants. RS is portable and easy to use in field-based public health nutrition settings. More research is needed to investigate validity and sensitivity in diverse populations.

**Keywords**  
Fruit  
Vegetables  
Carotenoids  
Health promotion

A diet rich in fruits and vegetables is associated with lower rates of obesity<sup>(1–3)</sup>, cancer<sup>(4)</sup>, CVD<sup>(5,6)</sup>, diabetes<sup>(7)</sup> and all-cause mortality<sup>(8,9)</sup>. However, in general, the US population does not consume the recommended amounts of fruits and vegetables<sup>(10)</sup>. This is especially true for rural<sup>(11)</sup> and disadvantaged<sup>(12)</sup> populations. Thus, examining and addressing the low consumption of fruits and vegetables, particularly in disadvantaged populations, is important to

prevent chronic disease. To effectively design and test interventions to increase fruit and vegetable consumption in these populations, valid and reliable measures are needed. Traditional methods of self-reported dietary assessment have limitations due to recall and social desirability biases<sup>(13,14)</sup>, and even greater limitations in underserved populations due to low literacy and numeracy<sup>(15)</sup>. The current reference measure for fruit and vegetable consumption,

blood carotenoid concentration, is an expensive, time-intensive and invasive measurement<sup>(16)</sup>. Thus, a critical gap in assessing the effectiveness of community- and policy-level interventions to promote fruit and vegetable consumption is the lack of a non-invasive, easy-to-use, valid and reliable measure of fruit and vegetable consumption in underserved populations.

Skin carotenoid status assessed by resonance Raman spectroscopy has emerged as a promising new biomarker of fruit and vegetable intake<sup>(17)</sup>. A wide distribution of skin carotenoid levels has been demonstrated<sup>(18,19)</sup> with high reproducibility over 6 months<sup>(19,20)</sup> and validity compared with plasma, skin biopsy and reported fruit and vegetable intake<sup>(19)</sup>, all necessary characteristics for a biomarker of nutritional status and food intake. Reflection spectroscopy (RS) is a potentially improved method of measuring skin carotenoid status<sup>(21)</sup>. However, to date, RS validation studies have been conducted in a laboratory setting and primarily in white populations<sup>(21,22)</sup>. Additionally, RS has not been tested in terms of feasibility, reliability and validity in a community-based field setting among racially and ethnically diverse individuals. Therefore, the present study's purpose was to assess the feasibility, reliability and validity of RS in assessing skin carotenoids in a racially diverse community sample recruited from a field-based food retail venue (small corner stores); moreover, to examine validity in greater detail among a purposefully selected group of African-American and Caucasian participants, we examined associations between RS-assessed skin carotenoids, plasma carotenoids and dietary data among participants recruited from a medical school listserv.

## Methods and materials

### Setting and participants

#### Study 1

This pilot study was part of a larger evaluation project designed to assess the effect of the North Carolina Healthy Food Small Retailer Program, a programme funded by the NC Legislature to improve the offerings of healthy foods in small corner stores in Eastern North Carolina, on dietary behaviours among rural and disadvantaged residents of Eastern North Carolina, USA<sup>(23)</sup>. As part of our evaluation project, in February–April 2017 we pilot-tested a customer intercept survey and the RS device (described below) among 466 customers in sixteen Eastern North Carolina small convenience/corner stores. Participants were eligible if they were over 18 years of age, a store customer and spoke English. Participants were given an information sheet describing the study and informed consent by signature was not required.

#### Study 2

In addition, to examine associations between plasma carotenoids and RS-assessed carotenoids in people

self-identifying as African American ( $n$  17) and non-Hispanic White ( $n$  13), in July–August 2017 we recruited and enrolled thirty participants from a medical school email listserv. Participants were screened for eligibility, which included not smoking, BMI between 18.5 and 29.9 kg/m<sup>2</sup>, healthy/no history of chronic disease, and not participating in any weight-loss or other lifestyle intervention programmes. After being screened and determined eligible, participants were invited to the East Carolina Diabetes and Obesity Institute lab in a fasting state. At the Institute, they read the informed consent materials, had the opportunity to ask questions and provided a signature signifying their informed consent. Participants had their blood drawn; had weight, height and skin carotenoids (RS-assessed) measured using standard procedures; and then completed an FFQ and Demographic Questionnaire (measures are described in detail below).

