



Weight Status and Visceral Adiposity Mediate the Relation between Exclusive Breastfeeding Duration and Skin Carotenoids in Later Childhood

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ABSTRACT

Background: Breastfeeding is associated with healthier weight and nutrient status in early life. However, the impact of breastfeeding on carotenoid status beyond infancy, and the influence of adiposity, are unknown.

Objectives: The aim of the study was to retrospectively investigate the relation between breastfeeding and carotenoid status, and the mediating effect of weight status and adiposity on this relation, among school-age children.

Methods: This was a secondary analysis of baseline data collected from a randomized-controlled clinical trial. Children 7–12 y old ($n = 81$) were recruited from East-Central Illinois. DXA was used to assess visceral adipose tissue (VAT) and whole-body total fat percentage (%Fat; i.e., whole-body adiposity). Weight was obtained to calculate children's BMI percentiles. Skin carotenoids were assessed via reflection spectroscopy. Macular carotenoids were assessed as macular pigment optical density (MPOD). Dietary, birth, and breastfeeding information was self-reported by parents.

Results: Skin carotenoids were inversely related to %Fat ($P < 0.01$), VAT ($P < 0.01$), and BMI percentile ($P < 0.01$). VAT and BMI percentile significantly mediated this relation between exclusive breastfeeding duration and skin carotenoids, after adjustment for dietary carotenoids, energy intake, and mother education.

Conclusions: Weight status and adipose tissue distribution mediate the positive correlation between exclusive breastfeeding duration and skin carotenoids among children aged 7–12 y. The results indicate the need to support breastfeeding and healthy physical growth in childhood for optimal carotenoid status. This trial was registered at clinicaltrials.gov as NCT03521349. *Curr Dev Nutr* 2021;5:nzab010.

Keywords: human milk, reflection spectroscopy, body mass index, adiposity, carotenoids, macular pigment, adolescents, pediatric

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Abbreviations used: cHFP, customized heterochromatic flicker photometry; MPOD, macular pigment optical density; SES, socioeconomic status; VAT, visceral adipose tissue; %Fat, whole-body total fat percentage.

Introduction

Carotenoids are fat-soluble plant pigments with antioxidant properties that can be found in green leafy vegetables (e.g., kale and spinach), orange and yellow fruits and vegetables (e.g., carrots, cantaloupe, and sweet potato), tomatoes, and egg yolk (1). Once absorbed, carotenoids stay in the circulation and some are deposited in the liver, skin, retina, adipose tissue, and more (2). Lutein and zeaxanthin are the only dietary carotenoids that selectively accumulate in the human retina. Along with *meso*-zeaxanthin, a derivative from lutein, the 3 carotenoids comprise

macular pigment. Macular pigment optical density (MPOD) is a measure of the density of macular pigment in the retina. Higher MPOD has been shown to correlate with better cognitive performance and better neural efficiency in children (3, 4). Major carotenoids in the skin include lycopene, β -carotene, α -carotene, β -cryptoxanthin, lutein, zeaxanthin, phytoene, and phytofluene (5). Skin carotenoids offer protection against erythema, sunlight damage, and premature skin aging (6).

Because the adipose tissue acts as a reservoir for fat-soluble carotenoids, an association between adiposity and carotenoid status has been proposed (7–9). Breastfeeding has been associated with child

weight in infancy and childhood (10). Prior longitudinal research indicates that breastfeeding contributes to maintenance of healthy weight status and lower fat mass or adiposity through childhood and into adulthood. Shorter breastfeeding duration has been linked to higher BMI and whole-body total fat percentage (%Fat; i.e., whole-body adiposity) in children ages 6–14 y (11–13). On the other hand, an inverse correlation has been found between weight status and adiposity and carotenoid concentrations of serum (14, 15), skin (16), and macula (7, 17). Given the previously observed interrelations between breastfeeding, carotenoids, weight status, and adiposity, it is possible that the long-term benefits of breastfeeding for carotenoid status may be mediated by children's weight or adiposity in later childhood. However, to our knowledge, no previous study has directly examined the potential mediating role of weight status and adiposity in breastfeeding effects on carotenoid accumulation in children. This is particularly important to study given the increase in childhood obesity prevalence in the United States and around the globe (18).

