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

by

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Objective: Examine differences in skin carotenoid levels (SCL) based on time, age, sex, and race 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 SCL 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 SCL assessed using the Veggie Meter® based on time, sex, race, and age

Analysis: Analysis of Variance (ANOVA) with a Greenhouse-Geisser correction to assess SCL at Time 1 between sex, race, and age (n=112). Repeated measures ANOVA to assess SCL over time (n=45) using Bonferroni correction[b].

Results: On average, children were 4 years old, African American (81.3%), male (57%) and mean SCL 266 (SD 82.9). SCL were significantly different over time ($p<.001$). Significant differences were observed between ages ($p=.01$) and sex ($p=.01$), but not between race.

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, age, and race are potential confounders which should be assessed in future studies using the Veggie Meter®.

Keywords: Skin Carotenoid Levels, Preschool Aged Children, Head Start, Veggie Meter®

SKIN CAROTENOID LEVELS OVER TIME AND DIFFERENCES BY AGE, SEX, AND RACE AMONG HEAD START CHILDREN (3-5 YEARS) LIVING IN EASTERN NORTH CAROLINA

A Thesis

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CHAPTER 1: INTRODUCTION

Low intake of fruits and vegetables (FV) among preschool-aged children (PSAC) (3-5-years old) is well documented[1]. On average, 8.3% of PSAC are consuming less than 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, as assessed using non-invasive methods such as resonance Raman Spectroscopy (RSS)[15], and pressure-mediated reflection spectroscopy are emerging as valid and reliable tools to assess intake. The Veggie Meter® (VM®) 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 among PSAC[19]. 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 SCL as measured by the VM®, particularly among low-income, minority PSAC who are at greatest risk for low FV intake. The current study aims to fill this gap in research by establishing SCL among low-income, PSAC enrolled in Head Start (HS) centers in North Carolina (NC) over a 6-month period. This study sought to answer four research questions. **#1**: Are there significant differences in 3-5-year-old PSAC's SCL over time (Time 1-3 [n=45])? **#2**: Are there significant differences in SCL by sex (Time 1 [n=112])? **#3**: Is there a significant difference in SCL by race (Hispanic, African American, White, and Other [Asian, Alaska native or American Indian, Native Hawaiian or other Pacific Islander]) (Time 1 [n=112])? **#4**: Are there significant differences in SCL by age (3-, 4-, 5-year olds) (Time 1 [n=112])? Researchers hypothesized SCL would change significantly over time with significant differences being observed by sex, race, and age.

CHAPTER 2: METHODS

Research Design

This study examined baseline SCL and changes in SCL among 3-5-year old low-resource, low-income PSAC between 3-time points over a 6-month period and differences with sex, race, 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-income, low-resource PSAC enrolled in HS. The sampling frame included approximately 250 children from 3 HS centers in NC. Exclusion criteria were children not enrolled in HS, if the child was not between 3-5 years, children with a cognitive impairment, children in which parental consent was not obtained, and/or non-English speaking individuals.

Data Collection

Researchers collected data from parents and children over the 2018-2019 academic year (October-February). 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 (one 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 SCL were measured at 3 times throughout the school year with 2-month separations between, as previous studies have indicated SCL reflect FV intake over the prior 4-8 weeks[16]. The use of multiple timepoints allowed researchers to establish changes in SCL 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' SCL, 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

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

Veggie Meter (VM)®: SCL were measured as an objective, non-invasive 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]. The VM® is useful for measuring foods high in carotenoids such as carrots, squash, tomatoes, and green leafy vegetables, but does not effectively measure FV low in carotenoids such as apples, bananas, pears, or potatoes[20]. For measurement, the child's finger was 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 SCL 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[21]. The measurement took approximately 45 seconds, for using the multiple measurement mode. In multiple measurement mode, the finger was inserted and retracted 3 times and an average score was determined for the 3 measurements.

Prior to the current study, a protocol to guide data collection was developed. The methods were piloted in summer 2018 during the HS open enrollment period in which approximately 500 children were assessed. From the pilot study, 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.

Height/Weight: For completing the VM® assessments, height/weight were measured as biomarkers for running the VM® assessment (**Table 1)**. Protocols were adapted and revised from the 2007 NHANES Anthropometry Procedure Manual[22].