### Self-reported fruit and vegetable consumption

#### Study 1

Corner store participants self-reported consumption of fruits and vegetables over the past month using the National Cancer Institute (NCI) 'all day' Fruit and Vegetable Screener (F&V Screener)<sup>(23)</sup> (scoring algorithms are located at <http://www.epi.grants.cancer.gov/diet/screeners/fruitveg/scoring/allday.html#how>). Frequency response options range from never, 1–3 times last month, 1–2 times per week, up to 5 or more times per day. The NCI F&V Screener queries the frequency and amount for the following items: 100% Juice (orange, apple, grape, or grapefruit); Fruit (fresh, canned, frozen, no juice); Lettuce Salad; French Fries/Fried Potatoes; Other White Potatoes (baked, boiled and mashed potatoes, potato salad and white potatoes that are not fried); Cooked Dried Beans; Other Vegetables; and Tomato Sauce (tomato sauce on pasta or macaroni, rice, pizza and other dishes). The F&V Screener also includes frequency for Vegetable Mixtures (foods such as sandwiches, casseroles, stews, stir-fry, omelettes and tacos). Daily servings of fruits and vegetables were calculated using the standard algorithm (that includes frequency and amount consumed) as provided on the NCI website<sup>(24)</sup>. While the F&V screener does not gather detailed information on specific carotenoid-rich fruits and vegetables servings consumed, it is a preferred tool for use in community-based nutrition studies because of its simplicity and brevity of administration and analysis<sup>(24,25)</sup>. For this pilot analysis, our goal was to determine if the RS device (a rapid assessment tool) agreed with the F&V Screener (another rapid assessment tool) to assess fruit and vegetable consumption.

#### Study 2

For the smaller validation follow-up study, our objective was to determine if intakes of specific fruits and vegetables or of total dietary carotenoids were correlated with RS device measures in a diverse population. For this study,

the Fred Hutchinson Cancer Research Center (FHCRC) FFQ was used to determine detailed information about dietary carotenoid intake and fruit and vegetable consumption. The self-administered FFQ asks participants to report the frequency and portion size of 125 food and beverage line items over a defined time period (1 month in our study)<sup>(26,27)</sup>. Dietary carotenoids and daily fruit and vegetable servings were calculated using the FHCRC standard algorithms<sup>(26,27)</sup>.

### **Skin carotenoid assessment**

In addition to self-reported dietary assessment, we also used the RS device to evaluate the use of skin carotenoids as a non-invasive, objective measure of dietary intake of fruits and vegetables. The RS device (Veggie Meter; Longevity Link Corp., Salt Lake City, UT, USA) was developed by those who originally developed resonance Raman spectroscopy as an objective indicator of carotenoid status<sup>(17,21,22)</sup>. RS involves measurement of the skin carotenoid absorption via reflection under application of topical pressure<sup>(21)</sup>. Applying a modest amount of topical pressure allows the carotenoid absorption spectra to be derived from the reflection spectra; the topical pressure provides an increase in the contrast between carotenoid absorption strength and the combined absorption background from other chromophores<sup>(21)</sup>.

Trained research staff directed participants to place the right or left index finger (the side most comfortable for the participant) on a lens on the top of the RS device. A lever was then lowered over the finger which applied a gentle pressure, which temporarily blanches the skin by restricting blood flow, as is necessary to minimize interference of blood Hb with the RS carotenoid measurements. The device is linked to a tablet computer and shows the participant where his or her skin carotenoid reading falls on a histogram of all other readings prior to their measure. Each participant's index finger was scanned three times and the average of the last two readings was used in this analysis. When time permitted, data collectors recorded the time three readings took using a stopwatch and recorded any qualitative comments made by participants specific to the RS device.

### **Plasma carotenoid measurements**

Plasma was isolated by centrifugation in EDTA-containing Vacutainer tubes. Plasma was aliquoted and stored at  $-80^{\circ}\text{C}$  and shipped on dry ice to the USDA/ARS Grand Forks Human Nutrition Research Center analytical lab. Human plasma carotenoid analysis was performed by LC-MS/MS. In brief, under reduced lighting,  $10\ \mu\text{l}$  plasma is mixed with  $990\ \mu\text{l}$  of methanol containing internal standard, centrifuged to remove protein, and  $25\ \mu\text{l}$  of the supernatant is injected on to a C-30 column (YMC Carotenoid). The gradient elution method was previously described<sup>(28)</sup>. Extraction efficiency was adjusted relative to

the internal standard and analytes were quantitated by external calibration curves.