Thus, the aim of the present study was 2-fold: 1) to investigate interrelations between breastfeeding, weight status, adiposity, and carotenoid status among children at school age; and 2) to investigate the mediating effect of weight status and adiposity on the association between breastfeeding and carotenoid status at school age. We hypothesized that breastfeeding duration would be positively correlated with carotenoid status among school-age children; however, these relations would be mediated by weight status and adiposity.

Methods

Participants

This was a secondary analysis of baseline data collected during a randomized-controlled clinical trial (NCT03521349). The study sample was comprised of children ages 7–12 y. The baseline measurements were collected during 3 laboratory visits. The mean number of days between the first and the last appointment was 15.9 d. All but 3 of the participants completed the visits within 1 mo. The study protocols were approved by the Office for the Protection of Research Subjects at the University of Illinois at Urbana–Champaign. Written informed consent from the parents of the children who served as subjects and assent from the child subjects themselves were obtained. Participants were recruited through flyers placed locally, social media, university and community webpages, and summer sports camps in the Champaign–Urbana and surrounding areas. Inclusion criteria included being between 7 and 12 y of age, and absence of physician diagnosis of autism spectrum disorder. Subjects received compensation for their participation. Payments were made in the form of e-code gift cards.

Skin carotenoid assessment

Skin carotenoid scores were obtained utilizing reflection spectroscopy via a device known as a “Veggie Meter” (Longevity Link Corporation). The reliability and validity of this technique have been supported in an adult sample (19). This device relies on a noninvasive technique that provides a rapid and quantitative estimation of skin carotenoid concentrations. The scores are indicative of the absorption strength of chromophores absorbed in the 460- to 520-nm wavelength range. This range encompasses α - and β -carotenes, β -cryptoxanthin, lycopene, lutein,

and zeaxanthin, while minimizing the potential interference effect induced by confounding chromophores, including oxy- and deoxyhemoglobin and melanin (20). Fingertips are the recommended tissue of measurement owing to their minimal melanin content, although no significant correlations have been observed between melanin and reflection spectroscopy carotenoid measurements in a sample of Japanese subjects (melanin indexes: 0.4–1.5) (19). One index finger free of dirt or cuts was placed in between a clear convex lens through which a light source was projected. Such a position temporarily pushes the blood away from the measured area in order to minimize the confounding effect of hemoglobin absorptions. The Veggie Meter scores are logarithmic scale values based on reflectivity (20). The device was calibrated and cleaned before each assessment. Three single scans were taken at all 3 laboratory visits; thus, the overall mean of 9 scores was included in the present analyses.

MPOD

MPOD was measured using customized heterochromatic flicker photometry (cHFP) via a macular densitometer (Macular Metrics Corporation). This technique was described in detail previously (21). The procedure has been modified to accommodate a pediatric sample and the modifications have been described previously (22). Briefly, subjects were instructed to judge the presence of a flickering light of 2 varying wavelengths (460 nm and 570 nm). Trained examiners manipulated the radiance of the flickering light with a shorter wavelength while the longer wavelength was held constant, until the subject indicated a null flicker zone to determine peak wavelength absorption. MPOD was measured at the fovea (maximal macular pigment) and para-fovea (minimal macular pigment). The reliability of the modified procedure has been previously demonstrated in preadolescent children (23). Three MPOD values were collected on nonconsecutive days for each subject. MPOD measurement was attempted at every laboratory visit. However, some participants reported they were unable to detect a null flickering zone (i.e., the stimulus never seemed to stop flickering). On very few occasions, the participants were not able to focus on the test. Therefore, no MPOD score was obtained in the aforementioned scenarios. The mean of all values was included in the final analyses. Mean MPOD values >1 or <0 were excluded.

