Using Skin Carotenoids to Assess Dietary Changes in Students After One Academic Year of Participating in the Shaping Healthy Choices Program

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Objective: to determine whether 4th-grade students participating in the Shaping Healthy Choices Program (SHCP), a school-based nutrition intervention, change vegetable and carotenoid intake measured by skin carotenoids and dietary intake

Design: single group pre-test, post-test with a self-selected, convenience sample of students recruited at the school

Participants: 30 students had both dietary intake data and skin carotenoid measurements pre- and post-intervention.

Intervention(s): included garden-enhanced education, family and community partnerships, increased regionally procured produce in the lunchroom, and school-site wellness committees. Lasted one academic-year (nine months)

Main Outcome Measure(s): dietary intake of vegetables and carotenoids as measured by Block food frequency questionnaire and skin carotenoids as measured by resonance Raman spectroscopy.

Analysis: paired t-test, ANOVA and Pearson's correlation were used. Statistical significance set at $\alpha=0.05$

Results: Reported vegetable intake decreased by 0.4cups ($P=0.06$). Reported carotenoid intake decreased by 1.5mg ($P=0.05$) and skin carotenoids decreased by 2247.9 RRS intensity units ($P=0.04$). Change in reported intake correlated with change in skin carotenoids ($r=0.43$, $P=0.02$)

Conclusions and Implications: While the reported decrease in vegetable and carotenoid intake was unanticipated, the RRS measurements confirmed this. RRS data can help evaluate changes in fruit and vegetable intake.

Using Skin Carotenoids to Assess Dietary Changes in Students After One Academic Year of Participating in the Shaping Healthy Choices Program

INTRODUCTION

Most children in the U.S. are not meeting the Dietary Guidelines for Americans' recommendations for fruit and vegetable intake.¹ The Shaping Healthy Choices Program (SHCP) was developed based on the Social Cognitive Theory² and the Social Ecological Model³ to encourage the consumption and enjoyment of fruits and vegetables. The SHCP is a multi-component, school-based nutrition intervention that aims to improve dietary behaviors and prevent childhood obesity by improving students' individual factors, as well as factors in the home and school environments, while simultaneously creating a community-based support system.⁴ In this study, changes in dietary intake of fruit and vegetable intake were evaluated using two methods, reported dietary intake and skin carotenoids.

While numerous intervention studies aimed at increasing fruit and vegetable intakes in children have been conducted, the assessment of changes in dietary behavior in children remains a challenge.⁵⁻⁷ Reported dietary intake is often used in community-based interventions, however it can be unreliable, and it is time and labor intensive.⁸⁻¹¹ Skin carotenoid status measured by resonance Raman spectroscopy (RRS) can be used as a biomarker to assess fruit and vegetable intake because carotenoids are predominately found in fruits and vegetables and their presence in the body can reflect cumulative dietary intakes of these foods.¹²⁻¹⁵ The RRS method is a quick, noninvasive, and non-biased method to assess skin carotenoids and has previously been validated in children.¹⁵ Skin carotenoid status using RRS has also been reported to change in response to dietary intake, making it possible to use this methodology to detect changes in dietary behavior.^{16,17} These reports, however, have largely been a result of feeding studies where there

are substantial changes in carotenoid intake. Few studies to date have used RRS to measure changes in usual dietary intake in children. Additionally, no studies have used this RRS instrument to measure skin carotenoid changes in response to a multicomponent, school-based nutrition program.

The objective of the current study was to determine whether students participating in the SHCP changed their consumption of fruits, vegetables, and carotenoids measured by Food Frequency Questionnaire (FFQ) and an RRS instrument that was developed for research purposes (a research-grade RRS). A secondary objective was to determine whether the RRS instrument could be useful to measure changes in fruit and vegetable intake after an intervention lasting one academic year. It was hypothesized that students participating in the SHCP for one academic year would increase consumption of vegetables and therefore have greater reported intake of vegetables and carotenoids and greater skin carotenoid concentrations. Additionally, it was hypothesized that an increased skin carotenoid intensity would be associated with a greater carotenoid intake.

MATERIALS AND METHODS

Study Design: This study was a sub-study of the larger SHCP study based on available dietary intake and skin carotenoid data. It was a single group pre-test, post-test design in which a self-selected, convenience sample of students was assessed pre- and post-intervention.

Participants (n=30): The students were recruited at the beginning of each academic year over two years from 4th-grade classrooms in a Northern California elementary school participating in the SHCP.⁴ The intervention was implemented twice, once over the course of the 2012-2013 academic year and repeated again in the 2013-2014 academic year. Exclusion criteria included living in a smoking household or having illnesses or chronic conditions known to change

carotenoid status.^{12,18} Under these criteria, no students were excluded from the study. The University of California, Davis Institutional Review Board approved the study protocol. Parents provided written informed consent and children provided oral assent.