Data Analysis

Data analysis was conducted using the Statistical Package for Social Sciences (SPSS) 24.0. Summary statistics are provided in frequency (proportion) or mean (SD) as appropriate. Oneway ANOVA was used to assess SCL at Time 1 between sex, race, and age (n=112). A multiple linear regression with sex, age, and race as factors was used to examine adjusted effects. To examine changes in children's SCL over the 3-time points, 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 which resulted in significant differences between the two groups ($F(2,76) = 3.98$, $p=.02$, $r=.10$)[23]. This difference was, in part, due to the nutrition education intervention, and not due to seasonal trends which were the focus of the current study. Interaction terms between sex, age, race and time were tested and removed if not statistically significant. Bonferroni correction[(b)] was used for multiple comparisons among 3 time points. All statistical differences are reported at $P<0.05$ level.

CHAPTER 3: RESULTS

One-hundred and twelve HS children were enrolled in the study and were present for the first round of data collection at Time 1 for all three sites. From Sites 2 and 3, 45 children participated in Time 2 and Time 3 data collection. The majority of participants were African American (81.3%), male (57%), and 4-years old (**Table 2**). Of the 112 participants, parents reported 10 (8.9%) children had food allergies.

Average SCL (n=112) was 266.00 (SD 82.9) at Time 1. Mean SCL 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, 58.22)=17.56, p=.001[b] and between Time 2 to Time 3 (p<.001[b]), however no significant differences were found from Time 1 to Time 2 (p=.496[b]) (**Figure 1. a.**). No interaction between sex, age, race, and time was statistically significant so they were removed from the model. Addressing research questions 2-4, SCL and its effects between sex, race, and age at Time 1 for all three sites are shown in **Table 2**. Significant differences were observed between sex with males having higher mean SCL (282 SE 9.3) than females (243 SE 12.8) (F(1,153.36) =.77, p=.01) (**Figure 1. b.**). Average SCL varied slightly among race groups, but the differences were not significant (F(1,153.36) = .14, p= 0.67) (**Figure 1. c.**). Hispanic children had the highest SCL (297 SE 19.7), followed by white/Caucasian (281 SE 37.4), African American (265 SE 8.8), and other races at (234 SE 35.8). Finally, significant differences were observed between ages (3-, 4-, and 5-year-olds) with mean SCL 3- (241 SE 13.6), 4- (267 SE 8.3), and 5-year-olds (340 SE 48.5) (F(1,153.36) = 1.48, p=.01) (**Figure 1. d.**). The multiple linear regression showed similar results that SCL differed significantly between sex and age groups but not significantly between race groups.

CHAPTER 4: 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. The current study fills this gap by establishing SCL among low-income PSAC over a 6-month period. The study also examined differences in SCL over time and between sex, race, and age. Average SCL differed significantly over time. Additionally, significant differences of mean SCL between sex and age groups were observed, however, no significant differences were observed among different race groups.

Average SCL measured in the current study were higher than previous reports of SCL 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 SCL 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 SCL among PSAC using the VM®[24]. In the current study, the geographic location was considered 25.4% rural[25], and children were from families who fell 125% below the poverty line[26]. Potentially explaining some of the variability between study reports, children enrolled in Ermakov[19] lived in an entirely urban area[25] and were from families of varying SES.

In the current study, significant differences between SCL measured at 3 time points over a period of 6-months were observed. SCL are directly influenced by intake of FV high in carotenoids[19]. SCL 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. Since young children do not have full control over the foods that are available in their home and school environments[27], SCL may vary naturally over the course of a year based on availability of FV.

Access in home and ECE may further by impacted by FV seasonality[28]. In theory, availability of FV increases throughout the summer months, with SCL being representative of dietary intakes 4-6 weeks[16], it was hypothesized to see higher SCL in the fall (Time 1) as opposed to the winter (Time 2 and 3). Supporting this hypothesis, significant differences in SCL were observed between Time 1 and Time 2 (October and December); and between Time 2 and Time 3 (December and February), with Time 2 having the highest average SCL. However, Time 3 likely represented only FV consumption that occurred away from the HS setting, as children were out of school for winter break over this period. While at school, children had the opportunity to consume FV during meal and snack times at HS. According to school menus, during the course of the study broccoli was served 8 times, sweet potato 3 times, tomato 3 times, and carrots 9 times[23]. More research is needed to explore FV intake in homes with young children and ECE cross-referencing SCL and observed intake.