### **Covariates**

Covariates thought to possibly influence self-reported fruit and vegetable consumption as well as carotenoid absorption and biodistribution<sup>(19,29–31)</sup> were queried and included in predictive models.

#### *Study 1*

On the customer intercept survey, we asked participants to self-report race/ethnicity, smoking status (yes/no), age (continuous in years), sex (male, female), and height and weight (to calculate BMI). Response options for race/ethnicity included American Indian or Alaskan Native, Asian, Black or African American, Native Hawaiian or Pacific Islander, and White; and for ethnicity Hispanic or Latino or non-Hispanic or Latino, or Other/Refused. We dichotomized race/ethnicity as African American and non-African American to maximize power in analyses.

#### *Study 2*

Participants were asked to self-report race/ethnicity, age (continuous in years), sex (male, female, other), income and education levels.

### **Data analysis**

#### *Study 1*

We assessed feasibility of the RS device by examining the time it took to obtain the three readings and anecdotal comments made by customers, as well as the ease of setting up and using the RS device in a small convenience/corner store. We examined reliability using the CV of the triplicate measure (also of the second two measures, assuming the first measure was a 'practice run'), calculated as the standard deviation of the three (or two) readings divided by their mean. We examined whether RS scores were normally distributed using the Kolmogorov–Smirnov test. We assessed agreement between RS scores and the easy-to-implement NCI F&V Screener tool (convergent validity) by using linear regression to examine associations between RS scores and self-reported NCI fruit and vegetable daily servings, controlling for race, smoking status, age and sex.

#### *Study 2*

We examined associations between RS measures and (i) intake of total carotenoids and servings of fruits and vegetables as calculated from the FHCRC FFQ and (ii) plasma carotenoids as determined from blood drawn in a fasting state, using Pearson's correlation coefficients and linear regression models, adjusted for race, age and sex. (All participants were non-smokers.) The statistical software package SAS version 9.4 was used for all analyses.

## Results

Table 1 includes participant (corner store customer) characteristics and indicates that the majority (65.9%) were African American and 42.7% had an annual household income of less than \$US 25 000. The mean age was 43.3 years, mean daily servings of fruits and vegetables were 1.8 and 2.0, respectively, and mean BMI was 30.0 kg/m<sup>2</sup>. The mean RS score (for three readings) was 234 RS intensities.

We recorded the time for the process of scanning the finger three times using the RS device for 151 of the total of

**Table 1** Characteristics of 479 corner store customers (Study 1), Eastern North Carolina, USA, February–April 2017

Characteristic	Frequency	%
<b>Race/ethnicity</b>		
African American	309	65.9
Non-African American*	160	34.1
Missing or refused	10	2.1
<b>Annual household income (\$US)</b>		
<25 000	176	42.7
25 000–50 000	146	35.4
>50 000	90	21.9
Missing	67	14.0
<b>Sex</b>		
Female	196	41.2
Male	280	58.8
Missing	3	1.0
<b>Smoking status</b>		
Yes	228	47.6
No	251	52.4
	<b>Mean</b>	<b>SD</b>
Age (years)	43.3	15.1
BMI (kg/m <sup>2</sup> )	30.0	7.9
RS score (mean of three readings)	234.2	86.2
RS score (mean of the last two readings)	235.5	87.5
Daily servings of fruit (NCI F&V Screener)	1.8	2.2
Daily servings of vegetables (NCI F&V Screener)	2.0	1.9
Daily servings of fruits and vegetables (NCI F&V Screener)	3.8	3.2

RS, reflection spectroscopy; NCI, National Cancer Institute; F&V Screener, 'all day' Fruit and Vegetable Screener.

\*The non-African American group included five American Indians/Alaska Natives, one Asian, fifteen Hispanics/Latinos, 126 Whites and thirteen Other racial groups. Nine were missing and one refused.

