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Skin carotenoid status measured by resonance Raman spectroscopy as a biomarker of fruit and vegetable intake in preschool children

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Abstract

BACKGROUND/OBJECTIVE—Dietary assessment in children is difficult, suggesting a need to develop more objective biomarkers of intake. Resonance Raman spectroscopy (RRS) is a non-invasive, validated method of measuring carotenoid status in skin as a biomarker of fruit/vegetable intake. The purpose of this study was to examine the feasibility of using RRS in preschool children, to describe inter-individual variability in skin carotenoid status and to identify factors associated with the biomarker in this population.

SUBJECTS/METHODS—We conducted a cross-sectional study of 381 economically disadvantaged preschoolers in urban centers in Connecticut (USA). In all, 85.5% were black non-Hispanic or Hispanic/Latino, and 14.1% were obese and 16.9% were overweight by age- and sex-specific body mass index (BMI) percentiles. Children had their skin carotenoid status assessed by RRS in the palm of the hand. Fruit/vegetable consumption was assessed by a brief parent/guardian-completed food frequency screener and a liking survey.

RESULTS—We observed inter-individual variation in RRS values that was nearly normally distributed. In multiple regression analysis, higher carotenoid status, measured by RRS, was positively associated with fruit/vegetable consumption (P=0.02) and fruit/vegetable preference (P<0.01). Lower carotenoid status was observed among younger children, those participating in the US Supplemental Nutrition Assistance Program, and those with greater adiposity (P<0.05 for all).

CONCLUSIONS—We observed wide variability in skin carotenoid status in a population of young children, as assessed by RRS. Parent-reported fruit/vegetable intake and several demographic factors were significantly associated with RRS-measured skin carotenoid status. We

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

recommend further development of this biomarker in children, including evaluating response to controlled interventions.

Keywords

carotenoids; biomarker; children; resonance Raman spectroscopy

INTRODUCTION

Dietary assessment is inherently difficult and involves additional complications in children. Self-report methods are subject to bias and measurement error due to problems with reporting, portion size estimation and inaccurate recall.[1,2] In preschoolers, dietary assessment requires the assistance of a proxy reporter, usually parents or teachers.[3] Direct observation (for example, measured plate waste or digital photography) techniques offer more valid estimation than questionnaires,[4,5] but these methods are laborious, limited to small populations and do not reflect usual dietary intake. Literature also exists supporting the use of food liking/preference scales to assess intake, assuming that individuals habitually consume what they like and avoid what they do not.[6,7] The invasiveness of more objective measures of nutrition status, typically involving plasma samples for biochemical analysis, limits their feasibility for young children. Thus, a need exists for a valid, rapid, and non-invasive method to assess nutritional status in children using biomarkers, such as carotenoid status for estimating fruit and vegetable intake.

Carotenoids are plant pigments responsible for red, orange and yellow coloring. Classified as antioxidants, carotenoids have a suggested role in the prevention of many diseases, including cancer,[8–11] heart disease[12–14] and age-related eye disease,[15,16] as well as all-cause mortality.[17] Because carotenoids are not found in significant concentrations in other foods, but are widely distributed in fruits and vegetables, they are considered to be the best current biomarker for fruit and vegetable intake.[18] Traditional approaches for carotenoid assessment often depend on self-reported intake of fruits and vegetables linked to food composition databases for estimation of carotenoid content in foods. However, the results of dietary intervention trials have suggested that self-reported carotenoid intake can have bias and measurement error.[19] Alternatively, biochemical analyses such as high-performance liquid chromatography can be used to measure carotenoids in plasma or tissue samples.[20,21]

The development of non-invasive, less expensive, but sensitive optical monitoring technologies provides an alternative to high-performance liquid chromatography for measurement of carotenoids in living tissues. In particular, resonance Raman spectroscopy (RRS) has emerged as an objective indicator of carotenoid status.[22,23] A novel, non-invasive technique for measuring carotenoid status in the skin using visible light, RRS utilizes a small probe with a laser at a blue wavelength (λ =488 nm) to measure total carotenoid concentration in skin.[24] The Raman scattered light produces a spectral fingerprint of the carotenoid molecules based on their unique molecular structure (alternating carbon double and single bonds) and vibrational energy levels. One of the preferred body sites for Raman scanning is the palm of the hand because the stratum corneum, the outer skin tissue layer where carotenoids concentrate, is relatively thick, and the melanin content is less variable among individuals of different races and ethnicities.[23]