Sex differences in SCL were observed with higher SCL observed in males. Other studies found females had 23% higher SCL (p<.001)[19]. However, in the current study, males had significantly higher SCL 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) males were more likely to meet the daily vegetable group guidelines while females consumed more energy per day from fat[29]. However, another study reported females (11 years old) had higher FV intake and a higher associated preference for FV compared to males[30]. Findings from the current study suggest that low-income preschool-aged males may have higher FV intake compared to females. However, more research is needed to understand this finding and its implications for long-term intake and health outcomes.

This study did not find significant differences between SCL and race groups, but slight variations were observed between race groups. Overall, Hispanic children had the highest SCL average, followed by white, African American, and other races. However, only a small percentage of Hispanic children were included in this study compared to African Americans. Previous studies have reported significant differences between SCL and race groups; however, these differences were observed in adult populations[18,19]. In those studies, Hispanic adults had an average of 299 (90% Hispanic study population)[19] for SCL with the highest found in Japanese groups at 335 (100% Asian study population)[19] and the lowest group being majority African American adults at 240 (SD 99.7) (87.2% African American study population)[18]. To the author's knowledge, no published studies have reported SCL for different race groups in PSAC. However, reports of FV intake among children may provide some insight into the slight variations observed in our sample. NHANES (2001-2004) reported Hispanic children generally meet the minimum recommendations for total fruit, whole fruit, dry beans and peas, and other vegetables compared to African American and white children[3]. Additionally, white children may be more likely to meet the minimum recommendations for orange and other vegetables and whole fruit, when compared to African American children[3]. It is possible cultural food differences in the types of FV consumed may eventually explain variations in SCL between race groups. Findings from the current study suggest there are only slight variations in SCL between race groups living in the same community, however more research is needed to explore skin carotenoid variations across race groups. Additionally, a larger sample including similar race distribution may be needed to effectively assess the impact of race on SCL.

Finally, age appears to have an effect on SCL, with older children (5 years) having the highest level of SCL. Younger children tend to have higher levels of neophobia ("fear of new") when trying new foods[31]. Neophobia peaks around 3 years and begins to decline in 4- and 5-year old children[31]. High levels of neophobia can result in avoidance of a specific food items, such as FV, due to a lack of exposure[32]. Younger PSAC may be less likely to consume FV because foods are still considered novel[31]. Due to the age of this study population, 3-year-olds (n=34), 4- (n=48), and 5- (n=10), the majority of the children may still avoid new food items, specifically FV. While, findings from the current study suggest that age may influence skin carotenoid level more research is needed to explore the impact of food neophobia on FV consumption in a larger sample size with equal sampling between age groups.

Limitations and Strengths

The study was not without limitations. The study sample size only included children of low SES and was confined to a specific geographic location. Therefore, study findings should not be generalized but may be used for comparison as research builds to inform our understanding of SCL 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 with a food-based learning intervention. The intervention had a positive effect on children's SCL[23]; therefore, these children had to be removed from analyses exploring SCL 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 colorless carotenoids such as apples and bananas[20]; current understanding of VM® scores does not allow for estimation of actual portions of FV consumed; 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. The study also had strengths. This study was the first step to fill the gap by providing a portable, non-invasive, objective measure to assess FV consumption among PSAC. To the authors knowledge, it is also the first study to include baseline SCL for low-income children over time and between sex, race, and age groups.

CHAPTER 5: 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. A promising non-invasive tool for objectively measuring FV consumption that uses reflection spectroscopy to assess SCL is the VM®. The VM® assessments were simple to administer on younger populations, minimum setup was required, and its ease of use was inherently simpler than completing blood plasma assessments. 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[24]. In the interim, understanding SCL 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 SCL 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[17,31] and sex[19,29] may have a significant effect on SCL. Other studies support that race[18,19] may also be important. While more research is needed to understand the influence of these variables on SCL, researchers should consider these variables as interventions are developed and or adapted for new populations.

