SKIN CAROTENOID ACCUMULATION IN RESPONSE TO A TWO-WEEK SWEET POTATO SNACK ADDED TO THE USUAL DIET

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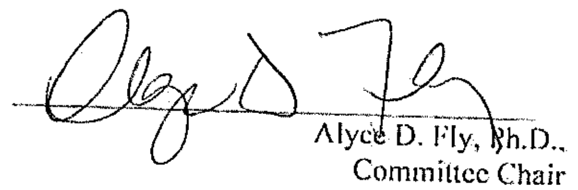

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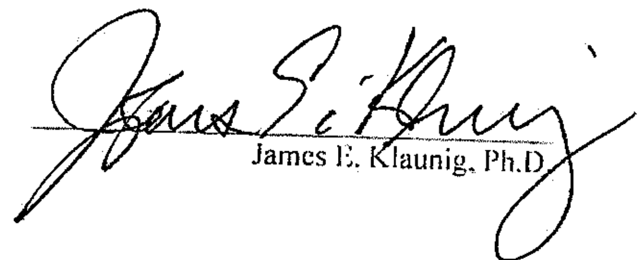

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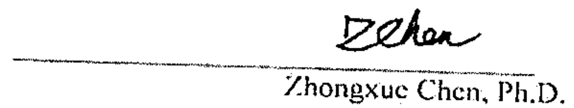

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This manuscript is dedicated to the memory of my aunt, Marisa-Rose Cashin, Ed.D. Through her graceful courage, she showed me how to push past my personal limitations and believe in myself. I am forever thankful for her guidance and love!

Taylor Erickson

Skin Carotenoid Accumulation in Response to a Two-Week Sweet Potato Snack Added to the Usual Diet

A lack of consistency exists in the literature regarding the most appropriate time to measure the effectiveness of nutrition interventions by way of pressure-mediated reflectance spectroscopy (RS). There is a growing use of non-invasive methods like pressure-mediated RS to objectively measure fruit and vegetable intake in different populations, particularly in the context of nutrition interventions. This study evaluated the changes in those measurements, skin carotenoid scores (SCS), from baseline SCS values both during and following a two-week intervention promoting increased red-orange vegetable consumption on top of a participant's usual diet. Specifically, it investigated a general time frame where measurement of SCS would be most advantageous for determining the effectiveness of a nutrition intervention.

To test the hypothesis that SCS values would be different from baseline SCS values both during and following the intervention, participants took part in a three-phase nutrition intervention that included a 1-week baseline period, a feeding period in which participants were fed a sweet potato snack 3 times a week for two weeks on top of their usual diet, and a 4-week monitoring period. SCS were measured through each of the three periods with pressure-mediated RS. The scores were analyzed using a linear mixed model with repeated measures for participants over time to determine whether skin carotenoids increased from baseline to the follow up points of the intervention and post-intervention periods. The results showed that change in mean SCS from baseline over time was significant. While SCS during the intervention period were not significantly higher than baseline, SCS at post-intervention were significantly higher than baseline on average.

Observations of these data suggest that 2 to 3 weeks after the beginning of a two-week intervention may be a period of interest when measuring the efficacy of such an intervention. On this basis, future studies should be designed for participant final measurements to be made approximately 2 to 3 weeks following a similar dietary intervention.

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INTRODUCTION

Carotenoids are lipophilic pigments most commonly found in plants like leafy green vegetables and red-orange-yellow fruits and vegetables (1, 2). There are over 600 known carotenoids, but fewer than 60 are found in the diet and less than 20 are found in human blood and tissues (2, 3). These compounds can be classified as either vitamin A precursors (provitamin A) or not. The carotenes, such as, α-carotene, β-carotene, and β-cryptoxanthin, possess provitamin A activity since they can be converted into retinol in the body (2). Other carotenes, such as lycopene, and the xanthophylls, such as lutein and zeaxanthin, do not possess provitamin A activity, but they still provide health benefits like antioxidant activity and photoprotection (3, 4). In fact, all carotenoids are known to be efficient quenchers of free radicals and potent scavengers of reactive oxygen species (ROS) (3, 5). Their antioxidant properties are of great interest in the field of public health, particularly in the prevention of chronic diseases like cardiovascular disease and cancers (1, 3).

Humans cannot synthesize carotenoids, so they must get them from their diet, usually in the form of fruits and vegetables. In fact, carotenoids comprise the majority of vitamin A intake in the U.S. (2). The most commonly consumed carotenoids in the U.S. are α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin (6, 7). After absorption in the intestine, most carotenoids are transported from the lymph into the blood by lipoproteins. As a result of their varying chemical composition, the bioavailability and accumulation of the different carotenoids in specific locations of the body is highly variable (3, 4, 6, 8-12). However, the carotenoids that are incorporated into the body can then be measured, from blood, tissue, or skin, as a biomarker of fruit and vegetable consumption (13-15). Carotenoids in the blood are

representative of short-term carotenoid availability, whereas the skin and other tissues serve as long-term reservoirs of these compounds (6, 9, 13, 16-18).

It is well-documented that carotenoid concentration in the blood and skin correlates with fruit and vegetable intake and other lifestyle factors like stress and tobacco/alcohol use (4, 6, 10, 14, 15, 19). Traditional carotenoid measurement techniques include extraction from blood or biopsy of skin, and detection through high performance liquid chromatography (HPLC), making them the "gold standard" as biomarkers for carotenoid status, and generally reflect fruit and vegetable intake (6). However, these methods for collecting samples are highly invasive to subjects and their subsequent analysis via HPLC is expensive (4, 13, 18). These issuess make these approaches impractical as biomarkers for large nutritional interventions and epidemiological studies (14). Additionally, samples collected for HPLC analysis have a short half-life and can degrade rapidly if not handled properly.

Due to the limitations of traditional methods, the literature supports the use of non-invasive techniques for the *in vivo* measurement of skin carotenoids, like resonance Raman resonance spectroscopy (RRS) and pressure-mediated reflectance spectroscopy (RS), which have been documented to correlate with blood concentrations (4, 9, 18-20). Non-invasive methods like RRS and RS are of interest to nutrition scientists and epidemiologists, as quantification of nutrition interventions necessitate simple, effective, and inexpensive biomarkers of fruit and vegetable intake.

While time to peak carotenoid accumulation in the blood (a time-course) has been established (8, 10, 17, 21), there is a lack of consistency across studies regarding an approximate time-course of skin carotenoid accumulation after consumption of fruit and vegetables. The growing interest in and use of non-invasive measures, particularly RS (7, 19, 22, 23), necessitate understanding the time-course of skin carotenoid accumulation. This is crucial to establishing RS

measurement and study design protocol for nutrition interventions that are often aimed at increasing FV consumption in the study population, particularly FV of concern like dark leafy greens and red/orange vegetables. Since many nutrition interventions aim to promote increased consumption of FV of concern, it is also important to establish whether the consumption of the recommended weekly intake of these vegetables can lead to a detectable difference in skin measurements. These findings will inform nutrition scientists and epidemiologists when designing and assessing intervention programs.

Therefore, the purpose of this investigation is: 1) to evaluate changes in skin carotenoid score (SCS) from baseline measured by pressure-mediated RS during a two-week long supplement promoting increased red/orange vegetable consumption per the USDA 2015-2020 Dietary Guidelines for Americans (24) on top of a subject's usual diet and 2) to evaluate changes in SCS from baseline measured by pressure-mediated RS following a two-week long supplement promoting increased red/orange vegetable consumption per the USDA 2015-2020 Dietary Guidelines for Americans on top of a subject's usual diet.

