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Original Research

Skin Carotenoids: A Biomarker of Fruit and Vegetable Intake in Children

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ABSTRACT

Background Studies of adult subjects have found a strong correlation between serum carotenoids and skin carotenoids measured by resonance Raman spectroscopy (RRS). No published studies have examined correlations between skin and serum carotenoids among children.

Objectives We aimed to validate skin RRS methodology against serum carotenoid measurements by high-performance liquid chromatography and to determine whether RRS can be used as a valid biomarker of fruit and vegetable (F/V) intake among children.

Design In our cross-sectional study, participants were 45 healthy children aged 5 to 17 years who provided three blood samples used to assess serum carotenoid concentrations and three RRS skin measurements of the palm within a 4-week period. Dietary intake of F/V was assessed three times within 4 weeks using a 27-item food frequency questionnaire (FFQ) and an automated multiple-pass 24-hour daily recall. Estimates of intake from three FFQs, completed at least 7 days apart, were averaged. Estimates of intake from 24-hour daily recalls were collected on 2 weekdays and 1 weekend day and averaged.

Results Levels of skin and serum carotenoids were highly correlated (R^2=0.62; P<0.001). A linear regression model, controlling for child's weight and scanner unit, predicted that for every unit increase of total F/V from FFQ and total F/V as assessed by 24-hour daily recall, RRS intensity was predicted to increase by 3,798 (P=0.001) and 3,504 (P=0.001), respectively. Similar results were observed for reported high-carotenoid vegetable intake. Total carotenoid and beta carotene levels from 24-hour daily recalls correlated to total serum carotenoids levels (P<0.01 and P<0.05, respectively). Total carotenoid, alpha carotene, and beta carotene levels from the 24-hour daily recalls correlated to RRS (P<0.01).

Conclusions Skin carotenoids measured by RRS were strongly correlated with serum carotenoid levels and were positively associated with estimates of intake from FFQ and an automated multiple-pass 24-hour daily recall among children aged 5 to 17 years. Skin carotenoids may be used as valid biomarker of F/V intake among children.
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ACCURATELY MEASURING FRUIT AND VEGETABLE (F/V) intake in children is imperative to assess changes in consumption behavior. Collecting accurate food recalls in children has historically been difficult due to their immature cognitive ability and reporting skills.[1] Current tools for collecting food recalls in children are time- and labor-intensive. Food frequency questionnaires (FFQs) are the easiest to implement. However, recalls in children younger than age 10 years have poor correlation to actual intake.[2] A more accurate method, 24-hour daily recall, is more time- and labor-intensive and not valid in children aged 9 years and younger.[3]

Carotenoids are pigments found in F/V that are important bioactive nutrients for human beings.[4] Numerous studies have demonstrated that F/V can be protective against oxidative damage[5] and people who eat high amounts of F/V have lower risk for mortality and many chronic diseases.[6] Concentration of serum carotenoids is correlated with F/V intake in adults.[7-13] The National Academy of Sciences[14] states, "Blood concentrations of carotenoids are the best biological markers for consumption of fruits and vegetables." However, measuring serum carotenoid levels is invasive, labor-intensive, and measures are sensitive to day-to-day variation in carotenoid consumption.[9] A noninvasive method to measure skin carotenoid levels uses resonance Raman spectroscopy (RRS) and may better reflect long-term carotenoid status compared with blood carotenoids.[15,16] Primary carotenoids detected in the skin are lycopene,

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beta carotene, alpha carotene, beta cryptoxanthin, lutein, zeaxanthin, phytoene, and phytofluene, with lycopene present in the highest amounts.[4]

Past research has validated this measure against serum/plasma carotenoids in adults[7,8] and against F/V consumption in adults.[7,8,10,11,17] Others have found concentration of carotenoids from serum[18,19] and RRS[20,21] positively correlated with F/V consumption in children. Currently there are no published studies looking at the comparison of carotenoids in blood to RRS in children, which is needed to validate this tool as a biomarker of F/V intake in children.

The purpose of this study was to measure the correlation between skin and serum carotenoid levels and the correlation of these biomarkers to reported F/V intake from 24-hour dietary recall and FFQ in children.