Intervention: The SHCP intervention is described in detail elsewhere.⁴ Briefly, the SHCP aimed to 1) increase nutrition knowledge and use of science processing skills; 2) promote availability, consumption, and enjoyment of fruits and vegetables; 3) improve dietary patterns and encourage physical activity; 4) foster positive changes in the school environment; and 5) facilitate development of an infrastructure to sustain the program. Intervention activities included an inquiry-based, garden-enhanced nutrition curriculum, *Discovering Healthy Choices* (DHC), taught in the classroom;¹⁹ cooking demonstrations in the classroom using the *Cooking Up Healthy Choices* curriculum;²⁰ family newsletters sent home to parents; health fairs or health screenings at the school; improvements to the school lunchroom including installing a salad bar and increasing procurement of regional produce; and development of a school-site wellness committee. In the 2012-2013 school year (August-May), the nutrition curriculum was delivered by an SHCP educator. In the 2013-2014 school year (August-May), the intervention was repeated with a new cohort of 4th-grade students and the teachers delivered the DHC curriculum after participating in a comprehensive professional development program. Because the overall results regarding dietary intake across both years were consistent, the students' data from both years were combined.

Data Collection and Analysis: Pre-intervention data collection occurred before the intervention activities began in August and post-intervention data collection occurred immediately after the intervention's completion in May. Measurements included:

Anthropometrics: height and weight were measured at the school site.⁴ The methods followed guidelines published by the Centers for Disease Control and Prevention (CDC). Height was measured using a transportable stadiometer (Seca 213, Chino, California) and body weight was measured using an electronic scale (Seca 803b, Chino, California). Body Mass Index (kg/m^2) was calculated and age and sex-specific BMI percentiles were derived. Classifications for adiposity were based on those determined by the CDC and the Expert Committee on Childhood and Adolescent Overweight and Obesity using the 2000 CDC growth charts.²¹⁻²³ Under these classifications, overweight was defined as at or above the 85th percentile and obese was defined as at or above the 95th percentile of the sex-specific BMI-for-age growth chart.²³

Demographics: race and ethnicity of the student were reported by the parents using a demographic questionnaire that was sent home.⁴

Dietary intake: dietary intake was self-reported by students with the help of their parents at home using the 2004 Block Food Frequency Questionnaire (FFQ) for Ages 8-17. Students were provided detailed classroom instruction on completing the FFQ and were given standardized bowls and a plate consistent with the diagram provided with the FFQ to use as a reference.⁴ Questionnaires were returned to the teachers for researchers to collect. Completed FFQs were analyzed by NutritionQuest (Berkeley, CA). In the analysis report, α -carotene, β -carotene, β -cryptoxanthin, lutein, and lycopene were quantified. Total dietary carotenoids were calculated by taking the sum of the individual carotenoids reported by the FFQ analysis. Total vegetables (minus white potatoes) was calculated by taking the difference between total vegetables and white potatoes reported by the FFQ analysis report.

Skin carotenoids: In the 2012-2013 school year, pre-intervention total skin carotenoids were assessed in November/December using RRS at a health fair event organized by SHCP

researchers at the school site. Student attendance at the health fair was optional but required for participation in the evaluation. In May 2013, a similar health fair event was hosted and students volunteered to return for post-intervention measurements. In the 2013-2014 school year, skin carotenoids were assessed in November/December using RRS and measurements were conducted during class time. Post-intervention skin carotenoid data was collected May 2014 during class time. The instrument and method used in this study were developed for research purposes by members of this group and have been validated in diverse populations and details are reported elsewhere.^{12,13,15,24-26} Due to the overlap on the absorption spectra for all carotenoids, individual carotenoid intensity is unavailable using this instrument.

Statistical Methods: Inclusion in this sub-study of students was based on available data and those without post-intervention data were excluded. Means and standard deviations were calculated for continuous variables and distributions were examined for normality. All variables followed a nearly normal distribution and were not transformed. Percentages were calculated for categorical variables. Change was measured by taking the difference between pre-and post-intervention measurements. Paired t-tests were used to determine differences in skin carotenoid intensity, BMI percentile, and dietary consumption of fruit, vegetables, total carotenoids, and energy between pre-and post-intervention time points. Student's t-tests were also used to determine whether there were differences between those who had only pre-intervention data and both pre/post-intervention data. Variables considered for this analysis were race/ethnicity, gender, age, RRS intensity, and reported dietary intake of vegetables and carotenoids. Pearson's correlation coefficients were used to determine the relationship between change in skin carotenoid intensity and change in reported dietary intake of carotenoids, change in reported energy intake, and change in BMI percentile. Because students who typically consume low

amounts of fruits and vegetables may show a different pattern of change following an intervention,²⁷ a post-hoc sub-analysis of the larger SHCP study was conducted using paired t-tests to evaluate differences between pre-and post-intervention in students who reported consuming less than one serving of vegetables/day pre-intervention. Stata 13 (StataCorp, College Station, Texas) was used for all statistical analyses. Statistical significance was determined using an $\alpha=0.05$.