METHODS

Study design

A longitudinal cohort study was conducted where participants were followed for 47 days. The study had three phases; a baseline period consisting of 3 measurement days over a week; a 2-week dietary intervention including 3 measurement days for each of 2 weeks; and a post intervention period with measurements twice a week for 4 weeks.

Participants

Participants were recruited by convenience sampling at Indiana University, e.g., email, flyers, and word-of-mouth, into 3 recruitment waves during the summer of 2020. Upon expressing interest in the study, participants were contacted through email to schedule a screening appointment. Eligibility requirements were modified from those proposed to include a wider age range due to a paucity of responses to recruitment as the university largely closed the campus due to the COVID-19 pandemic. Eligibility criteria were affiliation with IU as a student, staff, or faculty member, and between the ages of 18 to 65 years old. Exclusion criteria were outlined in the screening survey (Appendix C) and included: answered “yes” to any of the 5 COVID screening questions developed based on criteria used by CDC (25) and Indiana State Department of Health (26); had psoriasis; used self-tanner/spray-on tanning at the time; smoked or vaped tobacco products at the time; drank heavily at the time defined as “a pattern of drinking that brings blood alcohol concentration (BAC) to 0.08 g/dL… [which] typically occurs after 4 drinks for women and 5 drinks for men—in about 2 hours” (27); had been diagnosed with liver disease; unwilling to eat sweet potatoes; and/or had a fat malabsorption disorder. Pregnant women were also excluded from the study. These exclusions were chosen because skin carotenoid score and carotenoid metabolism can be confounded by pigment abnormalities,

interactions with alcohol, malabsorption disorders, and in the case of smoking, carotenoids can be metabolized due to excessive oxidative stress (19, 28, 29).

Due to a lack of similar studies in the literature, adequate participant number (sample size) was estimated through a calculation using G*Power (30) ver. 3.1.9.4 (Franz Faul, Universität Kiel, Germany), where, an effect size was selected as d=.5, a "medium" effect, where d is a standardized mean change from baseline, d=Mean change/SD(31), and 80% power to detect a significant change in SCS was selected, yielding 34 participants. Forty participants were recruited assuming at least a 10% loss in participants due to drop out over the 7-week study period. Participants were monetarily compensated for their participation at the end of Week 1 (day 5; $25), Week 3 (day 19; $60), and Week 7, on the final day of the study (day 47; $120).

Procedures

Participants were recruited to one of 3 waves for a 47-day study. Each wave contained 3 periods including, baseline, feeding intervention, and post-intervention. Participants in a wave started the study on the same day to ensure they were exposed to similar conditions, including, sweet potatoes from the same batch, environmental (season, time of the year), and quality of the LED bulb in the instrument that measured the primary outcome variable, Veggie Meter (Longevity Link Corporation, Salt Lake City, UT).

COVID-19 Screening Protocol

Lab personnel were screened before starting each shift and wore face masks, face shields, lab coats, and gloves when interacting with participants. Social distance (6ft) was maintained except for during the blood collection and measurement of skin carotenoids. Each of these procedures lasted less than 15 minutes. Counter surface areas where participant interaction took place were sanitized with 10% bleach before and after the participant's visit. Participants were asked to wash their hands before and after handling study documents, being measured with the

Veggie Meter, having blood drawn, and eating the sweet potato snack. At each visit, participants were screened for COVID-19 before being allowed to enter the building. Researchers met participants outside the building at the start of their visit to ensure that participants were wearing masks and feeling well. Temperatures were taken with an iHealth Infrared Thermometer (iHealth Labs Inc., San Diego County, California) and recorded on the daily checklist; participants were only allowed to enter the building if their temperature was below 100°F. Participants were then asked the following COVID screening questions:

> 1) Since your last visit, have you had shortness of breath, difficulty breathing, or a cough? Or have you had a combination of at least two of these symptoms: chills, fever, repeated shaking with chills, muscle pain, headache, sore throat, or new loss of taste or smell?; 2) Are you ill or caring for someone who is ill?; 3) Have you been practicing social distancing?; 4) Have you had contact with someone you know to be diagnosed with COVID-19?; 5) Have you traveled on a plane in the last two weeks?

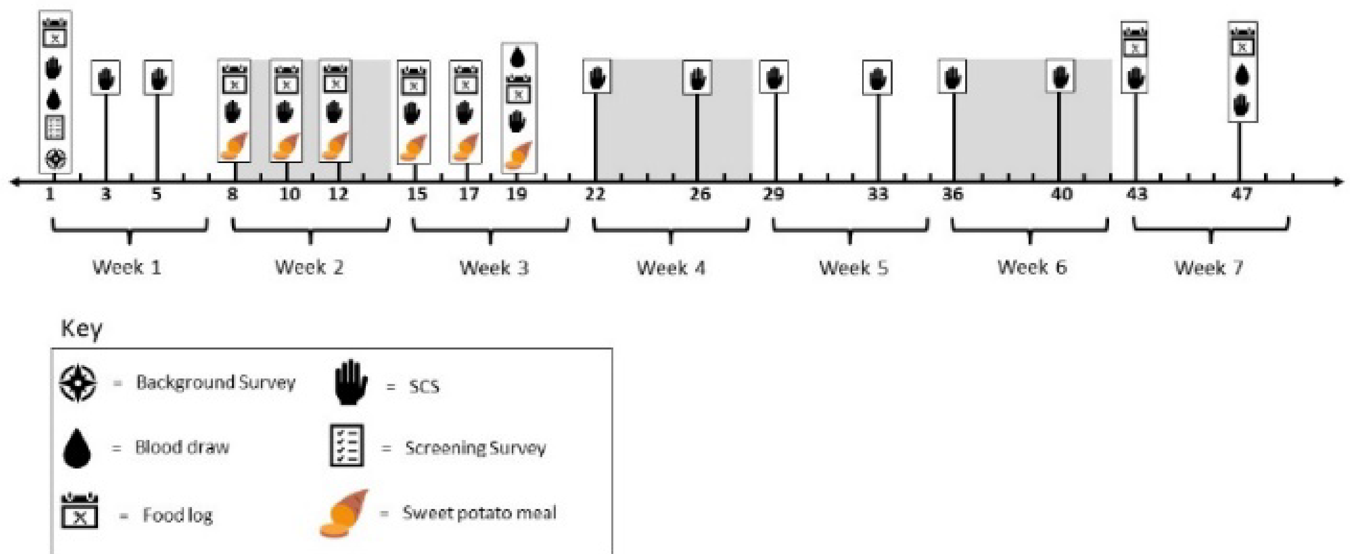
If participants had answered "yes" to any of the questions, they would not have been allowed to proceed further with the study and would be dismissed by the researcher.

