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Skin Carotenoid Status Over Time and Differences by Age and Sex Among Head Start Children (3-5 years) Living in Eastern North Carolina

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Abstract

Objective: Examine differences in skin carotenoid status (SCS) based on time, age, and sex of preschool-aged children (PSAC) enrolled in Head Start (HS) in North Carolina (NC).

Design: Data were collected using surveys from participating families. PSAC's SCS were measured 3 times over a 6-month period.

Setting: 3 HS centers in NC

Participants: 112 children aged 3-5 years old, enrolled in HS

Main Outcome Measure(s): Differences in SCS assessed using the Veggie Meter® based on time, sex and age

Analysis: One-way Analysis of Variance (ANOVA) to assess SCS at Time 1 between sex and age (n=112). Repeated measures ANOVA with a Greenhouse-Geisser correction to assess SCS over time (n=45) using Bonferroni correction(*b*).

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Results: On average, children were 4 years old, African American (81.3%), male (57%) and mean SCS 266 (SD 82.9). SCS were significantly different over time (*p*<.001). Significant differences were observed between ages (*p=.01)* and sex (*p=.01).*

Conclusions and Implications: The Veggie Meter® is a promising tool to assess fruit and vegetable intake but needs to be validated in PSAC as has in adults. Sex and age are potential confounders which should be assessed in future studies using the Veggie Meter®.

INTRODUCTION

Low intake of fruits and vegetables (FV) among preschool-aged children (PSAC) (3-5-years old) is well documented.[1] On average, only 8.3% of PSAC are consuming the daily recommended servings of FV,[2] with lower intakes reported among minority children[3,4] and children in low socioeconomic status (SES) groups.[5] Considering that eating behaviors developed in early childhood often persist into adulthood,[6] low FV intake among PSAC is concerning.[2,6] Unfortunately, few objective assessments are available to measure intake among young children. Instead, dietary behaviors are often assessed using subjective methods that lead to bias and error.[3] For these reasons, valid and objective methods are particularly needed to assess FV intake in this population.

When assessing diet among PSAC, many subjective methods lack accuracy[7] and generally rely on parent report as a proxy for child reports resulting in potentially flawed and/or biased data.[1] Since young children are not developmentally ready to respond to recalls and frequencies, some researchers have created valid pictorial tools that measure children's FV preference.[8,9,10] The foundation for these tools cites research demonstrating that children's measured preferences have the greatest impact of their eventual intake of FV.[5,11] However, while measures of preference are promising and have been associated with FV intake,[11,12,13] these tools are still subjective in nature as they do not directly measure intake.

Blood plasma carotenoid concentration is currently the gold standard for assessing FV intake as a biomarker.[14] Although blood plasma carotenoid concentration is the best available biomarker for carotenoid levels, blood plasma is not easily collected and assessed outside of a clinical setting and is an invasive assessment method.[14] Skin carotenoid status (SCS) is assessed using non-invasive methods such as resonance Raman Spectroscopy,[15] and pressure-mediated reflection spectroscopy are emerging as valid and reliable tools to assess intake. The Veggie Meter® (VM®) (Longevity Link, Salt Lake City, Utah) is a portable device that assesses skin carotenoids via pressure-mediated reflection spectroscopy.[16] It has shown validity and ease of use in community-based settings,[17,18,19] including PSAC.[19] The VM® is useful for measuring SCS over 4-8 weeks, and is reflective of intake of foods high in carotenoids such as carrots, squash, tomatoes, and green leafy vegetables, but does not effectively measure intake of FV low in carotenoids such as apples, bananas, pears, or potatoes.[20] While the VM® represents a promising tool for objectively measuring FV among young children,[17,19] limited research exists to describe its use in this population. More research is needed to understand SCS as measured by the VM®, particularly among low-resource, minority PSAC who are at increased risk for low FV intake.[21] The current study aims to fill this gap in research by establishing SCS among low-resource, PSAC enrolled in Head Start (HS) centers, federally funded preschools in which the Child and Adult Care Food Program (CACFP) dietary regulations are followed, in North Carolina (NC) over a 6-month period. This study sought to answer 3 research questions: #**1**: Are there significant differences in PSAC's SCS over time in regard to seasonality and access to FV? #**2**: Are there significant differences in SCS by sex? #**3**: Are there significant differences in SCS by age (3-, 4-, 5-years of age)? Researchers hypothesized SCS would change significantly over time since young children do not have full control over the foods that are available in their home and school environments,[22] SCS may vary naturally over the course of a year based on availability of FV in terms of seasonality. Researchers also hypothesized that SCS would have significant differences being observed by sex and age as previous studies have shown that SCS varied among sex and age.[17,18,19]

METHODS

Research Design

This study examined baseline SCS and changes in SCS among PSAC in a low-resource population between 3 timepoints over a 6-month period and differences with sex and age. The East Carolina University Institutional Review Board approved the study protocol involving human subjects (UMCIRB 18-001535). All subjects provided informed consent prior to participation.