RESULTS

Of the 249 students enrolled in the larger SHCP study who were eligible to participate in this study over the two years, 30 students (12%) who had both RRS and FFQ data (FFQ+RRS group, n=30) pre-and-post intervention were self-selected for this sub-study. Baseline characteristics of the participants are presented in Table 1. In this group, skin carotenoid measurements decreased by 2247.9 Intensity Units (IU) post-intervention ($P=0.04$, Table 2) and dietary intake of carotenoids significantly decreased by 1.5 mg post-intervention ($P=0.05$, Table 2). There was no significant difference regarding reported total fruit or vegetable consumption (Table 2).

Dietary intake of carotenoids correlated with skin carotenoids pre-intervention ($r=0.46$, $P=0.001$, data not shown) and post-intervention ($r=0.52$, $P=0.001$, data not shown). The change in dietary carotenoids correlated with the change in skin carotenoid intensity ($r=0.43$, $P=0.02$, Table 3). There was no correlation between the change in skin carotenoids with the change in reported energy intake and change in BMI percentile (Table 3).

In post-hoc analyses of students in the larger SHCP study with pre-and post-intervention FFQ data, there were 39 out of 93 students (42%) who reported consuming less than one serving

of vegetables per day at baseline. After participation in the intervention, these students reported a significant increase of 0.4 cups of vegetables ($P=0.01$, Table 2) and no change in fruit intake.

DISCUSSION

The decrease in reported dietary intake of vegetables and skin carotenoids was unanticipated given that the SHCP intervention aimed to increase consumption of fruits and vegetables. No change in fruit intake was anticipated as youth this age consume near to adequate amounts of fruit.²⁹ Other multi-component and school-based interventions that aim to increase fruit and vegetable intake have also reported little or no change in vegetable intake.²⁸⁻³⁰ One explanation for the decrease in skin carotenoids in this study may be the differences in offering of fruits and vegetables in the school at the beginning of the intervention (November/December) compared to the end (May). Vegetables offered in the lunchroom pre-intervention included autumn vegetables, such as sweet potatoes, squash, and butternut squash, which are more carotenoid-rich, compared to vegetables offered in the spring, such as celery, cucumbers, and snap peas. Lower skin carotenoids were also observed in studies that measured skin carotenoids over time.³¹ There was no correlation between the change in carotenoid intake and change in energy intake, so it is unlikely that the decrease in reported carotenoid intake is related to a decrease in total overall energy intake. Studies have indicated that there is an inverse relationship between skin carotenoids and BMI.^{12,13,15} However, there was no relationship between change in skin carotenoids and change in BMI percentile in this study population, so any changes in BMI do not appear to be influencing change in skin carotenoids.

The decrease in reported dietary intake of carotenoids was confirmed by the skin carotenoid measurements, indicating that the RRS instrument was able to detect this small decrease. This suggests that this subset of participants was consuming fewer carotenoid-rich

fruits and vegetables following the intervention. Regardless of the reason for a decreased reported carotenoid intake, the change in reported carotenoid intake correlated with the change in skin carotenoids. Thus, the RRS instrument was able to distinguish a change in skin carotenoids reflected by a small change (1.5mg) in reported intake. These results confirm that this method is an objective way to measure the carotenoid status of children and can be used to help evaluate changes in fruit and vegetable intake.

While there was an overall decrease in reported vegetable consumption, this was not the case for students who reported consuming less than one serving of vegetables per day pre-intervention. In post-hoc analyses of the original SHCP study, these students significantly increased consumption of vegetables post-intervention. This phenomenon of low consumers consuming more vegetables post-intervention compared to high consumers has also been observed in other studies that aim to increase intake of fruits and vegetables.^{27,32} This is noteworthy as this suggests that those students who are consuming very little vegetables at baseline are likely to benefit most from the intervention, therefore it is this group of students that should be targeted by interventions that aim to improve dietary behavior.