Period 1: Baseline

Period 1 was conducted to determine a baseline measure of skin carotenoids and included 3 visits during days 1-7 of the study. The initial appointment (day 1) was the longest of the 17 total visits. After the routine COVID-19 screening, potential participants were screened for eligibility for the study (Appendix C), signed consent documents (Appendix C), and were given a detailed overview of the study timeline (Figure 1). After the screening and informed consent process on Day 1, participants completed a brief background survey (Appendix C) and had their weight (in kg) and height (in cm) measured and recorded for descriptive measures. Weight was measured with an electronic scale (Tanita Model WB-110A NTEP III, Tokyo, Japan) and height

was measured with a free-standing stadiometer (Seca Model 225, Hamburg, Germany). Participants were asked if they wanted a copy of the informed consent for their records and were given a copy if elected. Participants filled out a food record, which was reviewed to fill in missing details by a trained investigator. Days 1, 3, and 5 of Week 1 established the baseline skin carotenoid levels of participants, as they consumed their usual diets. Data at baseline, for blood concentration of carotenoids (via venipuncture), were collected on Day 1. Blood outcomes were not utilized for this study.

Figure 1. Study Timeline



Primary Outcome Marker: Skin Carotenoid Measurements

The protocol for measuring skin carotenoids with the Veggie Meter was adapted from the protocol described by Ermakov et al. (19). Before measurement, participants washed hands with soap and water and dried their hands completely with a paper towel. The non-dominant index finger was measured three times at each visit; dominant index fingers were only used if the participant had a scar or stain on the non-dominant finger. Scores were recorded after each measure on the SCS data sheet (Appendix C) and later entered into a spreadsheet for statistical

analysis. After measurement, participants washed hands with soap and water for safety and the Veggie Meter lens and instrument were cleaned. Baseline skin scores were collected on Days 1, 3, and 5 of Week 1; then averaged to estimate a baseline skin carotenoid score (SCS). Each day that participants came into the lab to eat their sweet potato snack (Days 8, 10, 12 of Week 2 and Days 15, 17, 19 of Week 3), they were also measured for SCS. After the two-week intervention period, participants visited the lab twice a week to be measured for SCS (Week 4-7).

Blood Collection

The protocol for drawing blood via venipuncture was adapted from criteria established by the World Health Organization (32). Blood was collected from participants on Day 1, Day 19, and Day 47. Blood (10 mL) was collected into EDTA-treated lavender-top collection tubes and centrifuged at 1300 g for 10 minutes. The resulting plasma was aliquoted into 1-1.5 mL polypropylene cryotubes and stored at -80°C until analysis. Analysis of carotenoids in the plasma was not conducted as part of the thesis study.

Period 2: Feeding Intervention

Participants were provided with 1 cup-equivalent sweet potatoes at each of 3 visits to the lab during both Week 2 and Week 3 of the study; the recommended weekly level of red/orange vegetable consumption suggested for a 1800-2000 calorie diet in the USDA 2015-2020 Dietary Guidelines for Americans is between 3-5.5 cup equivalents (33, 34). The estimated dose of β-carotene in a single serving is 20,724 µg with very small amounts of α-carotene, based on recipe analysis with NDSR ver. 49 (Nutrition Coordinating Center, Minneapolis, MN) (Appendix D).

Figure 2. Jewel Sweet Potatoes

Preparation of Sweet Potato Snack

Sweet potatoes (Jewel variety, Harvestmark.com, Vardaman, MS), purchased from a grocery store in Bloomington (Kroger, Cincinnati, OH) were washed, wrapped in foil, placed in a glass pan and baked for approximately 2 hours in an oven (GE Café model, Boston, MA) at 350°F. After baking, potatoes were unwrapped, sliced open and flesh was scrapped free from the skin. Flesh was combined in a large mixing bowl and whipped for approximately 2 minutes until homogeneous and smooth (Figure 3). Mashed sweet potatoes from the mixing bowl were weighed into a Pyrex custard cup (180 g/cup). Cups were covered with plastic wrap and frozen at -20°F. Prior to feeding, cups were thawed overnight in the refrigerator, then reheated for 2.5 minutes in a microwave. Butter, unsalted, (Kroger, Cincinnati, OH) was sliced into 5 g pieces and frozen. Each cup with heated sweet potatoes had a 5 g slice of unsalted butter stirred in prior to serving. Butter was added to provide a lipophilic substance to support absorption of carotenoids. All potatoes fed to participants from a particular wave (1-3) came from the same lot.

Figure 3. Sweet Potato Mixture

Estimation of Participant Carotenoid Load

Participants completed 24-hour food records on each of the three visits during Week 1. Food records were entered into the Nutrition Data System for Research (NDSR) software version 49 (2018, Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN) to estimate the baseline carotenoid load of the participants. Food records were completed at each visit, 6 times, during the two-week intervention to provide a 24-hour food record of participant food consumption from the previous day. Participants filled out two final food records on Days 43 and 47 to ensure no changes in usual diet occurred throughout the study. Food records were reviewed for completion with a researcher certified in the conduct of dietary recalls.

Period 3: Monitoring

After the two-week intervention, participants visited the lab twice a week for skin measurements for four additional weeks (Day 22, 26, 29, 33, 36, 40, 43, and 47). In addition to baseline and intervention collection, blood samples were collected from participants on the last day of the study, Day 47. Participants had their weight (in kg) measured on the last day of the study to determine whether significant changes in body size occurred from baseline.

Statistical Analysis

Data Preparation

Data were cleaned to remove implausible values before analysis. One of the time points for a participant in Wave 1 was not included in the final analysis, as the SCS was substantially lower than previous measurements indicating that the Veggie Meter might not have been properly calibrated before use. On visit 13 for Wave 3 participants, the Veggie Meter did not calibrate correctly due to a damaged calibration tool. Measurements for participants on that day were substantially lower than previous values and were not included in the final analysis; 14 measurements were discarded. Over the course of the study, three of the participants in Wave 3 were not able to make 1 or 2 of their visits due to illness; there were 4 time points in total that did not have data and therefore were not used in the final analysis. After invalid or implausible data were removed, SCS triplicate measures for each individual at each time point were averaged to provide the SCS data by visit used in further analysis. The date of the second visit at baseline was set as zero (the midpoint of the 3 data points collected during week one) to determine number of days since baseline.

Participant Characteristics

Descriptive analysis was conducted to report frequency of males/females, mean and median age, weight before (kg), weight after (kg), height (cm), and BMI at baseline. Mean age and mean BMI at baseline were calculated. Frequency of race and Hispanic ethnicity was summarized from the background survey (Appendix C) and ages were summarized from the screening survey (Appendix C).

A paired samples t-test was conducted to determine if there were significant changes in the BMI of participants over the course of the study. Data from the food record analyses of 21 of the 39 participants were analyzed by a second paired samples t-test to determine if there were

significant changes in the diets of participants throughout the study (assessed by calculated carotenoid load). Significance was set at $\alpha=0.05$ for both t-tests.

An independent samples t-test was conducted to determine if there were significant differences in mean baseline SCS between the sexes. A second independent samples t-test was conducted to determine if there were significant differences in mean baseline SCS between participants with BMI > 25 and participants with BMI < 25. A third independent samples t-test was conducted to determine if there was a significant difference in mean SCS at baseline between younger and older females and younger and older males. Significance was set at $\alpha=0.05$ for all tests.

Data were examined to determine how mean SCS over time differed between younger males and females (20-35 years old) and older males and females (36-62 years old) in the study population. Mean SCS for all individuals at each time point was averaged to get a mean SCS over time for each group. Mean SCS for each group were plotted on a scatter plot with error bars for standard deviation.

Descriptive analyses for participant characteristics were performed using IBM SPSS Statistics, version 27 (IBM, Armonk, NY, USA).