Study Population and Eligibility

The primary participants for this study were low-resource PSAC enrolled in HS. The sampling frame included approximately 250 children from 3 HS centers in NC. Participation required that a child be 3-5 years old, enrolled in a participating HS center, and have written consent from their parent/guardian. Data were collected from children only if a child readily gave assent by agreeing verbally and physically to participate in the research process, regardless of parent consent. For this reason, children were excluded if they had identified disabilities and/or did not speak English.

Data Collection

Researchers collected data from parents and children October 2018-February 2019. Parents and children were informed of the study and recruited from 3 HS centers in NC during school registration in the summer, during the first parent meetings for each center, and through flyers sent home over the first weeks of the school year. Parents were then asked to provide consent for their child to participate and complete a survey. Interested parents were asked to return the completed packets to the researcher, their child's teacher, or to the HS director. Parents who completed and returned surveys were eligible to enter a drawing for a $100 gift card (1 per center).

Data collection timepoints along with type of data collection are illustrated in Table 1. Demographic information was collected from all 3 sites at Time 1. Height, weight, and SCS were measured at 3 times throughout the school year with 2-month separations between, as previous studies have indicated SCS reflect FV intake over the prior 4-8 weeks.[16–19] According to school menus followed by all three participating centers, during the course of the study broccoli was served 8 times, sweet potato 3 times, tomato 3 times, and carrots 9 times.[23] The use of multiple timepoints allowed researchers to establish changes in SCS between summer, fall, and winter.

Trained research assistants (RAs) assessed children, for whom parental consent was obtained, one at a time. RAs retrieved each consenting child from their respective classroom and led them to a pre-established private testing area free of distraction. To build rapport, the child was engaged in polite conversation unrelated to the pending assessment. Once the child was comfortable, the RAs began the data collection process. During a single assessment, children's' SCS, height, and weight were measured. Children received a sticker of their choosing after each session or if the child attempted to complete any of the proposed assessments (i.e. come with the RA to the designated data collection site).

Instrumentation

Instrumentation protocols were adapted and revised prior to the current study during a pilot study in summer 2018 during the HS open enrollment period. In the pilot study, approximately 500 children were assessed. From the pilot study, instrumentation protocols were revised to meet the needs of the PSAC. For example, due to children's limited attention span, the protocol was revised to use the multiple measurement mode in the VM® instead of completing 3 individual skin carotenoid measurements and averaging the 3 scores.

Parent-Reported Child Demographics: Parents were asked to report demographic information for their child including age, sex, and history of food allergies, as well as reporting similar demographic information about themselves through a form that was sent home. Parents were asked to complete this survey at Time 1 (Table 1).

Veggie Meter (VM)®: SCS were measured as an indicator of children's FV intake. The VM® uses reflection spectroscopy and is an objective, non-invasive measure of the level of carotenoid pigments in a person's skin.[19] For measurement, the child's finger was sanitized with a sanitizing wipe, then inserted into the instrument's finger cradle by a) bringing the pad of the fingertip in close contact with the light delivering and collecting contact lens, and b) by gently applying pressure to the finger such that the blood was temporarily squeezed out of the measured skin region.[19] The fingertip is an ideal region to be used for these measurements as there is an insignificant effect of melanin on SCS due to the limited pigment.[19] A laptop computer interfaced to the instrument analyzed the light that was reflected from the finger, and subsequently a carotenoid score was derived on a spectral range from 350-850 nanometers.[24] In multiple measurement mode, the finger was inserted and retracted 3 times and an average score was determined for the 3 measurements. The entire VM® assessment took approximately 45 seconds to complete.

Height/Weight: Height/weight were measured to assess BMI (Table 1). Protocols were adapted and revised from the 2007 NHANES Anthropometry Procedure Manual.[25]

Data Analysis

Data analysis were conducted using the Statistical Package for Social Sciences (SPSS) 24.0. Summary statistics are provided in frequency (proportion) or mean (SD) as appropriate. One-way ANOVA was used to assess SCS at Time 1 between sex and age (n=112). Levene's test was used to assess the equal variance assumption, residual QQ-plot was used to assess the normality assumption, and Welch's test was used to assess sensitivity to unequal variance. A multiple linear regression with sex and age as factors was used to examine adjusted effects. To examine changes in children's SCS over the 3 timepoints, a Repeated Measures ANOVA with a Greenhouse-Geisser correction was implemented using only children enrolled at Sites 2 and 3; as Site 1 was participating in a nutrition education intervention focused on improving FV consumption, and the current study focuses on seasonal trends only. Interaction terms between sex, age, and time were tested and removed if not statistically significant. Bonferroni correction[(b)] was used for multiple comparisons among 3 timepoints. All statistical differences are reported at $P<0.05$ level.