Studies around the world are now using the RRS technology to non-invasively measure skin carotenoid status as a promising biomarker of fruit and vegetable intake, but the evidence supporting its use as a biomarker comes primarily from studies of adult populations. Our group has previously shown that RRS is a valid biomarker of skin carotenoid status in healthy adults, compared with biopsies of excised human skin, with correlation coefficients

ranging from r=0.7 to 0.9.[25,26] RRS was also a reliable indicator of carotenoid status over time and across different body sites (three measured: palm, inner arm and outer arm).[25] In adults, RRS has also been found to correlate significantly with dietary intake of fruit and vegetable intake in cross-sectional studies.[23,25,27,28] Also, adult studies involving carotenoid or fruit and vegetable interventions have demonstrated that RRS values change accordingly.[29,30] Because of growing evidence supporting the validity of RRS as a biomarker of carotenoid status in adults, the purpose of this study was to examine the feasibility of using this biomarker in a population of children, where dietary assessment is notoriously difficult. Specifically, we aimed to use RRS in a field (non-clinical) setting to estimate the inter-individual variability, and to identify predictors of skin carotenoid status in a sample of preschool children.

SUBJECTS AND METHODS

Study population

A community-based sample of economically disadvantaged children was recruited from six federal and state-supported preschool programs in two urban centers in Connecticut, USA. A total of 381 preschool children, aged 3–5 years, from primarily black non-Hispanic and Hispanic/Latino ethnic backgrounds, were enrolled in the sample for RRS testing during August and September 2008. Written informed consent of their child's voluntary participation was obtained from a parent or caregiver, and oral assent was obtained from the children before RRS scanning. All procedures were approved by the Institutional Review Boards at the Yale University School of Medicine and the University of Connecticut.

Data collection

Participant characteristics—A baseline questionnaire (available in English and Spanish) was completed by parents to collect demographic information about their child, including date of birth, sex, race/ethnicity, parent/caregiver smoking status and current family participation in the US Supplemental Nutrition Assessment Program (SNAP). Children's date of birth and sex were confirmed with school enrollment records. Height and weight were measured by a registered dietitian using a platform scale and stadiometer (SECA, Hanover, MD, USA). Children were weighed with their shoes on to comply with school's fire safety regulations. These measurements were used to calculate body mass index (BMI) and age- and sex-specific percentiles (BMI %) from the 2000 CDC standards (with 'overweight' ≥85th percentile and 'obese' ≥95th percentile of BMI).[31,32]

Resonance Raman spectroscopy—Details of RRS instrumentation have been reported elsewhere,[24] with minor modifications in this study. Briefly, the instrument uses a compact, portable, continuous wave solid-state laser operating at a wavelength of 488 nm to shine blue visible light onto a tissue of interest (that is, skin). This laser is based on a frequency-double near-infrared semiconductor diode laser. The spectrograph is equipped with a linear charge coupled device array operating at room temperature. The array was interfaced to a laptop computer for data acquisition, processing and display. At a power of 0.2 W/cm^2 and an elliptical laser spot size of 2.5 × 1.5 mm, it is safe to expose skin to the laser light for 30 h. The light exposure is ~1000 times less than the exposure limit set by the ANSI z136.1-2007 standard, which assures that the instrument was safe for use in a population of young children. The palm of each child was scanned three times for reliability at an exposure time of 30 s per scan, and scans were performed immediately before or after the height/weight screenings. Protective goggles (Kentek Corporation, Pittsfield, NH, USA) were worn by the children and the research assistant to block any laser excitation light. Mean RRS values for total carotenoid status, measured as skin Raman intensity, were obtained to provide an assessment of total skin carotenoid status for each child.

Fruit and vegetable intake and preference—Parents completed a modified Block Kids Questionnaire (available in English and Spanish)–a brief, 2-page, 41-item food frequency screener (FFS) that inquired about their child's recent dietary intake in the past seven days (NutritionQuest, Berkeley, CA, USA). Intake of total fruits and vegetables was estimated (frequency × portion size, summed across foods). Additionally, intake of total and individual carotenoids was estimated based on the USDA Food and Nutrient Database for Dietary Studies[33] (Supplementary Appendix). Because adjusting for total energy intake (kcals) may yield more reliable estimates of self-reported diet,[34] we energy-adjusted intake of total fruits and vegetables and total carotenoids estimated from the FFS. Parents also completed the Preschool-Adapted Liking Survey, developed by our group. This two-page, 5 min survey asked parents to rate their child's likes or dislikes of 18 food and beverage items, as well as four non-food items (for example, a loud siren, taking a bath) on a continuous scale. Liking scores were generated by measuring the ±100 point line from the center rating ('he/she thinks it's ok' at 0) to the liking rating ('he/she really likes it' from 30 to 50 and 'he/she loves it' from 80 to 100) or to the disliking rating ('he/she really does not like it' from–30 to–50 and 'he/she hates it' from–80 to–100). An average liking score was computed for total fruits and vegetables, and for high carotenoid foods (Supplementary Appendix).