Primary Objective

The primary objective for the study was to describe if and when the skin carotenoid score (SCS) increased above the baseline SCS. Each participant's SCS data at the three baseline visits were averaged for an overall baseline score to include in analysis. Days from baseline were calculated for each visit by subtracting the "baseline" date, where the date of the second of the three baseline visits was used as the "baseline" date. To address the study objective, each time period (intervention and post-intervention) was compared to the average of the three baseline SCS measures with a linear mixed model to determine which time period showed a difference

from the baseline average. The null hypothesis was that there is no increase in SCS after the beginning of the intervention. All analyses were performed using the SAS System for Windows, version 9.4 (SAS Institute Inc., Cary, NC, USA).

The MEANS procedure was first used to compute descriptive statistics for the mean change in SCS from baseline at intervention and post-intervention. The MEANS procedure was also used to compute descriptive statistics for the mean SCS change from baseline by days since baseline and visit number.

Data were analyzed using a linear mixed model with repeated measures for participants over time (17 time points) to determine whether skin carotenoids increased from baseline to the follow up points of the intervention (Days 5-19) and post-intervention periods (Days 19-44). While data were included for SCS from each visit, each time point was coded with its time period: baseline/pre-intervention, intervention, or post-intervention. The model effectively compares the average of all data in the intervention period to baseline, and the average of all data in the post-intervention period to baseline to determine when SCS were significantly higher than baseline.

Data for mean change in SCS from baseline over time and mean SCS over time were depicted in scatter plots with a Loess line (locally estimated scatterplot smoothing). A loess line was fit to the data to visualize change over time since no theoretical models of the time-course from intervention to increased skin carotenoid score exist in the current literature.

The MIXED procedure was used to model both the means of the data as well as the variances and covariances in the linear mixed model. Period (baseline/intervention/post-intervention) was the main effect of interest. Covariates for this primary analysis were baseline BMI, baseline carotenoid load, and baseline SCS, as these factors may affect carotenoid absorption and accumulation (29). The correlation structure for all analyses was compound

symmetry indicating that a participant's SCS readings are correlated over time. The significance value was set at two-sided α=0.05 for all analyses.

Residuals of the mean change in SCS values were analyzed and plotted in a histogram to determine if the data met the assumptions of the analysis procedures.

Secondary Objective

Secondary analyses were conducted to determine if the baseline level of skin carotenoids affected the time to detect differences post-intervention. To do this, subgroups were created with tertiles of baseline carotenoid scores, where participants were categorized into Low, Median, and High tertiles. Data were then analyzed using a linear mixed model with repeated measures to determine change of SCS from baseline. Residuals of the mean change in SCS values across tertiles were analyzed and plotted in a histogram to determine the distribution of the data.

The main effect and covariates were fixed. The covariates include period (baseline/intervention/post-intervention), baseline BMI, baseline carotenoid load, group at baseline, and overall group effect (Period x Baseline Group). Simple differences in least square means were calculated for each group at each time point (baseline, intervention, post-intervention). The correlation structure for all analyses was compound symmetry. Significance was set at α=0.05.

Statistical analyses for primary and secondary objectives were performed by the Biostatistics Consulting Center staff at Indiana University.

RESULTS

Participant Characteristics

The study was conducted over an 11-week period (May 18, 2020- July 31, 2020). Forty participants were recruited into 3 waves. Wave 1 consisted of 12 participants, Wave 2 consisted of 12 participants, and Wave 3 consisted of 16 participants. Of the 40 recruited, 5 withdrew from the study for the following reasons: 1 participant became pregnant, 2 participants contracted COVID-19, 1 participant moved out of town, and 1 participant had a family health emergency. The analysis only includes 39 of the 40 original participants, as one of the individuals who withdrew only completed the first week of the study. The 4 other participants that withdrew from the study were included in the statistical analysis because they withdrew in Period 3 (post-intervention), which provided enough data points for the model. The linear mixed model that was used for analysis allows for the inclusion of partial participant data in order to provide unbiased results.

The age of participants ranged from 20-62 years old, with the average age equal to 32.5 ± 11.3 years and median age of 29 years. There were 2.5 times as many females (n=28) as males (n=11). The BMI of participants at baseline ranged from 18.6-47.0, with a mean of 25.5 ± 6.5. Twenty-one participants had a baseline BMI range between 18.5-25, which is considered healthy (35), and 18 participants had a BMI over 25, which is considered overweight or obese (35). Eleven participants were overweight and 7 were obese. Overweight and obese participants were placed in a single group during statistical analysis. None of the participants were underweight, that is, had a BMI less than 18.5. The majority of participants identified their race as “White” (n=27; 69.2%); 9 identified as “Asian” (23.1%); 2 identified as “Black/African American” (5.1%); and 1 participant identified as “Other- Latina” (2.6%). Five participants (12.8%) reported a Hispanic ethnicity.

There was not a significant change in the BMI of participants over the course of the study (P=0.361). The carotenoid loads calculated from the dietary data did not change from baseline to the last week (P=0.675), therefore it does not appear that the participants' diets changed over time.

Table 1 reports baseline mean SCS and baseline standard deviation of SCS for subgroups by sex, BMI, and age group. Males had a mean baseline SCS of 284.93 ± 113.71, which was about 25 points higher than the mean baseline SCS for females, 258.62 ± 93.57, a significant difference (P=0.027). There was not a significant difference in mean baseline SCS between older males and younger males (P=0.816) or between older females and younger females (P=0.738). Mean baseline SCS for participants with a BMI <25 was 262.14 ± 105.93) while baseline SCS for participants with a BMI >25 was 270.19 ± 90.04 which was not significantly different (P=0.433). The baseline mean SCS for all participants was 266.04 ± 100.20.

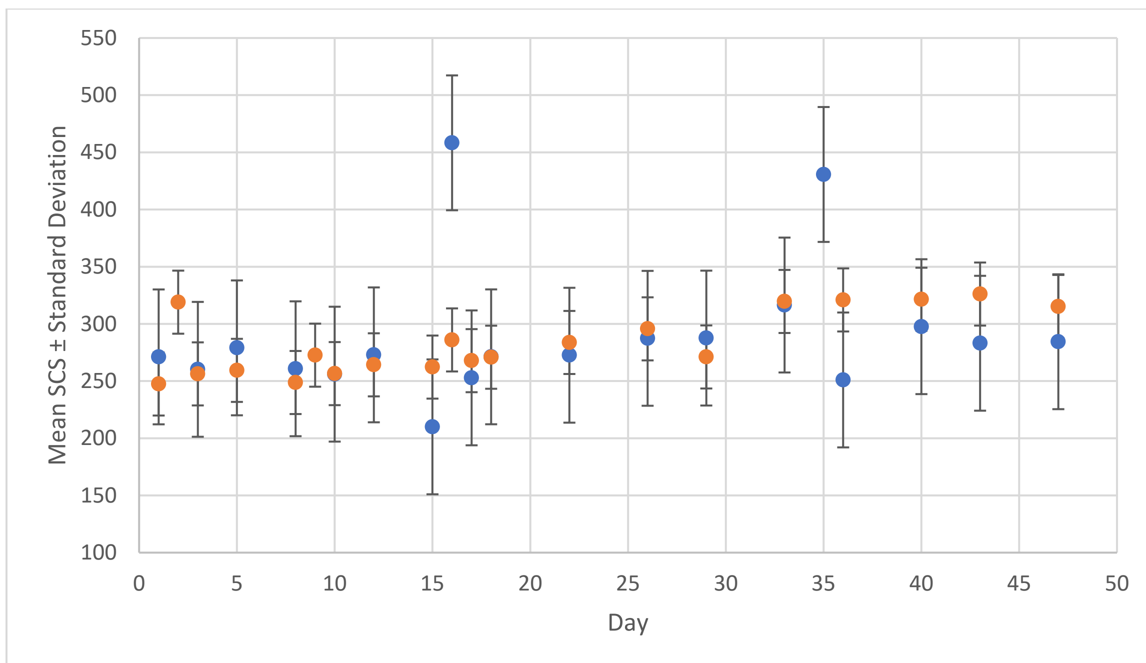
Table 1. Baseline mean SCS by participant characteristics