RESULTS

Of the 250 children eligible for the study, 112 HS children were enrolled in the study and were present for the first round of data collection at Time 1 for all 3 sites. From Sites 2 and 3, 45 children participated in Time 2 and Time 3 data collection. The majority of participants were African American (80.5%), male (57%), and 4-years old (60.7%) (Table 2). Participating children were fairly representative of all children enrolled across the participating Head Start program (79.5% African American, 52.7% 4-years old).[26] Among the 112 participants, parents reported 10 (8.9%) children had food allergies. Average BMI z-score was 0.73 (1.40) with 16% of children classified at overweight (85^{th}-95^{th} percentile) and 27% obese (95^{th} percentile and higher). No significant differences in BMI z-scores were observed between sites.

Average SCS (n=112) was 266.00 (SD 82.9) at Time 1. Mean SCS for Sites 2 and 3 (n=45) were 267.58 (SE 8.7) at Time 1, followed by 273.84 (SE 9.3) at Time 2, and 228.73 (SE 10.3) at Time 3. Addressing research question #1, significant differences were observed from Time 1 to Time 3 ($F(1.32, 58.22)=17.56$, p=.001[b], particularly, between Time 1 and Time 3 (p=0.001), and between Time 2 and Time 3 (p<.001[b]), however, no significant differences were found between Time 1 and Time 2 (p=.496[b]) (Figure 1. a.). No interaction between sex, age, and time was statistically significant so they were removed from the model. Addressing research questions 2 and 3, SCS and its effects between sex and age at Time 1 for all 3 sites are shown in Table 2. Significant differences were observed between sex with males having higher mean SCS (282 SE 12.84) than females (243 SE 9.39) (F(1,110 =6.34, p=.01) (Figure 1. b.). There was no evidence of unequal variance (Levene's test p = 0.86). There was no obvious violation of normality by normal QQ-plot, except a possible outlier with SCS > 600. Without the outlier, $p < .01$. Welch's test yielded a similar result. Finally, significant differences were observed between ages (3-, 4-, and 5-year-olds) with mean SCS 3- (241 SE 13.6), 4- (267 SE 8.3), and 5-year-olds (339 SE 43.5) ($F(2,109) = 5.77$, p=.01) (Figure 1. c.). There was some evidence of unequal variance (Levene's test p = 0.02) (likely due to the outlier). There was no obvious violation of normality by normal

QQ-plot, except a possible outliner with a SCS > 600. Without the outlier, p = .06 with Levene's test p = 0.64. Welch's test reported p = 0.08. The effect of age remained significant. The multiple linear regression showed similar results that SCS differed significantly between sex (p=.02) and age groups (p=.01). Without the outlier, however, the age groups became less significant (p=.16).

DISCUSSION

Valid, objective, and reliable methods are needed to assess dietary intake among PSAC. One promising non-invasive tool for objectively measuring FV consumption is the VM®, however, limited research exists to describe its use in PSAC.[27] The current study begins to address this gap by providing insight on SCS among low-resource PSAC over a 6-month period. The study also examined differences in SCS over time and between sex and age. Average SCS differed over time. Additionally, significant differences of mean SCS between sex and age groups were observed.

Average SCS measured in the current study were higher than previous reports of SCS in middle and high school students, and adults in a similar geographic location (219 [SD 68.1], 214 [SD 65.6], 229 [SD 71.7]) respectively.[17,18] However, this value is lower when compared to children of the same age group. Ermakov and colleagues reported a SCS of 380 for children 2-5 years of age living in San Francisco, California;[19] to the author's knowledge, this is the only study that has reported SCS among PSAC using the VM®.[27] In the current study, the geographic location was considered 25.4% rural,[28] and children were from families whose household income were below 125% of the poverty line.[29] Potentially explaining some of the variability between study reports, children enrolled in Ermakov[19] lived in an entirely urban area[28] and were from families of varying SES.

In the current study, differences between SCS measured at 3 timepoints over a period of 6-months were observed. SCS are directly influenced by intake of FV high in carotenoids.[19] SCS may have been significantly different from Time 2 to Time 3, but not from Time 1 to Time 2 due to Time 3 being reflective of the children's diet over winter break. To the author's knowledge, no studies have looked at SCS over time outside of an intervention focused on improving FV consumption.