Anthropometric and adiposity assessment

Height and weight were measured using a stadiometer (model 240; Seca) and a Tanita WB-300 Plus digital scale (Tanita), respectively, in order to compute subjects' BMI percentiles. Height and weight measurements were both repeated 3 times, and the mean of the 3 values was used in the present analyses. BMI percentile was calculated based on CDC growth charts for children and teens (ages 2–19 y), accounting for age and sex in addition to height and weight (24). Visceral adipose tissue (VAT) mass and %Fat were measured using DXA with a Hologic Horizon W (APEX Software version 5.6.0.5; Hologic). DXA has been validated as a reliable and accurate measure of adiposity in children (25).

Dietary assessment

Participants and their parents were provided with a 7-d diet record and detailed instructions by a trained member of the research staff. The diet records were returned at a subsequent laboratory visit. The record contained written instructions for recording food intake, includ-

ing how to describe food preparation methods, added fats, brand names, ingredients of mixed dishes and recipes, and visual estimation of portion size (i.e., 1 cup is approximately the size of an adult fist). Trained staff entered food records into the Nutrition Data Systems-Research version 2015 (Nutrition Coordinating Center, University of Minnesota) software. All subjects included in the current analyses provided diet records for ≥ 6 d, which captured their weekday and weekend eating history. Records that contained < 6 d or substantially missing information were excluded from the analyses. The primary dietary variables of interest included total energy (kcal) as well as the total carotenoids, which were calculated from the sum of lutein and zeaxanthin, β -carotene, α -carotene, β -cryptoxanthin, and lycopene.

Health history and demographics questionnaires

A pediatric health history survey was administered to 1 parent of each participant. This was a retrospective survey that included questions regarding early-life events including, but not limited to, gestational age, birth weight, breastfeeding duration, and formula-feeding duration. Exclusive breastfeeding was defined as “no formula or solids at all,” whereas nonexclusively breastfeeding was defined as “including formula and other foods” in the survey. Weight-for-gestational-age percentile was calculated based on the CDC growth charts, accounting for sex, gestational age, and birth weight (26). The 2013 Fenton chart was used for preterm infants (27). The application and validity of this survey have been described previously (28). Parents of participants also provided health and demographic information such as household income, parental education, race, and ethnicity.

Statistics

All statistical procedures were computed using SPSS version 24 (IBM). An α threshold of $P = 0.05$ was used to determine statistical significance. Two-tailed Pearson correlations were conducted to examine the relations between MPOD, skin carotenoids, weight status (BMI percentile), adiposity (%Fat and VAT), breastfeeding (exclusive and nonexclusive), diet (energy intake, total carotenoids), weight-for-gestational-age percentile, mother education, and household income. We did not conduct false discovery rate correlation because the bivariate correlations represent secondary analyses and were designed to confirm covariates to be included in the primary mediation analyses. An independent Student t test was used to compare the difference in Veggie Meter mean scores between subjects split by median exclusive breastfeeding duration. Mediation analyses were conducted using the PROCESS macro for SPSS developed by Preacher and Hayes (29, 30). The overall effect of the independent variable on the dependent variable is denoted by c (total effect). The c' coefficient represents the effect of the independent variable on the dependent variable adjusting for the mediating variable (direct effect). Path a represents the effect of the independent variable on the mediating variable and path b denotes the effect of the mediating variable on the dependent variable. Simple mediation analyses were conducted to determine the mediating effect of BMI percentile, %Fat, and VAT on the relation between breastfeeding duration and skin carotenoids. MPOD was not included in the mediation analyses given that no significant correlations were detected between MPOD, breastfeeding duration, breastfeeding exclusivity, BMI percentile, and adiposity measurements. The linearity, homoscedasticity, and estimation error assumptions were tested using the multiple regression standardized

predicted and residual values. All assumptions for mediation analyses were tested and met. Total energy intake is a known factor influencing body weight and thus was included in the mediation model as a covariate. Dietary carotenoids were adjusted for owing to their effect on skin carotenoid accumulation (31). Mother education was also included as a covariate to capture the potential impact of socioeconomic status (SES) on breastfeeding practice. An unadjusted model and a model adjusted for total energy intake, dietary carotenoids, and mother education were analyzed for each proposed mediator. Bootstrapping is a modern approach that overcomes the limitations of the Sobel test to estimate the significance of the indirect effect, which is calculated as the product of the a and b coefficients. It is recommended to perform bootstrapping with 5000 samples (32). Current analyses used 10,000 samples to increase the accuracy of the estimations. The indirect effect was interpreted as significant if the bootstrapping 95% CIs did not contain 0.