Participant Characteristics[4]	n	SCS[1] Mean ±SD[2]	P value[3]
Males	11	284.9±113.7	
Older	4	305.0±115.5	
Younger	7	288.9±102.5	0.816
Females	28	258.6±93.6	
Older	6	270.2±120.5	
Younger	22	255.5±86.9	0.738
			0.027*
BMI < 25	21	262.1±105.9	
BMI > 25	18	270.2±90.0	
			0.433
Total	39	266.0±100.2	

[1]SCS, skin carotenoid score
[2]SD, standard deviation
[3] * significant at 0.05 level
[4] "Older": 36-62 years old; "Younger": 20-35 years old

Figure 4 depicts the mean SCS ± standard deviation over time for older women versus younger women, coded as day number. The two outliers in the graph, near day 15 and day 35, are due to a schedule change by the same participant. This participant had to reschedule their appointment for those days, which were not planned as part of the original schedule and had an above average mean SCS in relation to the rest of their cohort, which explains why the data points are skewed.

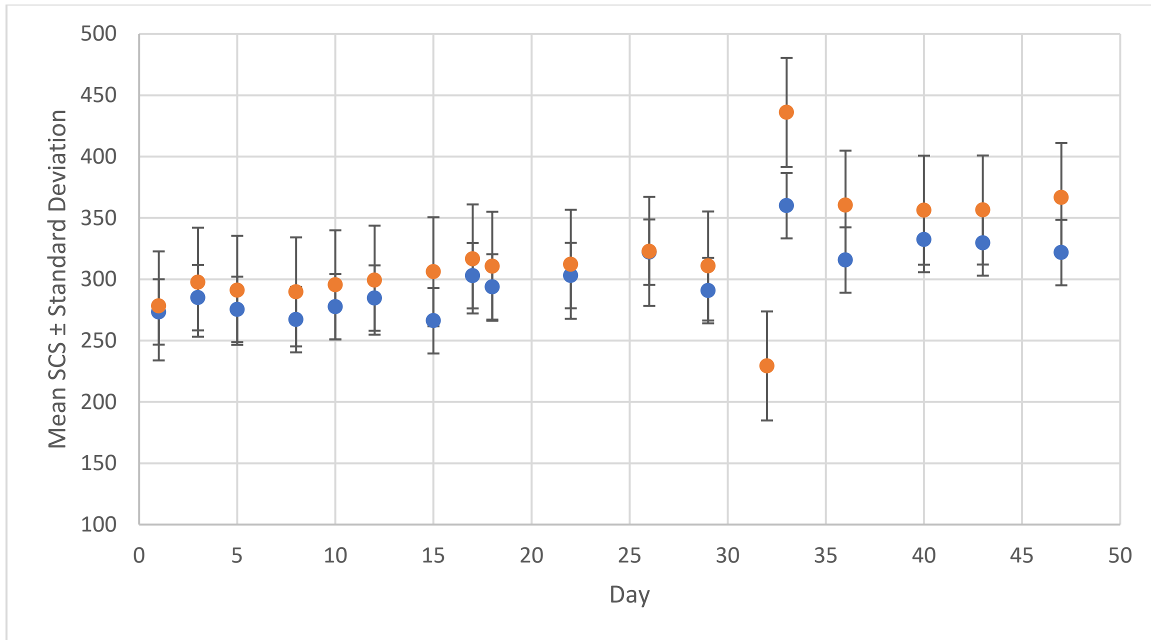
Figure 4. Scatter plot of mean SCS over time for older versus younger women[1]



[1]Older women (36-62 y) = ______ younger women (20-35 y) = ______

Figure 5 depicts the mean SCS over time for older men versus younger men, coded as day number. The outlier for younger men near day 30 was due to a participant in that cohort rescheduling a visit on a day that was not planned as part of the original schedule and had a below average SCS, which explains why there is a dip in the graph.

Figure 5. Scatter plot of mean SCS over time for older versus younger men[1]



[1] Older men (36-62 y) = ______ younger men (20-35 y) = ______

Table 2 reports mean SCS ±standard deviation at each period as well as minimum, maximum, and median values.

Table 2. Mean SCS at baseline, during intervention, and during post intervention period

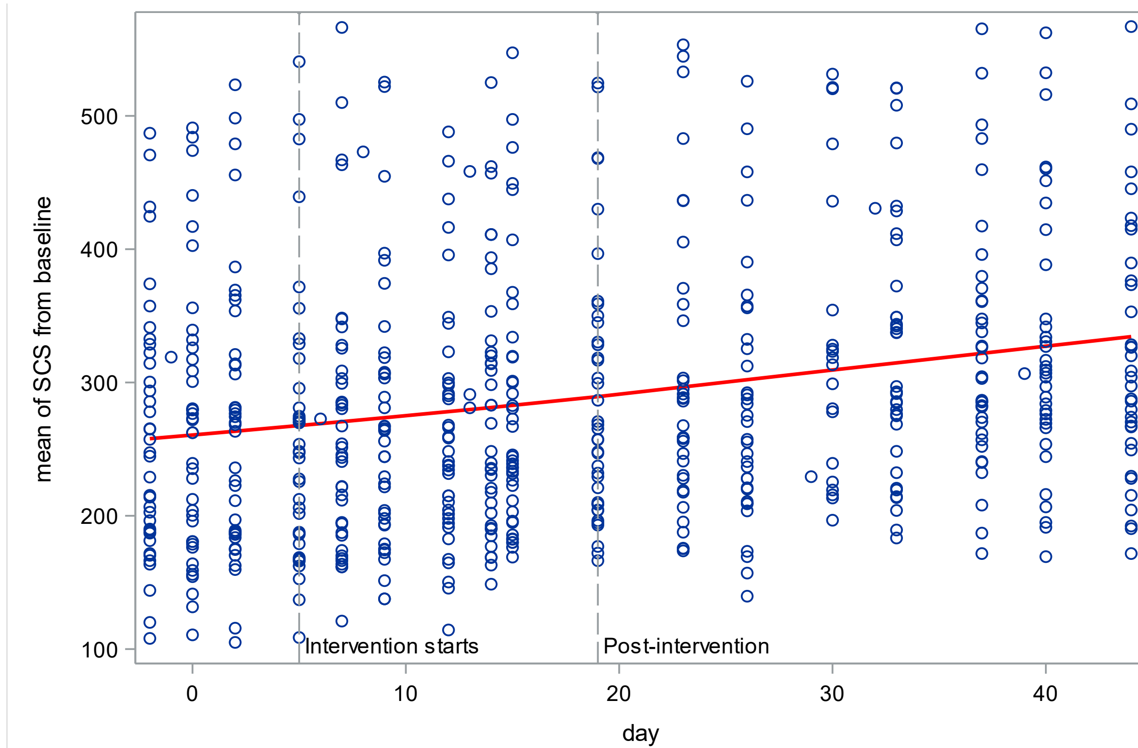
Period	n	Minimum SCS[1]	Maximum SCS[1]	SCS[1] Mean ± SD[2]	Median SCS[1]
Baseline	117	105.0	523.3	266.0±99.3	262.3
Intervention	234	108.7	566.3	271.6±97.2	248.3
Post Intervention	269	139.7	567.0	312.1±99.0	289.0

