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Feasibility of skin carotenoids as a biomarker of vegetable intake among Mexican-Origin children in a community setting: A cross-sectional pilot study

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ABSTRACT

Background and purpose: Studies in adults and children have demonstrated a strong correlation of skin carotenoids with fruit and vegetable (F/V) intake. There are few studies testing the usefulness of this methodology in a community setting. This study aimed to determine the feasibility of resonance Raman spectroscopy (RRS) skin carotenoid intensity scores in young children to assess F/V intake.
Methods: A cross-sectional study design was used to capture the RRS skin carotenoid intensity scores using the Pharmanex BioPhotonic Scanner. The RRS skin carotenoid intensity score ranges from 0 to 70,000+ with increasing scores indicating a higher carotenoid concentration. Children's dietary intake of F/V was assessed within one-month period using a 26-item food frequency questionnaire (FFQ) and multiple pass 24-hour dietary recalls (24HDRs) administered to the parent of the child. Participants were 51 Mexican-origin children (ages 3-8 years) living in two rural California school districts during July-August 2014. RRS skin carotenoid intensity scores; frequency of fruit and vegetable intake from FFQ; daily servings of fruit and vegetables and carotenoid intake from 24HDR. Spearman correlation was used to determine the correlation between RRS skin carotenoid intensity scores and average 24HDR F/V intake and FFQ F/V intake.
Results: The results of the study demonstrate a significant positive correlation between skin carotenoid levels and parent-reported 24HDR vegetable consumption (r = .41, p = .003) but not with 24HDR fruit, FFQ fruit, or FFQ vegetable consumption.
Conclusion: The use of skin carotenoid measurements may be more indicative of short-term vegetable consumption than overall fruit and vegetable or fruit-only consumption in this population of Mexican-origin children.

Key Words: Carotenoids, Children, Mexican, Fruits and vegetables, Resonance Raman spectroscopy

1. INTRODUCTION

There are a variety of tools that are used for dietary assessment, including food frequency questionnaires (FFQ), 24-hour dietary recalls (24HDR), and food records.[1] Though the FFQ and 24HDR tools pose less participant burden than food records, a major drawback of these two methods is their reliance on participant recall, which can lead to over-reporting of healthy foods and underreporting of unhealthy foods.[2] Dietary assessment is particularly challenging in children, where studies have demonstrated mixed

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results on the accuracy and reliability of child-reported or parent/caretaker-reported instruments.[3] Various factors such as age, gender, weight status, and social desirability affect participant reporting and introduce bias when using 24HDR and FFQ methods.[2] A systematic review of self-reported and parent-reported dietary assessments in children, compared to doubly labeled water, concluded that self-reported dietary recalls in children younger than nine years are unreliable. The use of parental or caretaker multiple-pass dietary recalls for children ages 4-9 years old is considered to provide an accurate measurement.[4] However, FFQs and 24HDRs rely on individual recall, which can introduce bias. Given these considerations, alternatives to FFQs and 24HDRs that can capture fruit and vegetable intake quickly, accurately, and objectively would prove to be valuable tools for research.

The Institute of Medicine considers serum carotenoids as the gold standard biomarker of fruit and vegetable (F/V) consumption since they are only found in high concentrations in these sources.[5] The use of serum carotenoids as a biomarker for fruit and vegetable consumption has been validated in adults.[6] Furthermore, a systematic review demonstrated the usefulness of carotenoids as a biomarker for fruit and vegetable intake.[7] Vegetables rich in carotenoids increase the level of carotenoids present in blood and in the skin compared to vegetables less rich in carotenoids.[8] However, the use of serum carotenoids presents many challenges including cost; participant burden; individual factors (e.g. bioavailability); limitations from nutrition databases; and practicality in community settings. In the past decade, a novel method of measuring carotenoid levels in the skin using resonance Raman spectroscopy (RRS) was developed.[9,10] The ability of RRS to measure carotenoid levels has been validated against serum and skin biopsy carotenoid levels in an adult population with various skin melanin levels.[10] Studies have demonstrated positive correlation between skin carotenoid levels and F/V consumption (r = .61, p < .001) in adults ages 18-30 years and children ages 5-17 years (R^2 = 0.18, p < .05).[11,12] In children ages 9-12, RRS skin carotenoids are correlated with plasma carotenoids (r = .62, p < .001) and total carotenoids as reported from an FFQ (r = .40, p < .0001).[13] However, there are no previous studies that have compared the usefulness of this methodology in a rural community with a large Mexican immigrant population.