Results

The final analyses included 81 participants (39 females). The mean age was 9.4 y old. Over 80% of mothers had a bachelor's degree or an advanced degree. The sample was comprised of predominantly white or Caucasian children ($n = 55$, 68%). Out of 81 participants, 5 identified as Hispanic. The majority (73%) of the participants were between the 5th and the 85th BMI percentiles (normal weight), whereas 22% of the participants had overweight or obesity (i.e., ≥ 95 th BMI percentile). The mean %Fat of the sample was $\sim 32\%$ which is consistent with the national average for this age group (33). The mean \pm SEM for total carotenoid intake was 6.5 ± 0.4 mg, with lycopene (3.8 ± 0.3 mg) being the most abundant, followed by β -carotene (1.5 ± 0.1 mg), lutein and zeaxanthin (0.8 ± 0.07 mg), α -carotene (0.3 ± 0.05 mg), and β -cryptoxanthin (0.08 ± 0.01 mg) being the least abundant carotenoid in the diet of this sample. The mean daily energy intake was 1739 kcal which falls within the estimated caloric needs for this age group (34). The mean duration the participants received any breast milk was 12.9 ± 9.1 mo; the durations for exclusively breastfeeding and nonexclusively breastfeeding were 4.8 ± 3.5 mo and 8.2 ± 8.1 mo, respectively. Approximately 90% of the participants received breast milk at some point during infancy and only 8 participants were exclusively formula-fed. Approximately 81% of the subjects were exclusively breastfed through 3 mo and 80% of the subjects had a total breastfeeding duration > 6 mo. The mean skin carotenoid score measured by the Veggie Meter was 304. The mean MPOD score was 0.56. Out of the 81 participants included in the analyses, there was 1 participant that only had 1 MPOD measurement, 9 participants that only had 2 MPOD measurements, and 6 participants that did not have any MPOD data. We excluded the 6 participants that did not have any MPOD data and 4 participants for having a mean MPOD score > 1 or < 0 . Therefore, the mean MPOD score was reflective of 71 participants in total. **Table 1** summarizes all descriptive data.

Table 2 presents the results from the 2-tailed Pearson correlations. BMI percentile, %Fat, and VAT were all inversely correlated with skin carotenoids. Age was positively correlated with VAT but not BMI percentile or %Fat. Male sex was correlated with lower %Fat but higher VAT. MPOD was positively correlated with age and inversely correlated with household income. Ma-

TABLE 1 Participant demographics and characteristics¹

Variables	Values
At school-age (age 7–12 y)	
Age, y	9.4 ± 1.6
Females	39 (48)
Mother education	
High school graduate	4 (4.9)
Some college	10 (12.3)
Bachelor's degree	34 (42.0)
Advanced degree	33 (40.7)
Race	
American Indian or Alaska Native	0 (0)
Asian	12 (14.8)
Black or African American	5 (6.2)
Native Hawaiian or Pacific Islander	0 (0)
White or Caucasian	55 (67.9)
Mixed or other	9 (11.1)
Hispanic	5 (6.2)
Household income, ² \$	
<40,000	16 (19.8)
40,000–70,000	25 (30.9)
70,000–100,000	18 (22.2)
>100,000	20 (24.7)
BMI-for-age percentile	58.4 ± 29.0
Underweight (<5th)	4 (4.9)
Normal weight (≥5th and <85th)	59 (72.8)
Overweight (≥85th and <95th)	7 (8.6)
Obese (≥95th)	11 (13.6)
Whole-body adiposity, %	31.5 ± 6.8
Visceral adipose tissue, g	185.9 ± 94.2
Total carotenoid intake, mg	6.5 ± 3.9
Lycopene, mg	3.8 ± 3.0
β-Carotene, mg	1.5 ± 1.3
Lutein and zeaxanthin, mg	0.8 ± 0.7
α-Carotene, mg	0.3 ± 0.4
β-Cryptoxanthin, mg	0.08 ± 0.09
Total energy intake, kcal	1738.8 ± 435.3
Skin carotenoid scores	304.1 ± 100.7
MPOD ³	0.56 ± 0.2
Retrospective response	
Total breastfeeding duration, mo	12.9 ± 9.1
Exclusive breastfeeding duration, mo	4.8 ± 3.5
Nonexclusive breastfeeding duration, mo	8.2 ± 8.1
Ever exclusively breastfed	73 (90.1)
Exclusively formula-fed	8 (9.9)
Birth-weight-for-gestational-age percentile	59.7 ± 29.0
Small (<10th)	5 (6.2)
Normal (≥10th and ≤90th)	60 (74.1)
Large (>90th)	16 (19.8)