[1]SCS, skin carotenoid score
[2]SD, standard deviation

At baseline, the SCS ranged from 105.0 to 523.3 with a median SCS of 262.3 and mean SCS of 266.04±99.3. During the intervention period, the SCS ranged from 108.7 to 566.3 with a median SCS of 248.3 and mean SCS of 271.6±97.2. At post-intervention, the SCS ranged from

139.7 to 567.0 with a median SCS of 289.0 and a mean SCS of 312.1±99.0. Figure 6 depicts the trend of change in mean SCS from baseline over time coded as number of days since baseline.

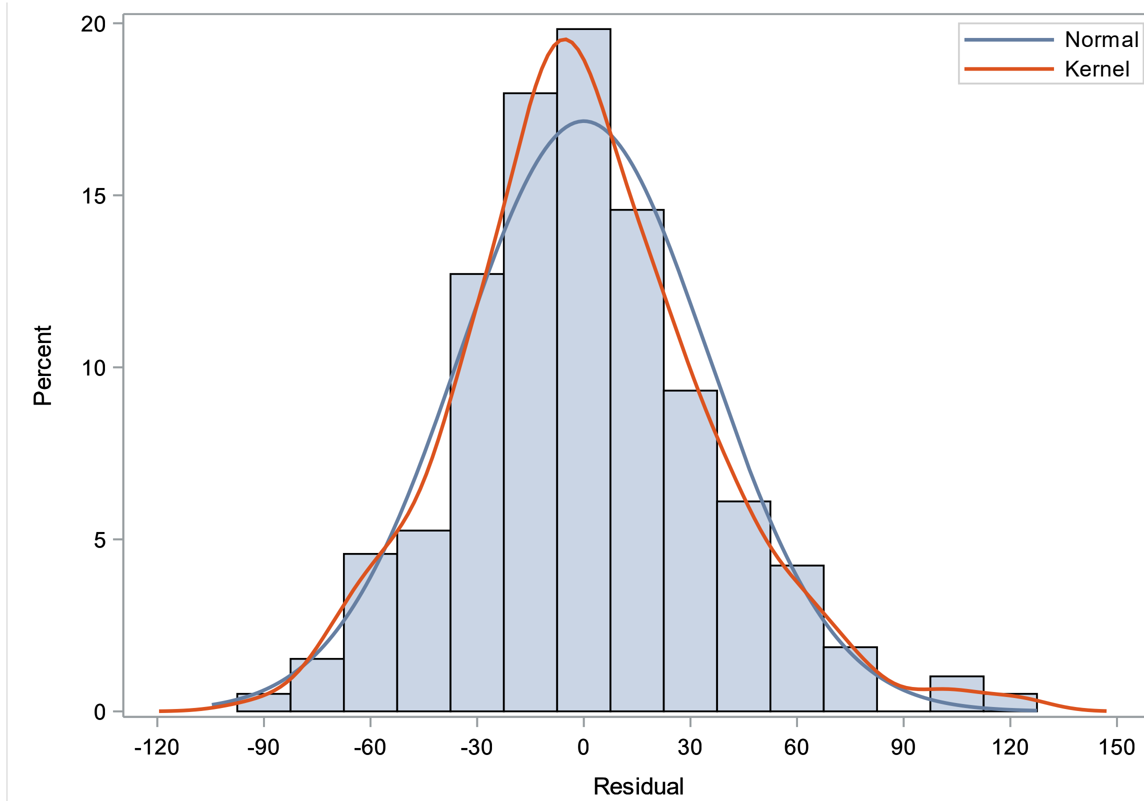
Figure 6. Scatter plot of mean SCS from baseline over time with linear regression line



Primary Objective

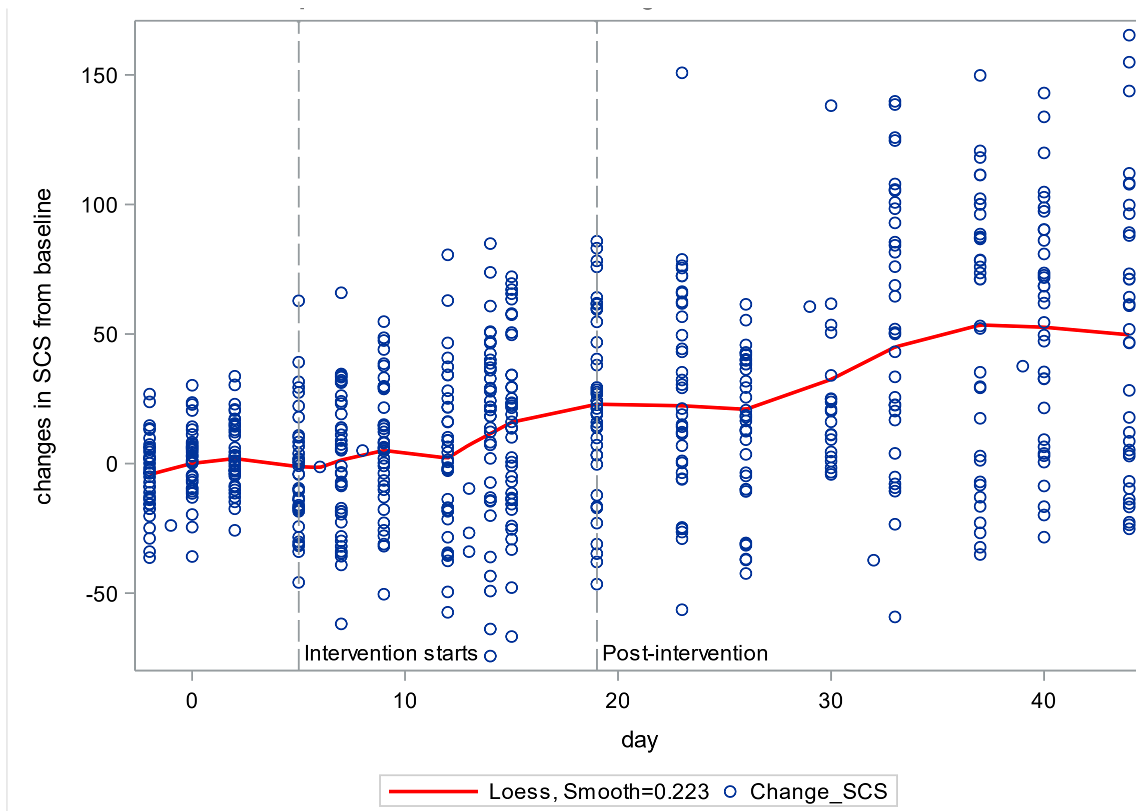
The distribution of residuals for mean change in SCS from baseline show that the data have a normal distribution, which meets the assumptions for the linear mixed model (Figure 7).