Statistical analysis

Descriptive statistics were obtained for the continuous response variable, mean RRS values for skin carotenoid status, as well as the main effect of demographic covariates and fruit and vegetable intake in relation to the response variable. To estimate variability in skin carotenoid status, a histogram of RRS values (measured in skin Raman intensity) was plotted for the total sample, using the mean of the three replicate values. RRS mean values and medians were estimated for five racial and ethnic groups (Hispanic, black non-Hispanic, white non-Hispanic, biracial and other) and by BMI percentile for sex and age, as a continuous and categorical variable for weight status (underweight (<5th percentile), healthy weight (5th to <85th percentile), overweight (85th to <95th percentile) and obese (≥95th percentile)). An analysis of variance was performed to test whether any of the two-group comparisons in RRS mean values was significantly different at the 0.05 level.

Several variables were selected for examination as the main predictors of total skin carotenoid status, measured by RRS in the preschool population: fruit and vegetable intake estimated from the parent-reported FFS and liking survey, BMI percentile for sex and age, current SNAP participation (yes/no) and age (months). Covariates of interest were specified *a priori* and included race/ethnicity, sex (male/female) and geographic school site (six-level categorical variable). The main effect of each predictor variable and covariate was examined in univariate linear regression analysis to determine the independent effects on total skin carotenoid status measured by RRS. Next, a multivariate regression analysis was conducted to identify determinants of carotenoid status in our preschool sample, with adjustment for covariates. Dietary intake and liking measures were modeled separately (for example, not included simultaneously in the same models) as possible predictors of RRS. All statistical computations were conducted using SAS v. 9.1 (SAS Institute, Inc., Cary, NC, USA) with a *P*-value of 0.05 considered statistically significant.

RESULTS

A total of 381 children had their skin carotenoid status assessed by RRS and dietary questionnaires completed by a parent or caregiver. Children were cooperative during measurement, with no unexpected difficulties encountered. Given the ease of use and rapid nature of RRS, we were able to scan up to 60 children in <2 h. Mean RRS palm measures of

total carotenoid status by demographic characteristics of the sample are reported in Table 1. Mean age of the children was 3.80 years (range: 2.75–4.83 years), and 85.5% identified as Hispanic/Latino or black non-Hispanic. Almost one third of the sample (31.0%) was overweight or obese, and 54.7% of parents reported their family was currently participating in SNAP. Mean RRS values for total carotenoid status were similar for boys and girls (20.59 vs 20.38; $P=0.77$). A marginally significant difference was observed in RRS mean values among five racial/ethnic groups ($P=0.08$), with white non-Hispanics having the highest RRS values. This group also had the highest carotenoid intake, as estimated from the FFS, but the difference was not significant. RRS mean values were significantly lower for children whose parent reported that their family was currently participating in the SNAP, compared with those who were not (19.34 vs 21.83; $P<0.01$).

Figure 1 shows the distribution of RRS values, measured as skin Raman intensity values (average of three readings), for the preschool sample. The mean RRS value was 20.48, with a standard deviation of 6.68. The data were approximately normally distributed, with a slight right-skew (Anderson–Darling test for normality, $P<0.01$; skewness =1.06), and RRS averages for four individuals measured greater than three standard deviations from the mean. RRS values for the total sample ranged from 2.93 to 53.55.