The Niños Sanos, Familia Sana (NSFS) study is a three-year, multi-component intervention study aimed at preventing childhood obesity in Mexican-origin children ages 3 to 8 years in rural California.[14] A secondary aim of the study is to increase fruit and vegetable consumption among children. Based on a subsample from the NSFS study, this study examines feasibility of RRS skin carotenoid measurements in comparison to an FFQ and 24HDR in young Mexican-origin children within a rural, largely-immigrant, community setting. It is important to evaluate the usefulness of new methods in groups with different cultural food habits and choices.

2. Methods

2.1 Study design and population

This study uses a cross-sectional design to compare fruit and vegetable consumption assessed by FFQ and 24HDR to RSS skin carotenoid levels, all of which were collected within the same month period during summer 2014. The study took place in a rural community in California. The Institutional Review Board at University of California, Davs approved the study protocol. Parents or legal guardians signed informed consent forms in their preferred language (Spanish or English).

2.2 Study population

The study sample included children of Mexican heritage in rural California. Children were eligible to be included in this study if 1) their families were enrolled in the larger NSFS study; 2) they were between ages 4-8 years; and 3) their parents consented to the collection of three dietary recalls, FFQs and RSS measures.[14] If families had enrolled more than one child in the study, only one child closest to the ages 4-5 years was selected to capture information before they enter the school system. In cases where a household had more than one eligible child between 4-5 years, the younger child was selected to participate in the study. Parents provided informed consent but assent from children in this age group was not required.

2.3 Sample size

The sample size for this study was computed using a lowest expected correlation of 0.35 between the FFQ and RRS skin carotenoid concentrations based on previous studies in adults and children.[10,12,15–17] A sample of 50 was estimated to detect a correlation of at least 0.35 with approximately 80% power (alpha = 0.05, two-tailed). To allow for incomplete data, we recruited 79 participants.

2.4 Dietary assessment

Bilingual, trained research assistants collected three 24HDR measurements from study participants. The 24HDR was parent-reported (in-person and via phone call) using the multiple-pass diet recall method for the diet of children for two weekdays and one weekend day were collected within one month.[1] The first diet recall was in-person and subsequent recalls were conducted over the phone. Multiple-pass diet recall methodology is deemed to be an appropriate approach to collecting accurate dietary information from individuals by reducing potential recall bias. The first dietary recall was scheduled in-person at a local office. The same research assistants collected subsequent dietary recalls over the phone using a standardized protocol. Participants were given a $10 gift card for completion of all three dietary recalls. The Nutrition Data System for Research (NDSR) visual portion handbook (Version 2012) assisted the parents in estimating servings of all foods. The 24HDR intake data were analyzed using the NDSR software (version 2012, Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN).[18] The resulting dietary intakes were an average of all days collected. Using definitions set by the 2015-2020 Dietary Guidelines for Americans, a serving of fruit was 1/2 cup of cut fruit (e.g. a medium banana or apple) or 1/2 cup fruit juice, and a serving of vegetable was 1 cup of raw leafy vegetables, 1/2 cup of cooked or raw vegetables, or 1/2 cup of vegetable juice.[19] During the dietary recall, parents were asked if the child consumed any supplements or vitamins. In addition to servings of fruit and vegetables, the NDSR database also provided estimates for carotenoid intakes.

The researchers administered a 26 item FFQ with a 5-category Likert response scale (1 = never or almost never; 2 = 1 time or less a week; 3 = 2-3 times a week; 4 = 1 time or less a day; and 5 = more than 1 time a day) to ask parents about their children's intake of fruit and vegetables and other foods over the previous 30 days.[20] The FFQ was collected within a month after first dietary recall administered. The FFQ F/V consumption was calculated by creating a sum of 7 items (fresh fruit, canned fruit, vegetable soup, other cooked vegetables, salsa, lettuce or cabbage, and other vegetables) to produce a score (range: 7-35). FFQ fruit consisted of 2 items (fresh fruit and canned fruit) with range of 2-10. The FFQ Veg variable consisted of 5 items (vegetable soup, other cooked vegetables, salsa, lettuce or cabbage, and other vegetables) to with a range of 5 to 25. The FFQ instrument used in this study has demonstrated construct validity and internal reliability in this population.[20] In another study, children's F/V consumption, as assessed by this FFQ, was correlated with household inventory of F/V.[21]