¹*n* = 81. Values are *n* (%) or mean ± SD. MPOD, macular pigment optical density.

²Preferred not to answer, *n* = 2.

³*n* = 71.

ternal education was positively correlated with total breastfeeding duration ($r = 0.24$, $P = 0.03$). In contrast, household income was not correlated with any of the breastfeeding measures. The total breastfeeding duration was inversely correlated with BMI percentile and VAT. Nonexclusive breastfeeding duration was not correlated with any of the adiposity and body weight outcomes. Skin carotenoid scores were positively correlated with dietary carotenoid intake. Subjects who were exclusively breastfed for <5 mo ($n = 40$, Veggie Meter score = 279) had significantly lower Veggie Meter scores than those who had an exclu-

sive breastfeeding duration ≥5 mo ($n = 41$, Veggie Meter score = 329; $P = 0.03$) (Figure 1).

Figure 2 presents the results from the unadjusted mediation analysis. The mediation effect through BMI percentile and VAT was trending but not significant without covariates. After the adjustment for dietary carotenoids, total energy intake, and mother education, there was a significant indirect effect of exclusive breastfeeding duration on skin carotenoids through BMI percentile (ab : 2.43; 95% CI: 0.21, 7.67) and VAT (ab : 2.13; 95% CI: 0.52, 4.63). Figure 3 presents the results from the adjusted models. %Fat did not appear to be a mediator for the relation between exclusive breastfeeding duration and Veggie Meter scores.

Discussion

The present study investigated the relation between breastfeeding and carotenoid status among school-age children, as well as the mediating effect of weight status and adiposity during school-age on this relation. BMI percentile and VAT showed significant mediating effects on the relation between exclusive breastfeeding duration and Veggie Meter scores, after the adjustment for dietary carotenoids, total energy intake, and mother education. No mediation effect was detected for %Fat. To our knowledge, no previous study has examined the relation among breastfeeding, skin carotenoids, weight status, and adiposity among school-age children.

Interestingly, MPOD was not associated with dietary carotenoid intake, BMI percentile, %Fat, VAT, skin carotenoid concentrations, total breastfeeding duration, or exclusive breastfeeding duration. Although reduced MPOD has been observed in adults with obesity or overweight compared with their healthy-weight counterparts (7, 35, 36), this relation has not been replicated in pediatric samples (37, 38). Similarly, no correlations were observed between MPOD and BMI percentile and adiposity measurements in our sample. It has been proposed that greater adiposity might induce greater low-grade inflammation and oxidative stress (39), which could reduce macular carotenoid concentrations. The absence of a correlation between MPOD and weight and adiposity in children might reflect lower absolute adiposity than in adults and therefore lower cumulative oxidative impact on macular pigment. In contrast, skin carotenoids appeared to be more sensitive to weight status and adiposity, as evidenced by the inverse correlations between Veggie Meter score and BMI percentile, %Fat, and VAT. The discrepancies between skin and macular carotenoids in relation to weight and adiposity might be explained in 2 ways. First, skin carotenoid concentrations are more responsive to dietary carotenoid intake than are macular carotenoid concentrations. Several intervention studies have found inconsistent improvement in MPOD after lutein and zeaxanthin supplementation (40–42). The identification of the lutein (steroidogenic acute regulatory domain 3) and zeaxanthin (glutathione S-transferase 1) binding proteins in the retina (43, 44) may suggest that macular carotenoid accumulation follows different kinetics than carotenoid deposit in other organs in the body. Second, skin carotenoids measured by Veggie Meter in the present study included α- and β-carotenes, β-cryptoxanthin, lycopene, lutein, and zeaxanthin, whereas macular pigment only includes lutein, zeaxanthin, and meso-zeaxanthin. The differences in these 2 carotenoid measurements might help explain why