Following a univariate linear regression analysis to determine independent effects on total skin carotenoid status measured by RRS (data not shown), multiple linear regression was used to identify the main predictors of skin carotenoid status in the preschool population, with adjustment for covariates (Table 2). In adjusted analyses, a significant positive association was observed between total skin carotenoid status and parent-reported fruit and vegetable intake, as measured by the liking survey ($P<0.01$), as well as energy-adjusted intake of total fruits and vegetables ($P=0.02$), estimated from the FFS. After accounting for diet, parent-reported SNAP participation was consistently inversely associated with total skin carotenoid status ($P<0.01$), while age in months and white non-Hispanic race/ethnicity were consistently positively associated ($P<0.01$), independent of the method used to estimate diet. Finally, an inverse association was observed between total skin carotenoid status and BMI percentile when the liking survey was used to estimate intake, but the relationship was only marginally significant when diet was estimated by the FFS. The partial correlation coefficients ($r_{partial}$) between RRS and diet ranged from $r_{partial} = 0.17$ for intake of total fruits and vegetables estimated by the FFS to $r_{partial} = 0.23$ estimated by the liking survey and intake of high carotenoid foods estimated by the liking survey. Because the distribution of RRS values was slightly right-skewed, we examined the effect of log transformation of the data. No differences were observed in the main outcomes of the study; thus, we retained the original untransformed RRS values for the final analysis.

DISCUSSION

In this study of underserved preschoolers, we had good acceptance of the technique, and observed an approximately normal distribution of skin carotenoid status, as measured by RRS. In addition to parent-reported fruit and vegetable consumption, age and white non-Hispanic race/ethnicity were also positively associated with skin carotenoid status in our preschool population, while parent-reported SNAP participation and measured BMI predicted lower carotenoid status. Both measures of dietary fruit and vegetable consumption (intake and preference) were significantly correlated with the objective indicator of skin carotenoid status, despite being fairly brief and limited estimates of intake.

Although the evidence that RRS is a reliable and valid biomarker of fruit and vegetable intake comes from many studies in healthy adults as discussed earlier, this was the first study to use RRS to estimate the variability of skin carotenoid status in a large sample of

children. This effort involved the use of a portable Raman scanner, developed specifically for use in this study. The Raman scanner used in our prior research was not portable, and the RRS values reported here use a different intensity scale compared with other results we have published, which were obtained using a different instrument (including the type of laser, spectrograph and optical components).[23,25,35] We observed that the children were cooperative during scanning, suggesting that RRS is a feasible, non-invasive method for assessing relative fruit and vegetable intake in young populations. The inter-individual variability we detected in this population was similar to that seen in adults and suggests that RRS may be used as a more objective measure of diet to identify children with poor carotenoid status, who may be at risk for nutrition-related diseases.

In addition to the significant correlation observed between the objective indicator of fruit and vegetable intake (RRS) and parent-reported diet in our preschool population, we also identified several demographic and anthropometric predictors of skin carotenoid status, measured by RRS, in this population. Age was positively associated with skin carotenoid status in these children. Data from the Third National Health and Nutrition Examination Survey (NHANES III) indicate serum carotenoid levels in the US population are relatively high in childhood, but decrease in the adolescent years and early adulthood.[18] Thus, we may be observing an age trend that is specific to the preschool population. Skin carotenoid status was inversely associated with obesity in our study. Additional studies have found similar results among serum carotenoids in children[36] and adipose tissue carotenoids in adults.[37] Possible explanations for the inverse association between obesity and carotenoid status include dietary differences compared with healthy weight individuals,[38] variability in body compartment size[39] and deposition of lipid-soluble carotenoids into adipose tissue for storage.[40] Because children identified as overweight or obese comprised almost one third of our sample, the observation that these children also had poorer nutrient status may have important health implications. Finally, we observed an inverse association between skin carotenoid status and reported current SNAP participation. This federal program provides funding assistance and nutrition education to improve the nutritional quality of low-income children and their families in the United States. While the SNAP encourages healthy food selection, growing evidence suggests that issues of access and affordability limit the purchase of fruits and vegetables by low-income consumers.[41,42] In our study, we only had information available about current SNAP participation during the time period when these preschool children were evaluated. Thus, we did not have the ability to examine the effect of duration of SNAP use on nutrient status. We also observed a significant positive association between white non-Hispanic race/ethnicity and RRS measures, although this subgroup represented only about 5% of our total population. From our results, it is difficult to disentangle the socioeconomic, racial and income effects that may be driving the inverse association we observed between skin carotenoid status and reported SNAP participation, but may suggest that SNAP participation is insufficient to overcome the adverse effects of poverty on children's nutrition status. This finding warrants further investigation.