The RRS skin carotenoid skin carotenoid levels of the children were measured during the first visit using the Pharmanex BioPhotonic Scanner (S2 Everest Edition, Pharmanex, Provo, UT, 2007). The scanner uses RRS to measure carotenoid levels at a point between the distal and proximal palm of the right hand. Skin carotenoid intensity scores in the palm area do not differ by skin tone and considered appropriate measurement location.[15] The scanner emits 473 nm beam of blue light that is then bent to 510 nm green light. Carotenoids are the only compounds known to bend a 473 nm light to 510 nm.[22] The RRS skin carotenoid measurement results in a continuous intensity score ranging from 0 to 70,000+ that indicates increasing carotenoids present in the skin with increasing intensity score. The palm area was exposed to the scanner for 90 seconds.[12,17] Two readings were collected. If the differences in measurements were greater than 5,000, a third measurement was collected. The final RRS skin carotenoid score was an average of the two closest measurements.

Trained researchers and staff collected anthropometric measurements of the children during home visits and at a school site using the NSFS study protocol from the Anthropometric Standardization Reference Manual.[23] Children were asked to remove their shoes and wear only light clothing. The child's height was measured in centimeters to the nearest 0.1 cm using a portable stadiometer (Model 213, Seca GmbH & Co. KG, Hamburg, Germany). The weight of each child was measured in kilograms to the closest 0.05 kg using a digital scale (Model 874, Seca GmbH & Co. KG, Hamburg, Germany). Child BMI z-scores were calculated using the CDC growth charts.[24] Detailed description of anthropometric methodology is described elsewhere.[14]

2.5 Statistical analysis

All data analyses were completed using SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA).[25] Data were visually inspected using scatterplots to examine linearity. T-tests and chi-square were used to examine differences in RRS skin carotenoid scores, FFQ F/V, and 24HDR F/V across subgroups (child age, child gender, BMI z-score). A Spearman correlation analysis among all study variables was performed to determine bivariate unadjusted relationships. Linear regressions were conducted with FFQ or 24HDR fruit and vegetable intake variables, controlling for child's age, gender, BMI z-score. The level of significance was set at $p < 0.05$ for all statistical analyses.

3. Results

Out of 79 recruited, the final sample included 51 children. Twenty-eight participants did not complete the study due to lack of interest or being unavailable for follow-up within the desired time period. Fifty-one participants completed at least one dietary recall. Of those who completed the first recall, 31 completed a second dietary recall, and 13 completed a third dietary recall. A skin carotenoid measurement and FFQ was collected from all 51 children who completed the study. Out of all dietary recalls collected, three were removed from the analysis due to caloric intake that was considered too low (below 800 kilocalories/day). Skin carotenoid measurements, FFQ, and dietary recalls were all completed within the same month. Summary characteristics of the sample are shown in Table 1. There were no statistically significant differences in demographic characteristics between those who completed the study and those who did not. There were no statistically significant differences in RRS skin carotenoid scores by gender (see Table 2). However, overweight and obese children had significantly lower RRS skin carotenoid intensity scores compared to normal weight children.

No significant differences in the consumption of recall reported fruits were observed by age, sex, and weight status for citrus juice, fruit juice, fruit (excluding citrus fruit), avocado, fried fruits, and fruit-based savory snacks (Data Not Shown). Although not significant, normal weight children had 0.18 servings of citrus juice compared to 0.04 servings in overweight children (p = .0619). Girls had 0.10 servings of starchy vegetables (not including potatoes) compared to 0.01 servings in boys (p = .0493). No other significant differences were observed among 24HDR vegetable intake by age, sex, or weight status.

Table 1. Baseline characteristics of participants in a study to determine if RRS skin carotenoids are useful among Mexican-origin children in rural California as a biomarker of F/V intake (n = 51)

Characteristics	Mean ± SD or %
Child age (years)	5.8 ± 1.5
Child sex (male)	56% (n = 29)
Child BMI-z score	0.93 ± 1.02
Maternal age (years)	35.7 ± 6.5
Maternal education (years), n = 50	8.9 ± 4.0
Maternal BMI (kg/m^2), n = 39	30.8 ± 4.5
Household income (US$/year), n = 46	19835 ± 1556

Table 2. Bivariate comparison of resonance Raman spectroscopy (RRS) skin carotenoids; food frequency questionnaire fruit and vegetable intake (FFQ F/V); and 24-hour dietary recall fruit and vegetable intake (24HDR F/V) in a study to determine if RRS skin carotenoids are useful among Mexican-origin children in rural California as a biomarker of F/V intake (n = 51)