SUBJECTS AND METHODS

Power calculations were computed using a correlation of 0.35 between the FFQ and the serum carotenoid concentrations. This is the approximate correlation identified in studies of adults[10] and is the lowest expected correlation of the comparisons in this research. A sample of 44 was estimated to give approximately 90% power (two-tailed α=.01) to detect a correlation of at least 0.35.

Subjects

A total of 50 healthy Cache County, Utah, schoolchildren aged 5 to 17 years were recruited through local elementary and secondary schools. The Institutional Review Board at Utah State University reviewed and approved the research protocol. A letter of information was sent via e-mail to parents of two local elementary schools. Older children were recruited by word of mouth and from siblings of elementary school children. Researchers recruited a purposeful sample of approximately 9% (n=5) children from each grade kindergarten through 12, 45% of whom were boys. The race/ethnicity of the participant population closely reflected the race/ethnicity of the local school-age population (16% Hispanic, 6% Asian, 2% Pacific Islander, and 76% white). Parents of children completed a qualifying online survey. If a child met the study inclusion/exclusion criteria, the parents were mailed/e-mailed and asked to complete a Center for Human Nutrition Studies health history questionnaire that includes past and present medical history, past and current medication, and nutritional supplement use. Children were excluded if they had a health history or habits that were known to affect carotenoid levels.[15,16,22] These exclusions included major illness during the 2 weeks before the study began, use of topical self-tanning lotion, chronic disease such as asthma or type 1 diabetes, and sun exposure >2 hours per day without use of sunscreen. Three children were excluded from the study due to a reported history of asthma.

Researchers obtained parent consent and child assent from participants in person. Participants were asked to maintain their normal lifestyle, including activity, nutritional supplement use, and dietary habits for the duration of the study. Participants received $20 at each of the three clinic visits and a $25 bonus for completing the study for a total of $85.

Protocol

Participants completed three clinic visits scheduled in the morning 7 days apart at the Center for Human Nutrition Studies clinic at Utah State University. Before a visit, the children completed a 10-hour overnight fast from eating or drinking anything except water. During the first clinic visit, height was measured using a Seca 223 digital stadiometer and weight was measured using a Detecto 758C digital scale. Height and weight were used to calculate body mass index (BMI) (ie, weight in kilograms/height in meters^2). BMI was categorized based on age and sex percentiles. At each clinic visit the following occurred: an 8-mL blood sample was collected from an arm vein into untreated glass vacutubes. Tubes were protected from the light to minimize light-induced degradation of serum carotenoids. Within 15 minutes of blood sampling, skin carotenoid concentrations were measured by trained researchers using the BioPhotonic Scanner (NuSkin Enterprises), a portable RRS device. The child placed his or her palm (between the distal and proximal palmar region) against the light window of the scanner and held it there for 90 seconds. The scanner emitted a light and displayed a score in Raman counts of 0 to 70,000+. Each child was scanned twice using the same scanner at all three clinic visits. At each visit, if there was a >2,000 Raman intensity count difference between scans, participants were scanned a third time. This was done to minimize the individual variation in RRS intensity counts. The two scores that were within 2,000 Raman intensity counts were averaged for the true score (see Figure 1). Three scanners were used in this research, and the units went through a self-calibration before each clinic visit. During the warm-up process, a black calibration cap was placed over the light window and the units self-calibrated using a patented process.[23] Children and parents were blinded to the results of their RRS score.

At each clinic visit, participants completed a computer-assisted 24-hour dietary recall (ASA24-Kids-2012 automated multiple-pass method[24]) and a 27-item 1 week look-back FFQ. In addition, they completed a health check list (yes or no answers) that reported lifestyle questions regarding the prior 7 days, including smoke exposure, nutritional supplement use, and illness since the previous clinic visit. Questions were asked on average daily hours of sun exposure and answers grouped into 0 to 29, 30 to 59, 60 to 90, and >90 minutes. Parents assisted participants younger than age 10 years to complete these forms.