Figure 7. Histogram of distribution of residuals for mean change in SCS from baseline



The mean change in SCS from baseline to intervention was 5.6 ± 30.3 and the mean change in SCS from baseline to post-intervention was 38.0 ± 46.3. Figure 8 depicts the mean change in baseline SCS to these periods, coded as number of days since baseline.

Figure 8. Scatter plot of mean changes in SCS from baseline over time with Loess line



The Loess line (locally estimated scatterplot smoothing) in Figure 8 depicts an increase in mean change of SCS from baseline over time, with a period of consistent increase from day 26 to day 36. The increase of mean change in SCS begins approximately 2 weeks after the beginning of the intervention period and approximately 1 week after the end of the intervention period.

Table 3 describes the statistics for the linear mixed model. Change in mean SCS from baseline over time was significant (P<0.001). While SCS during the intervention period were not significantly higher than baseline (Beta=4.5, SE=4.1, P=0.271), SCS at post-intervention were significantly higher than baseline on average (Beta=37.10, SE=3.98, P<0.001)

Baseline BMI of participants did not have a significant effect on mean change of SCS from baseline (P=0.993); neither did baseline carotenoid loads of participants (P=0.474).

However, baseline SCS may be a significant predictor of the amount of change in mean SCS over time (P=0.001).

Table 3. Results from Linear Mixed Model for change in mean SCS with baseline covariates

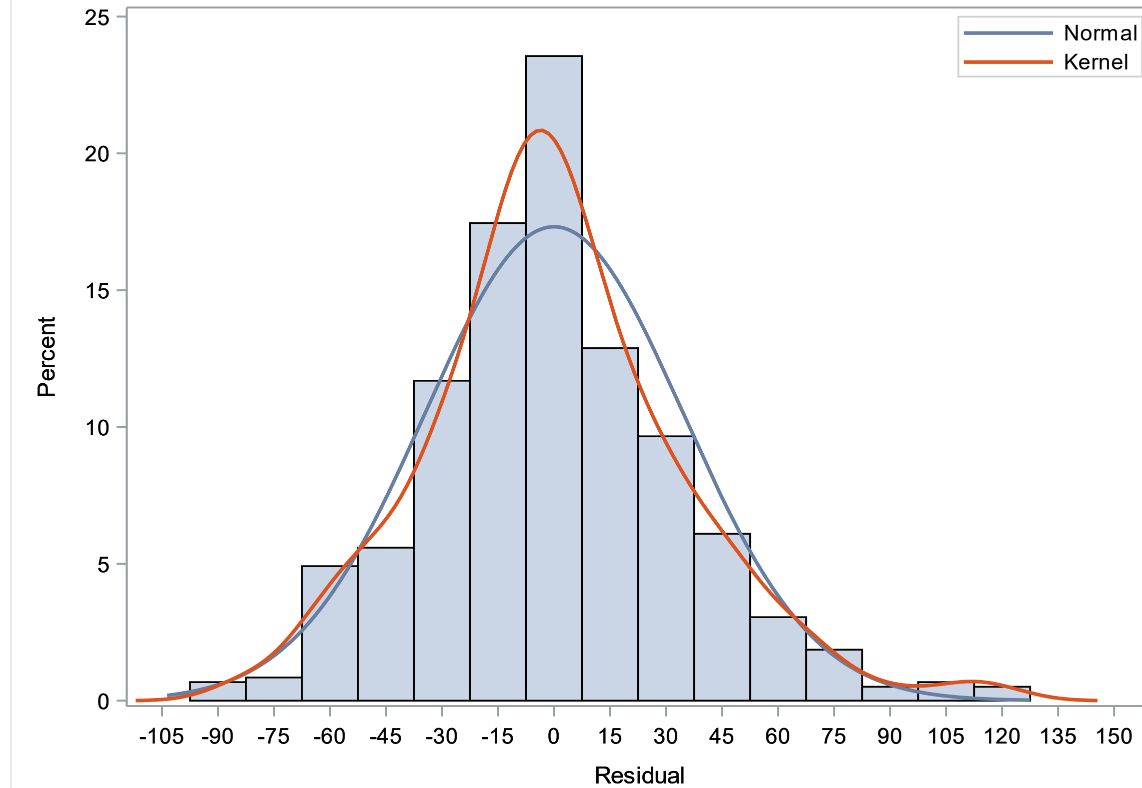
Effect		Estimate ± SE[1]	DF[2]	t value	P value[3]
Intercept		19.00 ± 7.80	33	2.44	0.020
Period	Baseline	0	-	-	-
	Intervention	4.52 ± 4.07	72	1.11	0.271
	Post-Intervention	37.10 ± 3.98	72	9.32	<0.001**
Baseline BMI		-0.002 ± 0.22	33	-0.01	0.993
Baseline Carotenoid Load		0.0002 ± 0.0002	33	0.72	0.474
Baseline SCS		-0.08 ± 0.02	33	-4.30	0.001**

[1]SE, Standard error
[2]DF, degrees of freedom
[3]**highly significant, < 0.01

Secondary Objective

The distribution of residuals for the mean change in SCS from baseline across tertiles show that the data for the secondary analysis follow a normal distribution (Figure 9).

Figure 9. Histogram of distribution of residuals for mean change in SCS over time for all tertiles



The main effect, or change in SCS across periods (baseline, intervention, post-intervention) for all tertiles was significant (P<0.001). Baseline BMI (P=0.826) and baseline carotenoid load (P=0.789) were not significant predictors of change in SCS over time across all groups. Group at baseline (Low, Median, High) may be a significant predictor in change of SCS from baseline over time (P=0.005). However, the overall group effect (Period x Baseline Group) was not significant (P=0.095). Table 4 describes the statistics for the LMM MIXED procedure applied to the covariates.

Table 4. Statistical Analysis of Linear Mixed Model for change in mean SCS for all baseline tertile groups (Low, Median, High)

Effect	Numerator DF[1]	Denominator DF[1]	F	P value[2]
Period	2	68	68.72	<0.001**
Baseline BMI	1	32	0.05	0.826
Baseline Carotenoid Load	1	32	0.07	0.789
Baseline Group	2	32	6.41	0.005**
Period x Baseline Group[3]	4	68	2.07	0.095

[1] DF, degrees of freedom
[2]**, highly significant, < 0.01
[3] High, Median, or Low tertile

Simple differences of the group effect least square means were calculated and analyzed for each tertile. The estimated difference in mean SCS between the intervention and the post-intervention period in the High group was -28.2 ± 5.5; this difference in mean SCS was significant (P<0.001). The estimated difference in mean SCS between the intervention and the post-intervention period in the High group was -0.2 ± 7.1; this difference in mean SCS was not significant (P=0.976). The estimated difference in mean SCS between the post-intervention and baseline periods was 28.0 ± 6.9; this difference in mean SCS was significant (P=0.001) (Table 5).