TABLE 2 Bivariate correlations for relations between skin carotenoid scores, MPOD, BMI percentile, adiposity, breastfeeding, demographics, diet, weight-for-gestational-age percentile, mother education, and household income¹

Variables	BMI percentile	%Fat	VAT	Skin carotenoids	MPOD ²
BMI percentile	—	0.75**	0.66**	−0.39**	−0.15
%Fat	—	—	0.55**	−0.40**	−0.11
VAT	—	—	—	−0.39**	−0.01
Skin carotenoids	—	—	—	—	0.08
Age	−0.06	−0.07	0.28*	−0.07	0.26*
Sex	−0.04	−0.38**	0.27*	−0.09	−0.02
Mother education	0.08	0.13	−0.11	−0.02	−0.18
Household income ³	0.09	0.15	−0.06	−0.05	−0.26*
Total breastfeeding duration	−0.24*	−0.17	−0.24*	0.14	−0.04
Exclusive breastfeeding duration	−0.21	−0.17	−0.22	0.21	0.02
Nonexclusive breastfeeding duration	−0.18	−0.11	−0.17	0.06	0.04
Total energy intake	−0.12	−0.09	0.01	0.02	0.23
Dietary carotenoids	−0.04	−0.09	−0.14	0.25*	0.13
Weight-for-gestational age-percentile	0.12	0.06	0.10	−0.17	0.04

¹MPOD, macular pigment optical density; VAT, visceral adipose tissue; %Fat, whole-body total fat percentage.

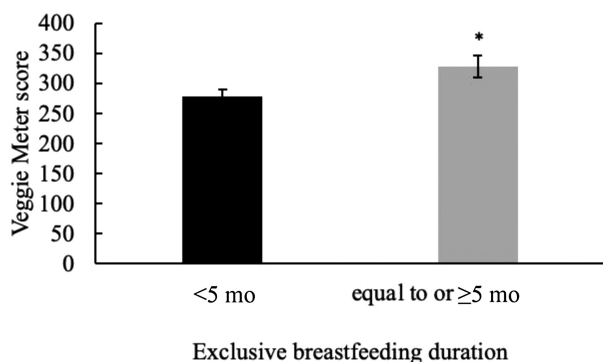
² $n = 71$.

³ $n = 79$ (2 preferred not to answer).

***Significant correlation: * $P < 0.05$ (2-tailed); ** $P < 0.01$ (2-tailed).

skin carotenoids measured by Veggie Meter and MPOD measured by cHFP resulted in incongruent correlations.

Evidence from epidemiological studies in recent years has shown that breastfeeding is related to a lower risk of childhood obesity (45). A meta-analysis including 25 studies and 266,508 subjects from 12 countries found a stepwise decrease of childhood obesity with increasing duration of breastfeeding, suggesting a dose-response effect. Specifically, children who were breastfed for ≥ 7 mo had a significantly lower risk of obesity (10). Although the underlying pathways for the protective effect of breast milk on childhood obesity are unclear, many possible mechanisms have been proposed to delineate this association. First, the intestinal microbiome profile of breastfed infants tends to be dominated by *Bifidobacteria* and *Lactobacillus*, whereas formula-fed infants tend to have more diverse microbiome communities that resemble those of older children. The differences might be due to the microbes in breast milk or on areolar skin, and components in breast milk that promote the growth of certain gut microbes in infants (46). The bifidogenic effect of breast milk has been studied in the past (47).

**FIGURE 1** Comparison of mean Veggie Meter scores between subjects split by median exclusive breastfeeding duration (5 mo).