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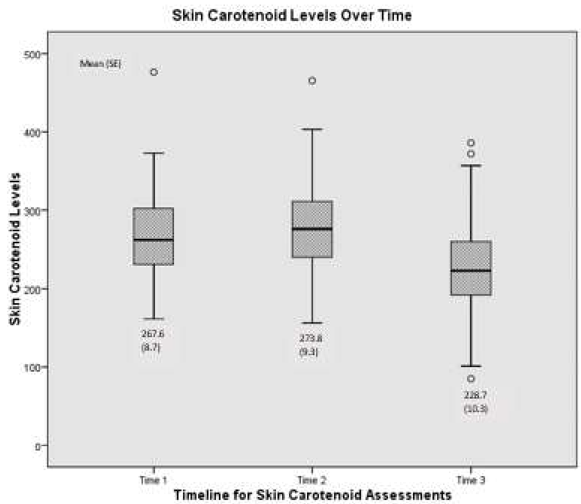
Table 1. Timeline for Data Collection and Assessments

Collection Timeline	**Activities/Assessments Completed**
<u>**Time 1** – Baseline Data Collection</u> 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
<u>**Time 2** – Mid Data Collection</u> December 2018 *Reflective of diet provided partially by Head Start (n=45)	• Veggie Meter® (skin carotenoid measurements) • Height & Weight Measurements
<u>**Time 3** – Post Data Collection</u> February 2019 *Reflective of diet during winter break (n=45)	• Veggie Meter® (skin carotenoid measurements) • Height & Weight Measurements

*Reflective of diet 4-6 weeks prior to measurement date

Table 2. Baseline Skin Carotenoid Levels Assessed by the Veggie Meter® and Differences with Sex, Race, and Age at Time 1 (n=112)

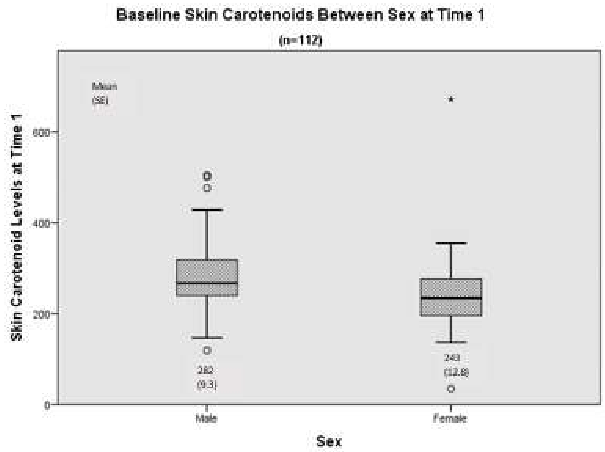
Characteristic	n (%)	Mean Skin Carotenoids	Standard Deviation	Standard Error	P-value for Difference
Sex					.01
Male	64 (57%)	282.53	75.14	12.84	
Female	48 (43%)	243.44	88.95	9.39	
Race					.67
African American	91 (81.3%)	265.23	84.39	8.84	
White	6 (5.4%)	281.00	91.55	37.37	
Hispanic or Latino	7 (6.3%)	296.71	52.10	19.69	
Other	7 (6.3%)	234.29	94.66	35.78	
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%)	340	145.60	48.53	



(F(1.323, 58.227)=17.564, p<.001)

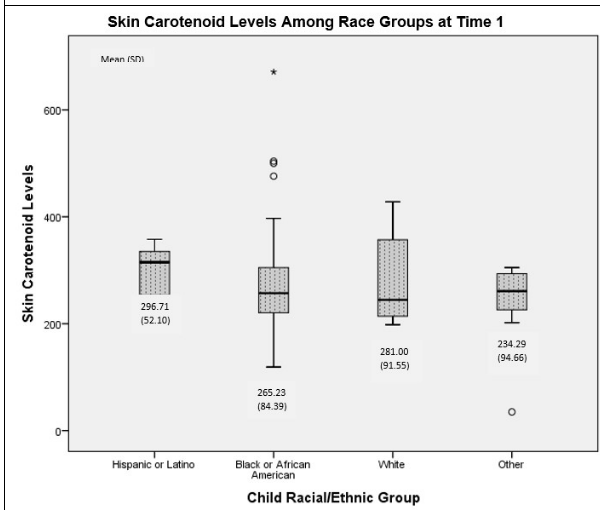
Figure 1. a. Mean Skin Carotenoid Levels Over Time for Head Start Children (n=45)