479 participants. On average, this process took 94.0 (SD 27.7) s. The RS device was generally received well by participants. Responses included positive feedback, including: 'This is interesting, this is cool stuff'; 'I want one of these. That's cool'. Some participants wanted to know more about what the score meant, and tried to relate the RS score to actual fruit and vegetable consumption, such as: 'I know my score is high because I eat a lot of fruits and vegetables'; 'My score is low – I need to eat more fruits and vegetables'. Not all responses were positive and some indicated hesitance, or a desire to get the readings done more quickly: 'Why are y'all doing this?'; 'Why do the numbers change?'

Intra-individual variation of the triplicate measurements was 6.8%. When the second two of the three readings were used, the variation of these two readings was 5.6%. Table 2 shows that among African American participants, the variation was 6.8% for the triplicate measurements and 5.6% for the second two readings. Among the non-African American participants, the variation was 6.7% and 5.7% for three and two readings, respectively.

### Study 1

We examined associations between self-reported daily servings of fruits and vegetables (NCI F&V Screener) and RS scores, adjusted for race, smoking, age, sex and BMI. There were no statistically significant associations between RS scores and NCI-assessed daily servings of fruits and vegetables (see Table 3). The  $R^2$  was 0.08 for each of the three models. When we conducted a sensitivity analysis, examining only African American and White participants, the results did not change.

### Study 2

For the small validation study, mean age of participants was 32.9 (SD 11.8) years, mean BMI was 25.1 (SD 2.7) kg/m<sup>2</sup>, mean skin carotenoid score was 296.4 (SD 110.3) RS intensities and mean carotenoids from food was 16954.6 (SD 12280.9) µg/d. Seven (24%) reported an annual household income less than \$US 19999 and nineteen (65%) reported having a college education or greater

**Table 2** Mean and standard deviation of age, BMI, reflection spectroscopy (RS) scores, CV of RS scores, and self-reported consumption of fruits and vegetables as reported on the National Cancer Institute (NCI) Fruit and Vegetable Screener (F&V Screener), stratified by African American v. non-African American (Study 1), Eastern North Carolina, USA, February–April 2017

	African Americans (n 309)		Non-African Americans (n 160)	
	Mean	SD	Mean	SD
Age (years)	44.6	14.2	40.6	16.4
BMI (kg/m <sup>2</sup> )	30.5	8.4	29.0	6.7
RS score (mean of three readings)	239.0	85.8	225.4	87.8
RS score (mean of two readings)	241.1	87.5	225.4	88.1
CV for the RS device (three readings)	6.8	8.2	6.7	8.1
CV for the RS device (two readings)	5.6	8.3	5.7	11.1
Daily servings of fruit (NCI F&V Screener)	1.8	2.3	2.1	2.2
Daily servings of vegetables (NCI F&V Screener)	1.9	1.8	2.2	2.2

**Table 3** Adjusted analyses examining associations between reflection spectroscopy (RS) scores for participants in Study 1 (*n* 479) and National Cancer Institute (NCI) Fruit and Vegetable Screener (F&V Screener)-assessed fruits and vegetables (adjusted for age, race, sex, smoking) and between RS scores for participants in Study 2 (*n* 30) and Fred Hutchinson Cancer Research Center (FHCRC) FFQ data and plasma carotenoids (adjusted for age, race and sex), Eastern North Carolina, USA, February–April 2017

Independent variable	Estimate	SE	<i>P</i> value	<i>R</i> <sup>2</sup>
NCI F&V Screener – fruit (Study 1)	–1.34	1.85	0.471	0.08
NCI F&V Screener – vegetables (Study 1)	1.88	2.09	0.370	0.08
NCI F&V Screener – fruit and vegetables (Study 1)	0.04	1.25	0.975	0.08
FHCRC FFQ – fruit and vegetables (Study 2)	31.60	11.27	0.010	0.27
FHCRC-FFQ – carotenoids (mg) (Study 2)	0.007	0.001	<0.0001	0.59
Plasma carotenoids (nmol/l) (Study 2)	0.07	0.01	<0.0001	0.55

**Table 4** Associations (Pearson's correlation coefficient and *P* values) between reflection spectroscopy (RS)-assessed skin carotenoids and self-reported fruit and vegetable consumption (daily servings), carotenoids from foods and plasma carotenoids, overall and stratified by African American *v.* non-African American (Study 2), Eastern North Carolina, USA, February–April 2017