In our previous study of adults, total carotenoids assessed by RRS were significantly correlated (r =0.7) with total carotenoids assessed by high-performance liquid chromatography analysis of skin biopsies and with plasma carotenoids.[25] As noted earlier, other groups have shown similar findings for adults. The purpose of this study was not to repeat our previous work in adults, but to assess the feasibility of the RRS biomarker in children, examine the distribution of skin carotenoid status in this population, and identify other factors in addition to diet that track with the biomarker. The correlation coefficients we observed between RRS and both measures of diet (intake and preference for fruits/vegetables) in our sample of children were lower than the correlations previously observed in adult populations. A number of factors could provide an explanation for these findings. First, the brief FFS used in our study was a crude measure that attempted to capture diet in

young children, which is especially difficult to measure. The FFS, which was completed by a parent/guardian for their child, assessed fruit and vegetable intake over 1 week only, whereas tissue levels reflect weeks to months of intake. Moreover, this study was an ancillary project to an ongoing study in preschool children, and the FFS was not specifically designed to assess carotenoid intake. It is known that common sources of carotenoids in children's diets include many foods besides fruits and vegetables, including lycopene from tomato-based products (for example, ketchup, spaghetti sauce and pizza sauce). While we attempted to incorporate these other food sources into our dietary measures, it is possible that we are not capturing all of the main sources of carotenoids in children's diets. To optimize correlations between self-reported diet and the RRS measure of carotenoid status in children, future research should utilize more comprehensive and quantitative dietary measures designed specifically for this population.

There is the possibility that melanin content in the skin could impact the RRS measures of carotenoid status, especially in this diverse population of children. In our previous work, we closely examined the RRS data from participants who self-identified as dark skin as compared with light skin tones.[25] When the RRS outer arm (area of most pigmentation) to palm (area of least pigmentation) ratios were compared between participants with dark vs light skin pigmentation, there was no evidence that RRS ratios varied significantly with skin pigmentation, as might be expected if a filtering effect on the laser and Raman scattered light was occurring. However, we had limited power to detect an effect of melanin with relatively few dark-skinned participants. In the present study, we scanned the palm of the hand, with one of the reasons being that melanin in the palm is less variable among children of different races and ethnicities. In future studies of RRS in adults and children, we plan to systematically examine the effect of melanin content on the RRS measures using established procedures for quantifying melanin in the skin.

A major strength of the present study was the use of RRS to objectively assess skin carotenoid status in a community-based sample of preschool children, where dietary assessment is difficult. We have used these data to gain an understanding of the inter-individual variability of skin carotenoid status within the population, since very little knowledge currently exists. We have also identified significant predictors of carotenoid status in our low-income preschool sample, so more targeted interventions can be created to better improve nutritional quality of children with poor carotenoid status. At this time, the RRS values measured are internally valid within a study, but external validity will depend on the use of an accepted external standard.

In summary, results from this study suggest that there is wide variability in skin carotenoid status in a population of young children, as assessed by RRS. In addition to parent-reported fruit and vegetable intake, which was positively associated with skin carotenoid status, several demographic factors also tracked with the biomarker, including some that have been shown to track with the biomarker in studies of adults (for example, obesity). In our experience, RRS was a feasible biomarker of fruit and vegetable intake that may facilitate research on diet and health in children. Future controlled intervention studies are needed in children to continue the development of RRS as a biomarker of nutritional status.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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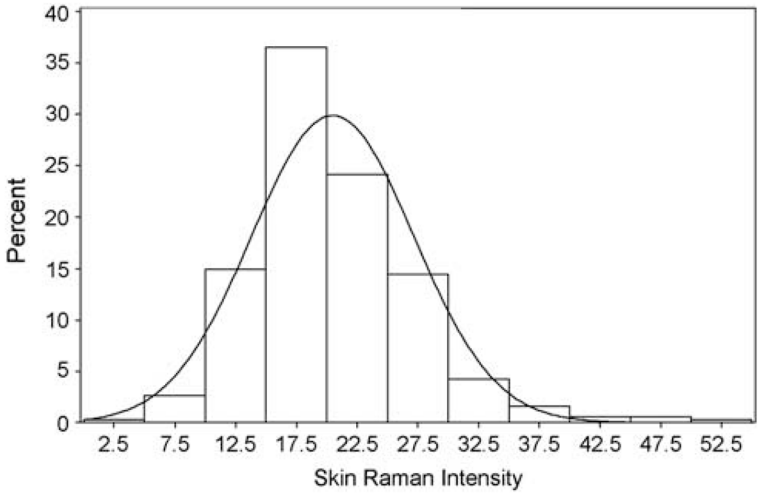

Figure 1.