In this study, vegetable intake was examined because the SHCP intervention focused on consuming more nutrient dense vegetables with less focus on fruit since children are already close to meeting the dietary recommendations for fruit.³³ Vegetable intakes were examined with and without white potatoes, because while they are the most highly consumed vegetable, most Americans consume white potatoes in a form that adds calories (such as French fries and potato chips) while being less nutrient dense, with less carotenoid concentration, compared to other vegetables.³⁴ This was true of this study population; white potatoes were consumed mostly as French fries and tater tots (data not shown). There was a slight difference in total vegetable

intake calculated with and without white potatoes, though this difference was not statistically significant. However, by examining vegetable intake with and without white potatoes, it can be determined whether increases or decreases in intake in total vegetables are due to changes in white potato consumption.

A strength of this study is that it used an objective, validated, research-grade evaluation tool to measure changes in skin carotenoids in children participating in a school-based, nutrition intervention. The instrument used in this study was extensively validated in controlled feeding studies and over extended periods of time against plasma and skin biopsies. Previous studies in children have used commercialized RRS technologies to measure changes in skin carotenoids resulting from provision of high-carotenoid foods or incentivizing children to consume high-carotenoid foods.^{17,27} Though the commercialized instrument is based on the research-grade instrument, there is no published data regarding the similarities or differences between the two instruments. While most studies have shown that RRS can measure increases in fruit and vegetable consumption, results from this study demonstrate that the tool can also reflect decreases in consumption, critical as a useful tool must be able to detect any change. This study shows that even small changes in dietary intake resulting from behavior change within an academic school year can be measured. This suggests that this tool can be used reliably to evaluate other school-based nutrition interventions that aim to change fruit and vegetable intake behavior. While the hypothesized outcome of increased vegetable intake was not achieved through evaluating mean intakes in this study population, it is still important to share these results. Too often, negative or unexpected outcomes are not published, yet these results are still important in order to maintain an unbiased body of scientific knowledge.³⁵ Additionally, many of

these studies may potentially yield interesting results. It is therefore important to evaluate the variation in the data, which this current study has done.

One limitation of this study is the lack of sun exposure data, which has been reported to affect skin carotenoid status.^{17,18,31} It is suggested that carotenoids have photoprotective effects and can be oxidized by sunlight, therefore increased sun exposure can diminish skin carotenoid status.¹² It is possible that participants in this study spent more time in the sun at follow-up given the warmer weather of the spring months in Northern California, and this could help explain a decrease in skin carotenoids. Additionally, skin carotenoids were measured at two different times of the year, when fruit and vegetable availability and intake may be different. The FFQ used in this study is not specifically designed to measure carotenoid intake and is restricted by a fixed food list and portion size options, which may affect the reporting of carotenoid intake. Regardless of this, reported dietary carotenoids correlated with skin carotenoid measurements, suggesting that the FFQ assessed carotenoid intake with some accuracy. Despite the small sample size, there was enough power for the instrument to detect changes, suggesting that this tool is sensitive enough to detect small changes in carotenoid intake.

IMPLICATIONS FOR RESEARCH AND PRACTICE

In regards to the changes in dietary intake in the larger SHCP intervention, more research is required to elucidate reasons for the decrease in reported vegetable intake. However, in post-hoc analysis of the SHCP, looking at those who consumed less than one serving of vegetables per day, a reported increase in vegetable intake was observed. This implies that the SHCP is a promising intervention that may have a greater benefit to those who are furthest from meeting dietary recommendations of vegetables. This can provide further insight into planning future nutrition education studies and help studies target the most vulnerable populations. This also

emphasizes the need to not just simply focus on means, but to separate the data in a meaningful way that might yield results that may otherwise be overlooked.

For the students in the RRS+FFQ group, it is evident that the decrease in reported carotenoid intake was reflected by the decrease in skin carotenoid intensity, implying that small changes in dietary intake of carotenoids is related to a measurable change in skin carotenoids. Biomarker data can help strengthen evaluations of future nutrition intervention studies and educational programs aimed at increasing fruit and vegetable intakes in children. RRS is a quick method that requires little effort on behalf of the participants and can be used to provide an objective measure to help evaluate changes in dietary intake. Future studies using RRS should consider sun exposure, timing of measurements, and larger sample sizes.

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336 **Table 1:** Participant Characteristics in the RRS+FFQ group (n=30) at Baseline.