Characteristic	N (%)	Mean RRS† Score (SD)	Mean FFQ F/V‡ (SD)	Mean 24HDR F/V servings (SD)	Mean 24HDR Daily caloric intake
Ages (years)					
3 to 5	16 (30.0%)	26,817 (9,021)	17.75 (3.7)	5.8 (4.6)	1,432 (507)
6 to 8	35 (70.0%)	29,010 (12,968)	16.9 (3.6)	4.2 (3.1)	1,369 (433)
Sex					
Male	29 (56.8%)	26,336 (9,832)	16.9 (3.9)	4.8 (4.1)	1,441 (545)
Female	22 (43.2%)	31,760 (13,834)	17.4 (2.7)	4.6 (2.9)	1,285 (233)
Weight Status					
Normal (<85%ile)	19 (44.1%)	32,522 (15,829)	18.2 (3.7)	4.6 (3.3)	1,192 (265)
Overweight/obese (≥ 85%ile)	24 (55.9%)	25,721 (7,021)*	16.1 (3.2)*	5.1 (4.3)	1,536 (535)***

*p < .10; ** p < .05; *** p < .01;

†Carotenoid intensity score (RRS): a measure ranging from 0-70,000+ with increasing score indicating higher carotenoid concentrations present in skin.

‡FFQ F/V consumption was calculated by creating a sum of 7 items (fresh fruit, canned fruit, vegetable soup, other cooked vegetables, salsa, lettuce or cabbage, and other vegetables) to produce a score (range: 7-35).

Skin carotenoid comparison to FFQ and 24HDR F/V consumption

The RRS carotenoid intensity scores were not correlated with FFQ total fruit and vegetable; FFQ fruit, and FFQ vegetable intakes. The 24HDR vegetable intake (r = .414, p = .003), but not total F/V (r = .188, p = NS) or fruit intake (r = .104, p = NS), was significantly correlated with RRS skin carotenoid intensity scores. Total carotenoid intakes, as reported by the 24HDR, were not correlated with RRS skin carotenoid scores (r = .251, p = .079). When controlling for child's age, gender, and BMI z-score, 24HDR F/V, 24HDR Vegetable, total 24HDR carotenoid intake, 24HDR lycopene, and 24HDR lutein + zeaxanthin were found to be significant (see Table 3).

4. Discussion

The results of this study suggest that noninvasive skin carotenoids can be a viable measure of vegetable consumption in Mexican-origin children in a rural community setting. Specifically, RRS skin carotenoid intensity scores may be more indicative of vegetable intake than fruit intakes in this population. Another study in children (n = 50) found that reported vegetable intake more strongly correlated (r = 0.32, p = .0001) with RRS skin carotenoid than fruit intake (r = 0.21, p = .02).[12]

Table 3. Linear regression of resonance Raman spectroscopy skin carotenoid intensity score (RRS) with fruit and vegetable (F/V) intake from food frequency questionnaire (FFQ) and 24-hour dietary recall (24HDR) controlling for child's age, gender, BMI z-score in a study to determine if RRS skin carotenoids are useful among Mexican-origin children in rural California as a biomarker of F/V intake (n = 51)

	RRS R^2	RRS ß (SE)	p-value
Frequency of consumption			
FFQ F/V†	0.27	754 (532)	.165
FFQ Fruit‡	0.30	3136 (1770)	.085
FFQ Vegetable#	0.25	713 (656)	.285
Servings			
24HDR F/V	0.30	941 (429)	.034
24HDR Fruit	0.24	812 (639)	.212
24HDR Vegetable	0.32	2135 (831)	.014
Total amount, mcg			
24HDR total carotenoids	0.32	0.48 (0.18)	.014
24HDR β carotene	0.24	0.69 (0.58)	.230
24HDR α carotene	0.22	1.12 (1.55)	.450
24HDR lycopene	0.31	0.57 (0.24)	.023
24HDR cryptoxanthin	0.25	11.1 (7.5)	.152
24HDR lutein + zeaxanthin	0.32	10.1 (3.9)	.015

†FFQ F/V = sum of responses (range: 7-30) for how frequently child consumes fresh fruit, canned fruit, vegetable soup, other cooked vegetables (not including potatoes), salsa, lettuce or cabbage, and other raw vegetables.
‡FFQ Food = sum of responses (range: 0-5) for how frequently child consumes fresh fruit or canned fruit.
#FFQ Vegetable = sum of responses (range: 5-25) for how frequently child consumes vegetable soup, other cooked vegetables (not including potatoes), salsa, lettuce or cabbage, and other raw vegetables.