Access in home and ECE may further by impacted by FV seasonality.[30] In theory, availability of FV increases throughout the summer months, with SCS being representative of dietary intakes 4-8 weeks,[16] it was hypothesized to observe higher SCS in the fall (Time 1) as opposed to the winter (Time 2 and 3). Partially supporting this hypothesis, significant differences in SCS were observed between Time 1 and Time 3 (October and February) and between Time 2 and Time 3 (December and February), with Time 2 having the highest average SCS. However, Time 3 likely represented FV consumption that mostly occurred away from the HS setting, as children were out of school for a one-month winter break from the end of December to late January. While at school, children had the opportunity to consume FV during meal and snack times at HS. HS programs are required to participate in CACFP per federal Program Performance standards.[31] While specific menu items may vary, centers are required to offer ½ cup servings of FV at both breakfast and lunch, ultimately providing 1 cup of FV daily. Much work has been done to describe the benefits of participating in CACFP, particularly as it relates to children's FV intake.[31]

Sex differences in SCS were observed with higher SCS in males. Other studies found females had 23% higher SCS (p<.001).[19] However, in the current study, males had significantly higher SCS compared to females. Our current understanding of sex differences for FV intake is not clear in the literature, with no published studies currently describing differences among PSAC. Among adolescents, (ages 7-14 years) males were more likely to meet the daily vegetable group guidelines, while females consumed more energy per day from fat.[33] However, another study reported females (11 years-olds) had higher FV intake and a higher associated preference for FV compared to males.[34] Findings from the current study suggest that low-resource preschool-aged males may have higher FV intake compared to females.

Finally, age may have an effect on SCS, with older children (5 years) having the highest SCS. Younger children tend to have higher levels of neophobia ("fear of new") when trying new foods. Neophobia peaks around 3 years and begins to decline in 4- and 5-year old children.[35] High levels of neophobia can result in avoidance of a specific food items, such as FV, due to a lack of exposure.[36] Younger PSAC may be less likely to consume FV because foods are still considered novel.[35] Due to the age of this study population, 3- (n=34), 4- (n=68), and 5-year-olds (n=10), the majority of the children may still avoid new food items, specifically FV.

Limitations

The study was not without limitations. The study sample size only included children from low-resource households and was confined to a specific geographic location. Further, the sample size was small and sample sizes in subgroups (i.e. age) were not well distributed. Therefore, study findings should not be generalized, but may be used for comparison as research builds to inform our understanding of SCS among PSAC. Due to the longitudinal nature of the first research question, researchers experienced challenges with attrition due to absenteeism (23.4%). Additionally, one participating center (n=67) was engaged in a food-based learning intervention. The intervention had a positive effect on children's SCS,[22] therefore, these children had to be removed from analyses exploring SCS changes over time. Furthermore, the VM® had its own limitations. For example, the VM® only measures consumption of FV high in carotenoids meaning it would not capture consumption of FV containing low amounts of carotenoids such as apples and bananas;[20] current understanding of VM® scores does not allow for estimation of actual portions of FV consumed, a higher score is considered better, but there is currently no established threshold; and finally, the VM® cannot determine if an individual's FV intake is adequate or meets recommendations.[20] More research is needed to fill these gaps in understanding.

IMPLICATIONS FOR RESEARCH AND PRACTICE

Low intake of FV among PSAC is well documented. In order to determine the most effective interventions, valid, objective, and reliable methods are needed to assess dietary intake. In the current study, the VM® served as a promising objective tool to measure SCS in PSAC.

However, as interest in the use of the VM® among young children grows, the tool should be validated using blood plasma and traditional subjective measures of intake (i.e. food frequency questionnaires, 24-hour recall) as has been completed in adults.[27] In the interim, understanding SCS among PSAC and how they change over the course of a year is critical. Intervention studies choosing to use the VM® as an objective outcome measure may observe SCS changes in intervention and control groups that are outside of the impact of the intervention. For example, the current study and others suggest that age[17,34] and sex[19,33] may have a significant effect on SCS. While more research is needed to understand the influence of these variables on SCS, researchers should consider these variables as interventions are developed and/or adapted for new populations.