Group	Total fruit and vegetable consumption (daily servings)		Total carotenoid intake from food (µg/d)		Total plasma carotenoids (µmol/l)	
		<i>P</i> value		<i>P</i> value		<i>P</i> value
All ( <i>n</i> 30)	0.48	0.009	0.69	<0.0001	0.71	<0.0001
African American ( <i>n</i> 17)	0.12	0.635	0.70	0.002	0.54	0.024
White ( <i>n</i> 13)	0.84	0.001	0.75	0.003	0.87	<0.0001

(data not shown). Table 4 shows correlations between FFQ-assessed carotenoids and fruits and vegetables, plasma carotenoids and skin carotenoids as assessed using RS, for the total sample (*n* 30) and stratified by race. There were significant correlations for all associations (ranging from  $r=0.48$  to  $r=0.86$ ) except for the association between FFQ-assessed fruits and vegetables and RS among African Americans. Table 3 shows that in models adjusted for age, race and sex, there were statistically significant associations between RS measures and (i) FFQ-estimated carotenoid intake ( $P<0.0001$ ); (ii) FFQ-estimated fruit and vegetable consumption ( $P<0.010$ ); and (iii) plasma carotenoids ( $P<0.0001$ ).

## Discussion

We found that the RS device was a feasible, reliable and potentially valid assessment tool for estimating carotenoid-containing fruit and vegetable consumption in this racially diverse sample. The device could be set up in a small corner store, in areas such as a fountain beverage counter or small table. It took, on average, 94 s to complete three readings in each participant, making it feasible for an intercept survey in a variety of community settings. In addition, in field-based community settings where time-efficient data collection is needed, two readings from the RS device should be adequate to evaluate and assess change.

Some participants were interested to know what the numbers meant, indicating that in future studies using the RS device a simple explanation should be used by data collectors, such as: 'The number is a measure of the level of carotenoids, or the colourful substances in some fruits and vegetables, in your skin'. Our data collectors usually

referred customers to the histogram on the laptop and told individuals that their line on the histogram represented where their values fell in relation to all the values ever taken by this machine.

The RS device had 6.8% variation in the triplicate measure in our field-based study, which was not substantively different between participants of different racial groups. Furthermore, in adjusted analyses, we found positive associations between self-reported fruit and vegetable consumption and RS scores in Study 2, indicating that the RS device is potentially valid for estimating fruit and vegetable consumption in a diverse community sample.

In Study 1, we did not find that NCI-assessed fruits and vegetables were associated with RS-assessed skin carotenoids; yet in Study 2, we found that FHCRC FFQ-assessed fruits and vegetables and carotenoids from foods were positively associated with RS-assessed skin carotenoids. This is perhaps due to the NCI F&V Screener being a validated tool for rapid assessment of total fruit and vegetable consumption *v.* carotenoid-rich fruit and vegetable consumption. In addition, it is likely that our population consumed a large proportion of potatoes and French fries, which are detected by the NCI screener but do not contain carotenoids. Study 2 results indicate that the RS device is a useful tool to assess non-potato and non-juice fruit and vegetable intake, which are the fruits and vegetables that are encouraged by the Dietary Guidelines for Americans. However, we did find that the association between self-reported total fruits and vegetables and RS-assessed skin carotenoids was not significant among African Americans, suggesting that there could be differences in genetics<sup>(31)</sup> or dietary patterns that account

for these differences. Our combined results from Studies 1 and 2 demonstrate the need to delve more deeply into understanding the RS device and associations with fruit and consumption given differences in race/ethnicity, ancestry and genetics, and regional food patterns, to further ready the RS device as a tool for nutrition surveillance and monitoring as well as evaluation of public health nutrition interventions.

The main limitation of the present study was the small sample size for our validation study examining correlations between RS scores and plasma carotenoids. This should be replicated in larger samples. In addition, nicotine could stain the skin of smokers, as could the powder from chilli peppers found on potato chips and other processed foods<sup>(32)</sup>, and this could potentially affect RS readings.

In the current study we found that the RS device holds promise for evaluation of community- and policy-level nutrition interventions in a variety of community settings. Further field validation is needed to examine associations between the RS device and the reference standard of blood carotenoids in diverse racial and ethnic groups and in a variety of community settings.

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