A possible explanation for the weak correlations between the RRS skin carotenoid scores and FFQ F/V index is due to the types of fruits and vegetables consumed by these children. The subgroups of fruit and vegetable in this sample that contributed the most to total intake were non-citrus fruits (including bananas and apples) and other vegetables (including vegetables in salads, soups, stews, stir-fry, and similar mixed dishes), which possess fewer concentrations of carotenoids. These children consumed relatively low amounts of dark-green vegetables, deep-yellow vegetables, and tomatoes, which are carotenoid-rich sources. Household food purchase data obtained using scanners at the point of purchase also provide evidence of this: the top ten fruits and vegetables purchased in this population were (in descending order): bananas, apples, tomatoes, carrots, avocados, jalapeno peppers, cucumbers, iceberg lettuces, cilantro, and strawberries (Blinded for Review: unpublished data, September 2015). This is similar to national data of most commonly purchased and consumed fruits and vegetables, which are apples, bananas, iceberg lettuce, onions, citrus (fruit and juices), and tomato products.[26, 27] Except tomatoes and carrots, fruits and vegetables most commonly purchased do not contain high concentrations of carotenoids.[5] If children were consuming more fruits than vegetables, this would increase the total fruit and vegetable intake but would not necessarily result in higher skin carotenoid scores. This observation may explain why there was no correlation between fruit intakes (by either the FFQ or 24HDR) with skin carotenoid intensity scores. Another reason that may explain the differences observed between 24HDR and FFQ is the items on each instrument. The FFQ used in this study is limited to only one fruit (all fresh fruit) item and seven vegetable items while the 24HDR does not have this same limitation. The FFQ may have been too limited to capture the variety of fruits and vegetables consumed by the population. Also, this FFQ only is designed to measure the frequency of consumption, whereas the 24HDR yields an estimate of amounts consumed as well.

There were challenges in collecting three 24-hour dietary recalls from participants in this population. Participant availability and dispersion across small, rural communities hindered obtaining all dietary recalls required to do standard analysis using this method. This posed an issue with the dietary recall, which requires multiple contact points, whereas the RRS skin carotenoid intensity scores and an FFQ measurements can be completed at one contact point with no loss of participation. Furthermore, the skin carotenoid intensity scores can be easily captured in a centralized location such as a school campus or community center and do not require the presence of the parent/guardian, as FFQ or dietary recalls do. Given these challenges, using RRS skin cartenoid intensity scores to measure vegetable intake in this population is may be a viable option. Although there is an initial lease or purchase of the appropriate equipment for RRS skin carotenoid measurements, they do not require further software, specific technical training, or data analysis processing costs as FFQ or 24-hour dietary recalls.

This study has several strengths and limitations. The study population includes children of Mexican-origin from rural California, a difficult to reach population. The current study is one of the few studies to implement the use of skin carotenoid measurements in a rural Mexican immigrant community setting with young children. Two dietary assessment instruments (FFQ and 24HDR) were used to compare skin carotenoid intensity scores as a measure of fruit and vegetable intake. Conversely, the study did not compare skin carotenoids to serum or skin carotenoid levels, measures that would have been challenging to obtain in this community-based study setting. The FFQ in this study only used limited number of items to capture fruit and vegetable intake frequency and may have biased reporting. Since this study included a small sample size from a difficult to reach population, the results of this study may not be generalized to other Mexican-origin populations. Due to challenges in this hard-to-reach population, it would be important to over recruit due to a higher dropout rate. Lastly, three 24HDR were not obtained from all participants. The lack of three complete dietary recalls from all participants likely introduces a bias in the food group and nutrient estimates. Although two 24HDR are deemed appropriate for larger population studies such as the NHANES, three complete dietary recalls are best for comparisons of nutrition assessment tools and individual nutrient intake. Therefore, the findings from this study should be interpreted with these limitations. However, it is important to note the challenges in obtaining three 24-hour dietary recalls from populations similar to the one in this study and the value of RRS skin carotenoid measurements provides when fruit and vegetable intake is the variable of interest.

Implications

RRS skin carotenoid score may be a useful biomarker for vegetable intake, but not total fruit and vegetable intake, in young Mexican-origin children in a community setting. There is potential to use RRS skin carotenoid scores as a quick and easy measure for interventions aiming to increase vegetable intake in young children. Future studies should further investigate how carotenoid levels change throughout the year to determine the effects of seasonality on dietary patterns in this population as well as how to effectively translate these findings to enhance fruit and vegetable consumption in these high risk communities. A cost-analysis of RRS skin carotenoid intensity scores versus other methods would also be beneficial in future studies.

CONFLICTS OF INTEREST DISCLOSURE

The authors declare that they have no competing interests.

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