Dietary Assessment

The FFQ was a modified version of a beverage and snack questionnaire (BSQ) developed and validated by Neuhouser and colleagues.[25] The BSQ asks children to report how often they consumed items from a list of fruits, vegetables, snacks, and beverages during the past week. For purposes of our study, this BSQ was modified by adding additional questions about high-carotenoid vegetables (HCV) and condensing snack items into more general categories. This modified BSQ tool contained 27 items compared with the 19-item tool used by Neuhouser and colleagues.[25]

Multiple 24-hour dietary recalls were collected to assess total habitual dietary intake, including 2 weekdays and 1

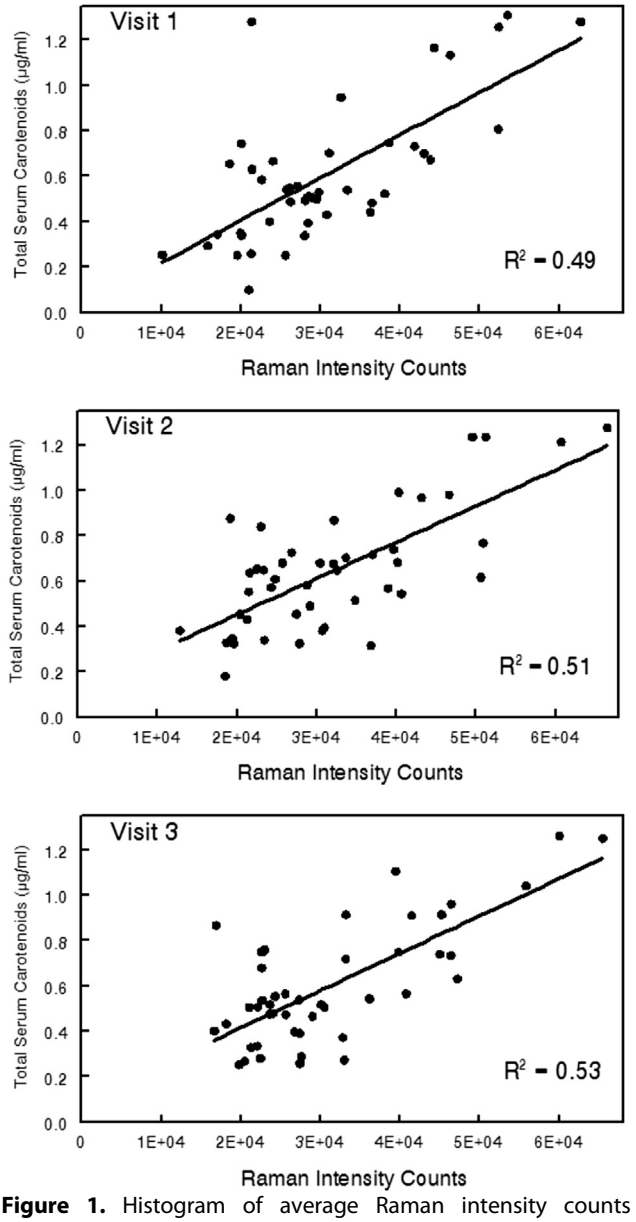

Figure 1. Histogram of average Raman intensity counts measured at the palm of the hand at each of three clinic visits in a study to determine whether resonance Raman spectroscopy can be used as a valid biomarker of fruit and vegetable intake among children. An average score was computed from all measurements (n=45).

weekend day, at least 7 days apart, within the 4-week study. The tool to collect the 24-hour dietary recall was the National Cancer Institute ASA24-Kids-2012.[24] This tool is based on the US Department of Agriculture Automated Multiple-Pass Method system involving five requests for intake data across the same 24-hour dietary recall, which has been validated in children aged 10 years and older.[2,3,26] Participants aged 10 years and older and parents of younger children completed the first recall during a clinic visit. Subsequent recalls were completed at home and the completion verified during clinic visits.

RRS

Skin carotenoid level was determined by calculating the average height of the peak Raman absorbance signal obtained and quantified from excitation of skin carotenoids using a low-intensity blue (λ=473 nm) LED light with green light (510 nm) detection[6] with the Biophotonic Scanner skin carotenoid levels reported as Raman intensity counts. The higher the count, the higher the concentration of carotenoid molecules detected at the site of measurement. The scanner reports total carotenoid value, not individual carotenoid counts, because there is overlap on the absorption spectra of each carotenoid.

Serum Carotenoid Levels by High-Performance Liquid Chromatography

Serum carotenoids were measured in the laboratory using HPLC.[27] Serum samples were extracted with hexane after the addition of α-tocopheral acetate as an internal standard. Extracts were dried under nitrogen and resuspended in mobile phase. Carotenoids were quantified using a Shimadzu AT-20A HPLC device equipped with an autosampler and diode array detector. Separation of the carotenoids was achieved by employing a Kromasil C18 5 μmol/L 25×4.6 cm column. The resulting chromatograph allows for the quantification of beta carotene, lycopene, and lutein.