Time 1 to Time 3 (p=.001a)
Time 2 to Time 3 (p<.001a)
Time 1 to Time 2 (p=.498a)
a. Adjustment for multiple comparisons: Bonferroni



(F(1.941,153.360) =.766, p=.01)

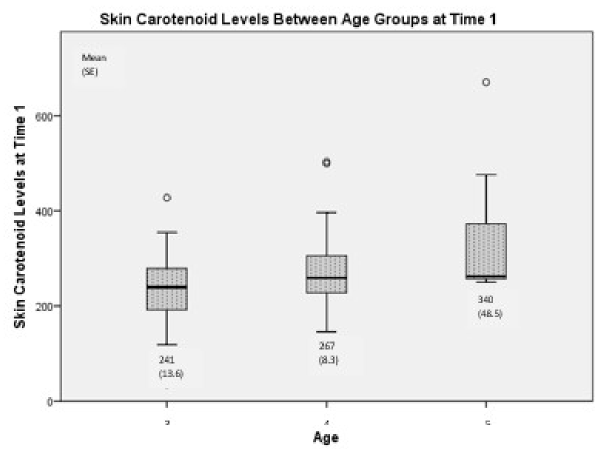
Figure 1. b. Mean Skin Carotenoid Levels Between Sex for Head Start Children (n=112)



Note: "Asian" is included in "Other" ethnic groups

(F(1.941,153.360) = .136, p= 0.67)

Figure 1. c. Mean (SD) Skin Carotenoids Among Racial Groups at Time 1 (n=112)



(F(1.941,153.360) = 1.475, p=.01)

Figure 1. d. Mean (SE) Skin Carotenoids Among Age Groups at Time 1 (n=112)

Figure 1. Skin Carotenoid Levels Over Time and Differences by Sex, Race, and Age.

APPENDIX A: IRB PERMISSION LETTER

EAST CAROLINA UNIVERSITY
University & Medical Center Institutional Review Board
4N-64 Brody Medical Sciences Building· Mail Stop 682
600 Moye Boulevard · Greenville, NC 27834
Office **252-744-2914** · Fax **252-744-2284** ·
www.ecu.edu/ORIC/irb

Notification of Amendment Approval

From: Social/Behavioral IRB
To: Virginia Stage
CC: Virginia Stage
Date: 7/15/2019
Re: Ame4_UMCIRB 18-001535
UMCIRB 18-001535
Food-based Learning in Head Start

Your Amendment has been reviewed and approved using expedited review for the period of 7/15/2019 to 7/14/2020. It was the determination of the UMCIRB Chairperson (or designee) that this revision does not impact the overall risk/benefit ratio of the study and is appropriate for the population and procedures proposed.

Please note that any further changes to this approved research may not be initiated without UMCIRB review except when necessary to eliminate an apparent immediate hazard to the participant. All unanticipated problems involving risks to participants and others must be promptly reported to the UMCIRB. A continuing or final review must be submitted to the UMCIRB prior to the date of study expiration.The investigator must adhere to all reporting requirements for this study.

Approved consent documents with the IRB approval date stamped on the document should be used to consent participants (consent documents with the IRB approval date stamp are found under the Documents tab in the study workspace).

The approval includes the following items:

Document	Description
Parent Survey-Research-Consent-Letter-Template_[Comparison] 070219 vcs.docx(0.01)	Consent Forms
Parent Survey-Research-Consent-Letter-Template_[Intervention] 070219.docx(0.01)	Consent Forms
PEAS Pilot Research Protocol - UPDATED 070219.docx(0.02)	Study Protocol or Grant Application

The Chairperson (or designee) does not have a potential for conflict of interest on this study.