Further, breast milk contains oligosaccharides which, although they are indigestible for the purpose of energy production by humans, promote the growth of *Bifidobacteria* (48, 49). *Bifidobacteria* has been associated with antiobesity and lipid-lowering effects in children (50). In addition to the physiological mechanisms proposed, environmental factors such as SES and stress have an impact on children's weight later in life. Higher SES has been associated with a longer duration of breastfeeding, which was in turn associated with a lower BMI in childhood (51, 52). Maternal education has been shown to have a larger impact on breastfeeding duration than have maternal income or maternal employment (53). Similarly, in the present study, we found that maternal education was positively correlated with total breastfeeding duration. In contrast, household income was not correlated with any of the breastfeeding measures. Nonetheless, the impact of SES factors on breastfeeding practice is complex. For instance, although more advanced maternal education might be linked to a better knowledge of the benefits of breastfeeding, higher education is linked to higher maternal employment, which has been shown to decrease breastfeeding (53).

The relation between body composition and skin carotenoid concentrations has been investigated in adults. A cross-sectional study in a group of ethnically diverse adults in New Zealand found an inverse correlation between skin carotenoid concentrations and BMI (54). This correlation was confirmed in a sample of Japanese adults (55). Few studies have examined this association in pediatric populations. In a sample of preschool-age children from low-income households, an inverse association was observed between skin carotenoid status measured by Resonance Raman spectroscopy and BMI percentile (8). To our knowledge, the present study is the first investigation of the association between skin carotenoid concentrations measured by Veggie Meter and adiposity measures in children. Given that adipocytes are the major reservoirs for carotenoids, it is possible that a higher degree of adipose tissue accumulation may limit the availability of carotenoids for other tissues. However, evidence has also indicated that individuals with obesity tend to have lower carotenoid concentrations in adipose tissue

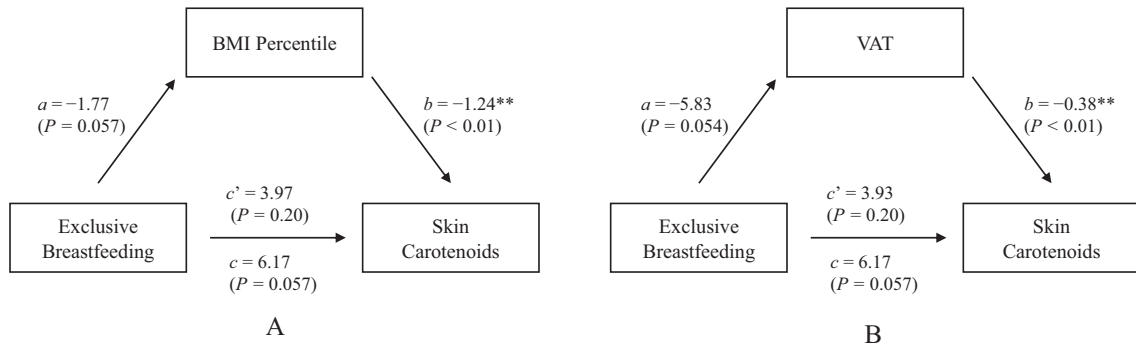


FIGURE 2 Results of mediation regression analyses for (A) BMI percentile and (B) VAT without covariates. VAT, visceral adipose tissue.

(56); thus, additional work is needed to understand the metabolism and sequestration of carotenoids across body tissues among persons with obesity. The exact mechanism behind the inverse association between skin carotenoid concentrations and body weight or adiposity is unclear. Similar to the potential effect of adiposity on MPOD, individuals with increased VAT may have a greater demand for carotenoids to combat the associated oxidative stress. Further, a study examined adipose tissue biopsy samples of 25 healthy adults and observed that abdominal adipose tissue had the highest concentration of carotenoids, resulting in fewer carotenoids being available in other body tissues (i.e., skin and macula) (57). Intriguingly, the inverse associations between skin carotenoid concentrations and body weight and adiposity measures held in our predominantly healthy-weight sample.