The samples were prepared in a room with reduced light to minimize photodegradation of the carotenoids. The samples were measured in duplicate. All chromatographic runs included quality control material to ensure comparability between analytical runs.

Statistical Analysis

Statistics, including *t* tests and analysis of variance, were used to examine differences, to describe the distribution of assessments and characteristics of the study population, and to compare means across subgroups (ie, age, sex, race/ethnicity, and BMI-for-age percentiles). Association of potential confounding factors were examined, including smoke exposure, reported illness, supplement use, and hours of sun exposure to both skin and serum carotenoid levels at each of the three data collection points. Linearity was tested by visual inspection of the scatter plots. To determine the normality of the data, Levene's tests were conducted for all descriptive statistic calculations. For the linear regression analysis, skewness and kurtosis statistics were confirmed to be less than or equal to ±1. Outliers within independent variables were top-coded to the next closest value. This was done by converting the scores to *z* scores.[28] Findings of *z* scores that were above the range of 1.96, or 1 standard deviation, were changed to be the next value closest to 1.96. This was done to be able to retain the outliers in the data set. Two to four results were top-coded for each independent variable (averaged total F/V from FFQ and 24-hour dietary recall; averaged total HCV from FFQ and 24-hour dietary recall; beta carotene, alpha carotene, lycopene, and total carotenoids from 24-hour dietary recall; averaged summed serum carotenoids, serum lycopene, and serum beta carotene; weekly and total averaged RRS intensity and serum carotenoids). Intraclass correlation coefficients (ICCs) were used to assess how well the RRS intensity counts were correlated over multiple scannings obtained at one visit

and the ICC of RSS and serum carotenoids taken at multiple visits.

Linear regression models were used to control for the variation in RRS counts between scanner units. Potential factors were explored to refine this model, including age, sex, race/ethnicity, BMI, and weight. Weight, but not BMI, was significant so weight was included in the model along with scanner unit when exploring associations between independent and dependent variables. Average RRS intensity counts or serum carotenoids served as the dependent variables. This linear regression model was created to examine associations between assessments, including weekly RRS intensity counts and the concentration of weekly serum carotenoids, total averaged RRS intensity counts and averaged concentration of serum carotenoids, RRS intensity counts and serum carotenoids, and diet assessment of F/V consumption and serum carotenoids. Statistics were conducted using SPSS software version 20.0 (2011) with a P value of 0.05 or below to be considered statistically significant.

RESULTS

Of 47 children who started the study, two were missing one or more assessments and were excluded. One child was unable to complete the study due to time conflicts and one was unable to complete two of the three blood draws. The average age for participants was 10.5 years.

There were no differences in mean RRS intensity counts or serum carotenoid levels by sex, age groups, or BMI groups (see Table 1). Among the different race/ethnicities, Asians had higher mean serum carotenoid levels ($P<0.05$) and consumed more HCVs as determined from 24-hour dietary recall ($P<0.01$). In addition, children from grades 6 through 8 reported significantly more HCV intake on FFQ than children in

Table 1. Resonance Raman spectroscopy (RRS), serum carotenoid (SC), fruit and vegetable (F/V), and high-carotenoid vegetable (HCV) intake by baseline characteristics of participants (N=45)[a] in a study to determine whether RRS can be used as a valid biomarker of F/V intake among children