Histogram showing distribution of total carotenoids in the palm, as assessed using RRS (*N* =381). Note bell-shaped appearance of histogram indicating a normal distribution, with a slight right-skew (Anderson–Darling test for normality, $P<0.01$; skewness =1.06).

Table 1

Resonance Raman spectroscopy measures for total carotenoids by baseline characteristics of the preschool sample (N=381)

	n (%)	RRS score[a]	*P*-value[b]
Age			0.23
<3.8 years	189 (49.6)	20.08±6.82	
≥3.8 years	192 (50.4)	20.90±6.53	
Sex			0.77
Male	193 (50.7)	20.59±6.45	
Female	188 (49.3)	20.38±6.93	
Race/ethnicity			0.08
White non-Hispanic	22 (5.8)	23.95±7.55	
Black non-Hispanic	98 (25.7)	19.84±5.85	
Hispanic/Latino	228 (59.8)	20.57±6.73	
Biracial (White/Black)	22 (5.8)	20.27±7.92	
Other	11 (2.9)	18.12±6.99	
SNAP participation[c]			<0.01
Yes	208 (54.7)	19.34±6.04	
No	172 (45.3)	21.83±7.17	
Current smoking in home			0.10
Yes	62 (16.5)	19.18±6.96	
No	314 (83.5)	20.72±6.62	
BMI percentile for sex and age			0.37
Underweight (<5th percentile)	15 (4.1)	22.13±7.17	
Healthy weight (5th to <85th percentile)	235 (64.9)	20.95±6.94	
Overweight (85th to <95th percentile)	61 (16.9)	19.48±4.97	
Obese (≥95th percentile)	51 (14.1)	20.50±7.09	

Abbreviations: ANOVA, analysis of variance; BMI, body mass index; RRS, resonance Raman spectroscopy; SNAP, Supplemental Nutrition Assistance Program.

[a] Mean±s.d. RRS palm measures of carotenoid status obtained with portable scanner (unstandardized measures).

[b] P for difference in means derived by Student's t tests (binary variables) or ANOVA (multilevel variables).

[c] SNAP participation defined as parent-reported family participation in the federal Supplemental Nutrition Assistance Program.

Table 2

Main predictors of skin carotenoid status, with adjustment for covariates[a]

Model	β	*P*-value	Model	β	*P*-value
Preference for total fruits and vegetables	0.05	<0.01	*Intake of total fruits and vegetables*[b]	0.87	0.02
Age (months)	0.15	<0.01	Age (months)	0.18	<0.01
SNAP participation (yes vs no)	−2.78	<0.01	SNAP participation (yes vs no)	−2.57	<0.01
BMI percentile for sex and age	−0.03	0.03	BMI percentile for sex and age	−0.02	0.04
Sex (male vs female)	−0.72	0.29	Sex (male vs female)	−0.38	0.59
Race			Race		
Hispanic/Latino	Reference		Hispanic/Latino	Reference	
White non-Hispanic	4.07	0.01	White non-Hispanic	3.72	0.02
Black non-Hispanic	−1.16	0.16	Black non-Hispanic	−0.82	0.33
Biracial (White/Black)	1.15	0.45	Biracial (White/Black)	1.20	0.45
Other	−2.50	0.22	Other	−2.57	0.23
Preference for high carotenoid foods	0.04	<0.01	*Intake of total carotenoids*[b]	<0.01	<0.01
Age (months)	0.15	<0.01	Age (months)	0.16	<0.01
SNAP participation (yes vs no)	−2.73	<0.01	SNAP participation (yes vs no)	−2.55	<0.01
BMI percentile for sex and age	−0.03	0.03	BMI percentile for sex and age	−0.02	0.07
Sex (male vs female)	−0.69	0.31	Sex (male vs female)	−0.38	0.59
Race			Race		
Hispanic/Latino	Reference		Hispanic/Latino	Reference	
White non-Hispanic	4.05	0.01	White non-Hispanic	4.12	0.01
Black non-Hispanic	−1.28	0.12	Black non-Hispanic	−0.95	0.26
Biracial (White/Black)	0.95	0.54	Biracial (White/Black)	1.30	0.41
Other	−2.72	0.19	Other	−2.00	0.35

Abbreviations: BMI, body mass index; SNAP, Supplemental Nutrition Assistance Program.

[a]Additional covariate: school site (non-significant in all models).

[b]Adjusted for average daily kilocalories. Units are as follows: preference is a continuous scale (−100 to 100); intake of total fruits and vegetables is servings/day; and intake of total carotenoids is μg/day.