	RRS+FFQ group
Average Age $\pm$ SD (years)	9.9 $\pm$ 0.6
Sex, n (%)	
Females	21 (70%)
Males	9 (30%)
Mean BMI percentile $\pm$ SD	74.5 $\pm$ 25.3
BMI-for-age Percentile	
Normal weight (5 th - <85 th percentile)	13
Overweight (85 th - <95 th percentile)	12
Obese ($\geq$ 95 th percentile)	5
Race/Ethnicity, n (%)	
African American/Black, not of Hispanic origin	3 (10%)
Asian/Pacific Islander	11 (37%)
Caucasian/White, not Hispanic origin	8 (27%)
Latino/Hispanic (Mexican-American, Puerto Rican, Cuban)	1 (3%)
Other	
Multiple reported	3 (10%)
Not reported	1 (3%)
	3 (10%)

Table 2: Comparison of Pre-and Post-Intervention Means $\pm$ SD of Skin Carotenoid Intensity, Reported Dietary Intakes, and BMI Percentile for the RRS+FFQ Group (n=30) and comparison of Pre-and Post-Intervention Means $\pm$ SD of Reported Dietary Intakes and BMI Percentile of Participants Who Reported Less Than One Serving of Vegetables Per Day Pre-intervention (n=39).

	Pre-Intervention	Post-Intervention	Mean difference	P-Value
RRS+FFQ group (n=30)				
	← Mean $\pm$ SD →			
Skin carotenoids , intensity units	32580.4 $\pm$ 11629.1	30332.4 $\pm$ 10329.7	-2247.9 $\pm$ 5671.3	0.04*
Dietary carotenoids , mg	7.7 $\pm$ 5.0	6.2 $\pm$ 4.1	-1.5 $\pm$ 4.0	0.05*
Vegetables , cups	1.2 $\pm$ 1.1	0.9 $\pm$ 0.5	0.4 $\pm$ 1.0	0.06
Vegetables (excluding white potatoes) , cups	1.1 $\pm$ 0.9	0.8 $\pm$ 0.1	0.3 $\pm$ 0.9	0.12
Leafy green vegetables , cups	0.3 $\pm$ 0.4	0.3 $\pm$ 0.3	0.1 $\pm$ 0.4	0.38
Orange vegetables , cups	0.1 $\pm$ 0.2	0.1 $\pm$ 0.1	0.1 $\pm$ 0.2	0.17
Fruit , cups	1.5 $\pm$ 1.0	1.8 $\pm$ 1.3	0.2 $\pm$ 1.5	0.43
Total energy , calories	1437.3 $\pm$ 888.2	1256.9 $\pm$ 545.6	-180.3 $\pm$ 831.4	0.25
BMI percentile	74.5 $\pm$ 25.3	71.3 $\pm$ 25.7	-1.8 $\pm$ 10.1	0.10
<1 serving vegetables/day (n=39)				
	← Mean $\pm$ SD →			
Dietary carotenoids , mg	4.3 $\pm$ 21.7	6.1 $\pm$ 4.1	1.9 $\pm$ 1.0	0.06
Vegetables , cups	0.5 $\pm$ 0.2	0.9 $\pm$ 0.8	0.4 $\pm$ 0.9	0.01*
Vegetables (excl. white potatoes) , cups	0.4 $\pm$ 0.2	0.8 $\pm$ 0.5	0.4 $\pm$ 0.8	<0.01*
Leafy green vegetables , cups	0.1 $\pm$ 0.1	0.2 $\pm$ 0.1	0.1 $\pm$ 0.3	0.01*
Orange vegetables , cups	0.0 $\pm$ 0.0	0.1 $\pm$ 0.2	0.1 $\pm$ 0.2	0.01*
Fruit , cups	1.4 $\pm$ 1.0	1.7 $\pm$ 1.2	0.3 $\pm$ 1.6	0.20
Total energy , calories	1072.0 $\pm$ 503.5	1362.6 $\pm$ 778.0	290.6 $\pm$ 892.5	0.05*
BMI percentile	74.7 $\pm$ 26.1	63.2 $\pm$ 30.3	-11.5 $\pm$ 20.0	0.002*

FFQ = Food Frequency Questionnaire

RRS = Resonance Raman Spectroscopy

*Statistically significant using an $\alpha=0.05$

Table 3: Pearson's Correlation Between Change in Skin Carotenoid Intensity and Change in Reported Intake of Carotenoids, Change in Total Energy Intake, and Change in BMI Percentile in the RRS+FFQ Group (n=30).

Change in Skin Carotenoid Intensity vs:	Correlation coefficient r	P-Value
Change in Reported Intake of Carotenoids	0.43	0.02*
Change in Total Energy Intake	0.28	0.13
Change in BMI Percentile	0.03	0.87

FFQ = Food Frequency Questionnaire

RRS = Resonance Raman Spectroscopy

*Statistically significant using an $\alpha=0.05$