Characteristic	N (%)	RRS counts (counts)	SC value (μg/mL)	Food frequency questionnaire F/V (servings)[b]	Food frequency questionnaire HCV (servings)[b]	24-Hour daily recall F/V (c)	24-Hour daily recall HCV (c)
		← *mean±standard error* →					
Grade/age (y)							
Kindergarten -5th/5-10	25 (55)	33,162±2,627	0.59±0.05	3.0±0.26	1.1±0.10	1.9±0.21	0.41±0.07
6th-8th/11-13	12 (25)	29,161±2,091	0.66±0.04	3.5±0.69	1.3±0.21	2.3±0.37	0.42±0.10
9th-12th/14-17	8 (19)	28,676±3,397	0.49±0.06	3.9±0.34	1.5±0.21	2.7±0.40	0.63±0.15
Sex							
Male	20 (45)	30,909±1,809	0.53±0.04	3.5±0.35	1.3±0.13	2.2±0.26	0.43±0.07
Female	25 (55)	31,743±3,072	0.67±0.06	3.0±0.32	1.1±0.13	2.0±0.18	0.47±0.09
Ethnicity							
White	34 (76)	30,575±1,667	0.55±0.04	3.1±0.23	1.1±0.09	2.0±0.19	0.34±0.05
Hispanic	7 (16)	31,547±4,673	0.67±0.05	3.7±0.93	1.5±0.28	2.5±0.43	0.73±0.13
Asian	3 (6)	43,283±13,406	0.95±0.16	4.2±1.20	1.8±0.33	2.0±0.50	1.1±0.16
Pacific Islander	1 (2)	18,866	0.31	5.4	1.9	4.6	0.35
Body mass index							
>85%	7 (16)	29,180±3,620	0.52±0.07	3.5±0.68	1.4±0.36	2.6±0.57	0.36±0.09
<5%	4 (8)	29,015±1,593	0.55±0.06	2.9±0.52	0.96±0.22	1.7±0.45	0.18±0.08
5%-85%	34 (76)	31,797±2,023	0.61±0.04	3.3±0.29	1.2±0.1	2.2±0.19	0.49±0.06
Weight (kg)							
16.3-29.9	15 (33)	36,511±3,759	0.68±0.08	3.3±0.37	1.1±0.15	1.7±0.22	0.36±0.08
31.0-43.8	15 (33)	28,142±2,192	0.55±0.05	2.5±0.21	1.0±0.10	2.0±0.27	0.45±0.09
46.7-75.0	15 (33)	29,348±2,679	0.58±0.05	4.1±0.53	1.5 ±0.19	2.6±0.33	0.54±0.11

[a]The group mean was calculated from the average of assessments at three time points.
[b]Serving size was based on what would fit in a participant's cupped hand (approximately ½ c).

kindergarten through grade 5 ($P<0.05$). There were no differences in mean RRS values or summed serum carotenoid values in participants who reported illness, nutritional supplement use, or levels of sun exposure and no child reported having exposure to tobacco smoke (data not presented).

Skin Carotenoid Levels Compared with Serum Carotenoid Levels

The duplicate or triplicate (if scores differed by >2,000 units) RRS intensity counts from each clinic visit were highly correlated (Week 1 ICC=0.99 [$P<0.001$]; Week 2 ICC=0.98 [$P<0.001$]; Week 3 ICC=0.99 [$P<0.001$]) and so the two RRS intensity counts that were within 2,000 units were averaged. In addition, averaged RRS intensity counts from visit 1, 2, and 3 were highly correlated (ICC=0.98 [$P<0.001$]) so they were averaged to create the variable average RRS intensity counts. Serum carotenoids were also highly correlated between visits (ICC=0.88 [$P<0.001$]) so these scores were averaged to create the averaged serum level. Linear regression models calculated skin carotenoid levels to be highly correlated with serum carotenoid levels at each clinic visit (see Figure 2). The averaged skin and averaged serum carotenoid levels were also highly correlated ($R^2=0.62$ [$P<0.001$]). With this model, averaged serum carotenoid levels and scanner unit accounted for 50.1% and 8% of the variance, respectively.

Skin Carotenoid Levels Compared with Diet

The results of multivariable linear regression models that controlled for scanner unit and weight of child, found that self-reported F/V and HCV consumption using FFQ correlated significantly with skin carotenoid levels. The results are listed in Table 2. Each serving of averaged total F/V and HCV reported from the FFQ was associated with a 3,798 ($P=0.001$) and 6,355 ($P=0.03$) increase in RRS intensity counts, respectively. From the 24-hour dietary recall averages, for each cup of averaged total F/V and HCV intake, RRS intensity increased 3,504 ($P=0.001$) and 10,820 ($P=0.027$), respectively. Carotenoids consumption was also estimated from the 24-hour dietary recall. For every milligram of consumed beta carotene, total carotenoids, and alpha carotene there was an increase in RRS intensity counts of 3,354 ($P=0.01$), 4,556 ($P=0.008$), and 12,299 ($P=0.002$), respectively. Lutein and zeaxanthin results were reported combined and did not correlate with averaged RRS intensity counts. Lycopene from 24-hour dietary recall was not significantly associated with RRS.