A few limitations of the current study are worth noting. First, the breastfeeding information was collected through self-report questionnaires ≤ 12 y after childbirth. Generally, the validity and reliability decrease as the time between breastfeeding and data collection increases. A review article found that the recall is most accurate during the first 3 y after childbirth (58). However, an investigation ($n = 374$) of maternal recall of breastfeeding practices 20 y after childbirth found a strong correlation between recorded and recalled breastfeeding duration (intraclass correlation coefficient = 0.82, $P < 0.001$) (59). Although discrepancies were reported between recorded and recalled dates, no apparent pattern of underestimation or overestimation has been identified. Ow-

ing to the retrospective nature of the present study, we were unable to examine the validity and reliability of the breastfeeding dates of the current sample. Nevertheless, further investigation of breastfeeding practices should aim to collect such data as close to childbirth as possible. Further, we were unable to obtain information regarding the nutrient composition and exact amount of breast milk owing to the retrospective study design. In addition, because only 1 subject in our sample was exclusively formula-fed, we did not have enough statistical power to test the effect of formula feeding on carotenoid status. In addition, the majority (73%) of the subjects were of healthy weight. Additional research is needed to test this relation in samples of children with higher overweight and obesity. However, the results from the present study suggest that greater adiposity is associated with lower skin carotenoids even when a healthy weight is maintained, which provides support for taking preventative actions toward childhood obesity before its onset. Further, the nature of the retrospective study does not support causality. Finally, the sample consisted of predominantly Caucasian and socioeconomically advantaged families. Indeed, the total breastfeeding duration and the exclusive breastfeeding duration of the present sample were notably longer than the national averages (60). For infants born in 2017 in the United States, 58% were breastfed at 6 mo and 47% were exclusively breastfed through 3 mo, whereas the rates in our sample were 80% and 81%, respectively. Therefore, further investigations should be conducted among a larger and more demographically diverse

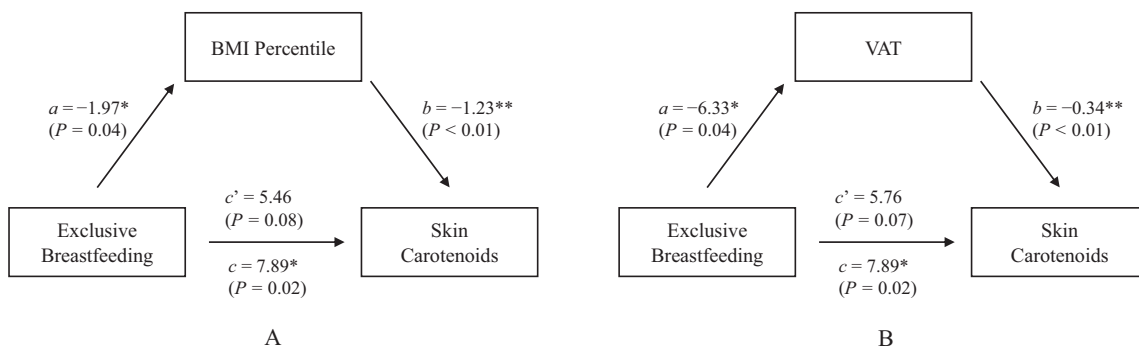


FIGURE 3 Results of mediation regression analyses for (A) BMI percentile and (B) VAT adjusting for total energy intake, total carotenoid intake, and mother education. VAT, visceral adipose tissue.

population. Nevertheless, the limitations notwithstanding, there are several strengths of the present work. To our knowledge, this is the first investigation of the impact of breastfeeding on carotenoid status in school-age children, albeit in a retrospective manner. In addition, anthropometric information was collected through measurements in the laboratory, which prevented the likely bias and inaccuracy from self-report data. We also used high-quality adiposity measurement techniques (i.e., DXA) to assess VAT and %Fat, in lieu of waist circumference. Finally, we assessed carotenoid status at multiple time points in 2 different tissues. The noninvasive nature of Veggie Meter and MPOD is particularly advantageous when used in children.

Our results indicated a positive correlation between exclusive breastfeeding duration and skin carotenoids mediated by weight status and VAT in children aged 7–12 y. These findings provide further support for the WHO recommendations to promote exclusive breastfeeding. Furthermore, body weight and adiposity play an important role in the interplay between breastfeeding practices and skin carotenoid concentrations in childhood.

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