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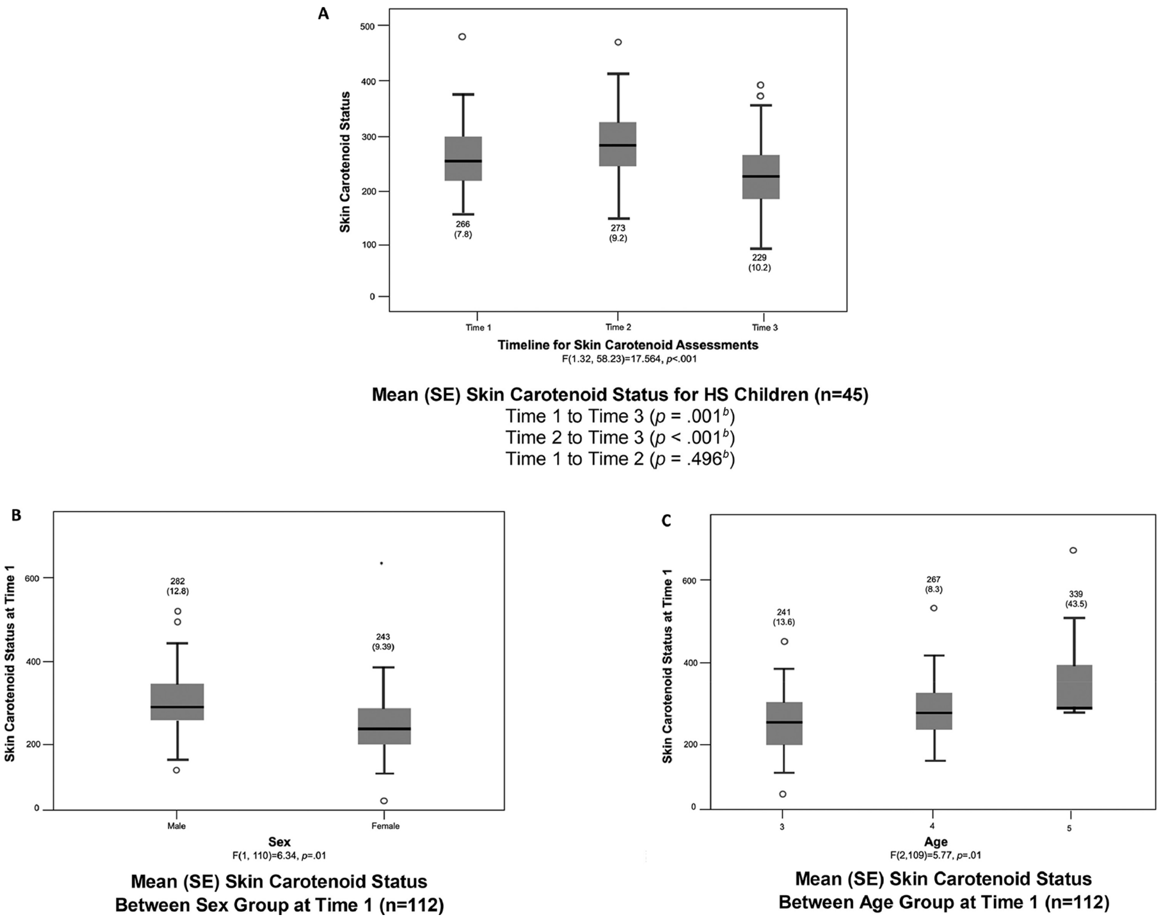

Figure 1. Skin Carotenoid Status Over Time and Differences by Sex and Age.

Table 1.

Timeline for Data Collection and Veggie Meter® Assessments

Collection Timeline	Activities/Assessments Completed
Time 1 – Baseline Data Collection October 2018 *Reflective of diet in summer prior to Head Start (n=112)	• Parent Survey (age, sex, ethnicity/race, history of food allergies) • Veggie Meter® (skin carotenoid measurements) • Height & Weight Measurements
Time 2 – Mid Data Collection December 2018 *Reflective of diet provided partially by Head Start (n=45)	• Veggie Meter® (skin carotenoid measurements) • Height & Weight Measurements
Time 3 – Post Data Collection February 2019 *Reflective of diet during winter break (n=45)	• Veggie Meter® (skin carotenoid meas urements) • Height & Weight Measurements

*Reflective of diet 4–8 weeks prior to measurement date

Table 2.

Baseline Skin Carotenoid Status Assessed by the Veggie Meter® and Differences with Sex and Age at Time 1 (n=112)

Characteristic	n (%)	Mean Skin Carotenoids	Standard Deviation	Standard Error	P-value for Difference
Sex					.01
Male	64 (57%)	282.53	75.14	12.84	
Female	48 (43%)	243.44	88.95	9.39	
Age					.01
3 Years Old	34 (30.4%)	241	79.42	13.62	
4 Years Old	68 (60.7%)	267	68.84	8.35	
5 Years old	10 (8.9%)	339	137.48	43.48	