Table 2. Multivariable linear regression analysis of the R^2 and beta values representing the relationship between total carotenoids measured by resonance Raman spectroscopy (RRS), summed serum carotenoids (SC), and fruit and vegetable (F/V) and high-carotenoid vegetables (HCV) intake estimated from food frequency questionnaire (FFQ) and 24-hour daily recall (24HDR) in a study to determine whether RRS can be used as a valid biomarker of F/V intake among children

	RRS model R^{2a}	RRS β	SC Model R^{2b}	SC β
FFQ F/V	0.32***	.49	0.18*	.39
FFQ HCV	0.21*	0.39	0.14	.33
24HDR F/V	0.31***	0.48	0.08	.24
24HDR HCV	0.21*	0.33	0.13	.30
24HDR total carotenoids	0.25**	.40	0.23**	.46
24HDR lycopene	0.19	.29	0.09	.37
24HDR alpha carotene	0.30**	.45	0.18	.38
24HDR beta carotene	0.24**	.39	0.23*	.28
24HDR lutein+zeazanthin	0.13	.14	0.09	.23

[a]Variable plus scanner units and RRS count were included in the multivariable linear regression model.
[b]Variable plus summed serum carotenoids were included in the multivariable linear regression model.
*$P<0.05$.
**$P<0.01$.
***$P<0.001$.

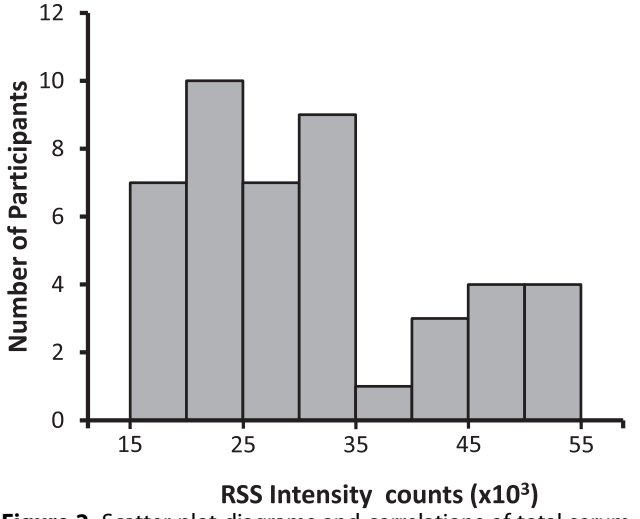

Figure 2. Scatter plot diagrams and correlations of total serum concentrations of carotenoids (μg/mL) and Raman intensity measured at the palm of the hand (n=45) in a study to determine whether resonance Raman spectroscopy (RRS) can be used as a valid biomarker of fruit and vegetable intake among children. Data for Raman intensity have been adjusted for differences between scanners.

Serum Carotenoid Levels Compared with Diet

Averaged summed serum carotenoid levels were significantly correlated with the averaged total F/V, but not HCV, intakes from FFQ. Averaged summed serum carotenoid levels did not significantly correlate with total F/V or HCV from 24-hour dietary recall. Individual dietary and total carotenoid intakes were not estimated from the FFQ.

Total carotenoid and beta carotene intake levels from the 24-hour dietary recall correlated significantly to total serum carotenoid levels. However, serum lycopene did not. Correlations of blood carotenoid levels with dietary lutein were not assessed because it is only reported combined with zeaxanthin on the ASA24-Kids-2012. Serum beta carotene level significantly correlated to beta carotene intake from the 24-hour dietary recall ($R^2=0.27$ [$P<0.05$]); serum lycopene

level did not correlate significantly to lycopene from 24-hour dietary recall.

DISCUSSION

These findings indicate that the RRS may be a valid and reliable indicator of serum concentrations of carotenoid and F/V intake among children. Skin carotenoid levels were positively correlated to serum carotenoid concentrations measured by HPLC among children. Similarly, skin carotenoid levels were positively correlated to total and carotenoid containing F/V intake as assessed from both an FFQ and multiple 24-hour dietary recalls. Measurement of skin carotenoid levels by RRS may provide a useful objective indicator of usual F/V intake among children, a group for which it is difficult to obtain accurate information about usual dietary intake.

This magnitude of correlation observed for skin carotenoid levels measured from RRS and serum carotenoid levels measured with HPLC is similar to that observed among adults.[7,8] This is the first report to date that looked at these correlations among children. In adults, skin carotenoid levels are less influenced by daily changes in dietary F/V intake than serum carotenoid levels.[6] Using RRS to assess skin carotenoid levels is fast, inexpensive, compact, and noninvasive, making it possible to use this technology outside laboratory settings. Thus, RRS may be a preferred method of assessing carotenoid levels and may better represent average F/V intake among children.

Significant correlations between skin carotenoid levels and total F/V and HCV from FFQ and 24-hour dietary recall were observed. A published study[20] in children comparing skin carotenoid levels that used FFQ as a method of assessment reported lower, yet significant associations for alpha carotene, beta carotene, lycopene, and lutein plus zeaxanthin (R^2= 0.13, 0.12, 0.08, and 0.03, respectively [$P<0.01$]). Research comparing plasma carotenoids with diet assessments found significant associations with alpha carotene, beta carotene, lycopene, but not lutein.[18] These studies used FFQ data converted to nutrient-level data, which potentially introduces more random and systematic error as well as restrictions imposed by a fixed list of foods and portion size estimations.

In adults using 24-hour dietary recall[12] and in adolescents using FFQ[29] researchers compared serum carotenoid levels and reported smaller but significant associations for alpha carotene and beta carotene but not for lycopene. Natarajan and colleagues[30] suggest that the higher validity for assessing beta carotene intake compared with lycopene and lutein is likely a reflection of higher quality food content data for provitamin A compounds, including, alpha carotene and beta carotene, compared with nonprovitamin A carotenoids.

Other studies in children comparing F/V intake to serum carotenoid levels[8,20,29] and skin carotenoid levels[21] found significant associations after adjusting for BMI. However weight was a stronger positive predictor in this study, possibly because it better reflects the volume of distribution of a given amount of carotenoid intake.

Our study had strengths and limitations. Most important, this is to our knowledge the first study to directly compare RRS measures of skin carotenoid levels against HPLC analyses of serum carotenoid levels in children and serves as a validation of the RRS method as an indicator of carotenoid status among children. Reliability of this method was assessed by testing multiple measures of skin carotenoids at each assessment period and over time. Similar to previous studies in adults,[31] little within-person variation was observed, which suggests the method is reliable over repeated measures. There was variation between our scanning units (250 and 5,190 counts), which was controlled for in the linear regression model.

ASA24-Kids-2012 was used to collect 24-hour daily recalls, and historically, 24-hour daily recalls have been a more accurate way to collect dietary carotenoid data.[29] A study investigating the validity and biases of food recall methods in measuring carotenoid intake over time compared carotenoid intake from 24-hour daily recall and FFQ with serum carotenoids.[30] The results found that 24-hour daily recall had lower error correlation over time, although both methods were subject to biases and error.[30]

No significant mean differences were found in skin or serum carotenoid levels between sexes, BMI, sun exposure, supplement use, or illness. This could be due to several factors: few children were in the high or low BMI groups, the study took place in late fall when sun exposure was minimal, illness was not described as to the severity or duration, there was limited diversity of the sample in terms of race/ethnicity, and there was no reported tobacco exposure. The serum carotenoid assay included three of the most commonly consumed carotenoids (ie, beta carotene, lycopene, and lutein) and were reported as summed, not total, serum carotenoid levels.

As discussed earlier, collecting accurate food recalls in young children has historically been difficult due to their immature cognitive ability and reporting skills.[2] Obtaining food recalls is also challenging in a school setting due to the time and resources required. The results from this study suggest that RRS may be a reliable biomarker of F/V intake and could be used in place of or in addition to food records. This may be especially important and useful in school-based studies where F/V intake is the outcome of interest or where interventions aim to increase F/V intake over a period of time. Based on the results of our study, RRS is a valid and reliable biomarker of nutritional status for children, supporting its future use as a biomarker for translational research studies.

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STATEMENT OF POTENTIAL CONFLICT OF INTEREST

After the conclusion of this study, M. Lefevre received funding from NuSkin, LLC, to conduct an unrelated study, and S. S. Aguilar was the study coordinator for that study. No potential conflict of interest was reported by the other authors.

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