Table 5. Simple differences of least square means in High baseline tertile

Period	Estimate ± SE[1]	DF[2]	t value	P value[3]
Intervention vs. Post Intervention	-28.2 ±5.5	68	-5.15	<0.001**
Intervention vs. Baseline	-0.2 ±7.1	68	-0.03	0.976
Post Intervention vs. Baseline	28.0 ±6.9	68	4.09	0.001**

[1]SE, Standard Error
[2]DF, degrees of freedom
[3]**highly significant, < 0.01

The estimated difference in mean SCS between the intervention and the post-intervention period in the second tertile, or Median group, was -31.1 ± 5.6; this difference was highly significant (P<0.001). The estimated difference in mean SCS between the intervention and baseline in the Median group was -1.6 ± 7.1, which was not significantly different (P=0.822). The estimated difference in mean SCS between the post-intervention and pre-intervention period in the Median group was 29.5 ± 7.0. This difference in the Median group was highly significant (P<0.001). Table 6 describes the simple differences of least square means in the Median group between periods.

Table 6. Simple differences of least square means in Median baseline tertile

Period	Estimate ± SE[1]	DF[2]	t value	P value[3]
Intervention vs. Post Intervention	-31.1 ±5.6	68	-5.50	<0.001**
Intervention vs. Baseline	-1.6 ±7.1	68	-0.23	0.822
Post Intervention vs. Baseline	29.5 ±7.0	68	4.22	<0.001**

[1]SE, Standard error
[2]DF, degrees of freedom
[3]** highly significant, < 0.01

The simple differences of the Low tertile group between periods shows greater changes between periods than the High and Median groups. The estimated difference in mean SCS between the intervention and post-intervention periods in the Low group was -37.9 ± 5.5; this difference was highly significant (P<0.001). The estimated difference in mean SCS between the intervention and baseline periods in the Low group was significant (P=0.037) with a change of 14.5 ± 6.8. The estimated difference in mean SCS between the post-intervention and pre-intervention periods in the Low group was 52.5 ± 6.8; this difference in mean SCS was also significant (P<0.001). Table 7 reports the simple differences least square means in the Low tertile group between periods.

Table 7. Simple difference of least square means in Low baseline tertile

Period	Estimate ± SE[1]	DF[2]	t value	P value[3]
Intervention vs. Post Intervention	-37.9 ±5.5	68	-6.92	<0.001**
Intervention vs. Baseline	14.5 ±6.8	68	2.13	0.037*
Post Intervention vs. Baseline	54.5 ±6.8	68	7.77	<0.001**

[1]SE, Standard error
[2]DF, degrees of freedom
[3]** highly significant < 0.01; *significant < 0.05

DISCUSSION

The primary objective of the study was to determine if and when the skin carotenoid scores (SCS) of study participants increased above the baseline SCS during and after a two-week period of supplementing their usual diets with the minimum weekly recommended level of red-orange vegetables. SCS increased over time, though not significantly between each time period. The data also show that the mean change in SCS increased significantly over time and that SCS at baseline may be a significant predictor of how much SCS changes over time. We observed an increase of 5.6 units in mean SCS from baseline to the mean of the intervention period and an increase of 38 units in mean SCS from the mean of the intervention to the mean of the post-intervention period. However, there was not enough statistical power to detect a significant accumulation of carotenoids in the skin during and after this intervention compared to baseline values. Additionally, there was no ability to identify the particular day that the increase occurred due to low power.

The secondary objective of the study was to determine if SCS at baseline affects the change in SCS over time. There were significant differences in SCS in the High and Median baseline tertile groups between the baseline and post-intervention periods, as well as between the intervention and post-intervention periods. There were significant differences in SCS in the Low baseline tertile group between all periods. These results indicate that the difference in mean SCS between periods may depend on the group at baseline and that it may be easier to detect significant changes if the SCS is low at baseline. However, the overall group effect (Period x Group at baseline) was not significant.

The research hypotheses stated, "there will be a significant difference in mean skin carotenoid measurements from baseline values <u>during</u> and <u>after</u> consumption of red-orange vegetables at recommended weekly levels for two weeks". A significant difference in the mean

changes of SCS of the Low baseline tertile group was observed during the intervention (pre-intervention to intervention period) but was not observed in either the Median or High baseline tertile groups. A significant difference in the mean changes of SCS was observed for all groups after the intervention (intervention to post-intervention) and over the entire study (baseline to post-intervention). The data cannot support the original research hypothesis since the bioavailability of carotenoids in red-orange vegetables differs substantially. However, the data show that there will be a significant difference in mean skin carotenoid measurements after consumption of 180 g of baked and mashed sweet potatoes three times a week for two weeks. The data also support the findings of Ermakov et. al that supplementation of the diet with a source rich in β-carotene significantly increases SCS over time as measured by pressure-mediated RS (36). The research hypothesis stating there will be a significant change in mean SCS during the intervention can neither be supported nor refuted since there was not enough power to detect a difference during the intervention period.

There were two apparent increases in the mean changes of SCS over time, however, there was not enough statistical power to detect a significant accumulation. The graph (Figure 8) appeared to increase in mean SCS between days 29 and 40 of the study, part of the post-intervention period, where mean SCS values were significantly higher ($P < 0.01$) than both the baseline and intervention periods. Day 29 was in week 5 of the study and was approximately 3 weeks after the start of the intervention period on day 8. This apparent increase in mean changes of SCS spanned a period of 2 weeks before plateauing to a level that was still higher than mean SCS at baseline. These results should be considered when determining post-intervention measurement time points as measuring on the last day of an intervention may not capture the true increase of SCS values. The results suggest that investigators should wait about 14-20 days after an intervention before measuring skin carotenoids with pressure-mediated RS for an intervention

using this amount of baked sweet potatoes in order for the process of carotenoid absorption and skin accumulation to occur. Our observations of the apparent increase in mean changes of SCS approximately 3 weeks after the start of the intervention period align closely with the findings of Jahns et al. (13) and Prince and Frisoli (10). A major difference between our study and the two mentioned is that SCS was measured by pressure-mediated RS instead of RRS, although the two methods are highly correlated (22). Jahns' study was longer than this study (28 weeks) and consisted of 4 phases: a 6-week depletion diet, an 8-week high-FV diet, another 6-week depletion diet, and then an 8-week period where participants consumed their usual diet. The high-FV diet consisted of a 7-day rotating menu in which participants received all meals from the researchers; the diet provided an average of 62 mg of mixed carotenoids from an average of 6-cup equivalents (1046 g) of raw, cooked, and juiced fruits and vegetables daily whereas the sweet potatoes in this study provided an estimated average of 60 mg of β-carotene weekly. Prince and Frisoli's experiment was similar to this study in that participants supplemented their usual diets with carotenoids, rather than changing their diets completely. However, Prince and Frisoli supplemented participant diets with β-carotene capsules containing 17 mg β-carotene per capsule, rather than whole fruits or vegetables. The participants took part in a series of multi-dose regimens: 51 mg once a day with breakfast, 17 mg three times a day with meals, 34 mg three times a day with meals, and 102 mg three times a day with meals. Prince and Frisoli found that serum and tissue accumulation of carotenoids was much greater when the capsules were administered over three meals. In Jahns' study, skin carotenoids were measured about 2 times a week during the non-intervention phases (phases 1, 3, and 4), which is same frequency that SCS was measured in the post-intervention phase of this study but were measured 5 times a week during the intervention phase (phase 2), which is a greater frequency than the intervention SCS measurements of this study. Prince and Frisoli only measured skin carotenoids for three of the

subjects during their final regimen. The ages of participants in Jahns' study were similar to this study, as recruited participants were between the ages of 18-65 years old; the average age of participants in Prince and Frisoli's study (31.8 years) was similar to the average age of participants in this study (32.5 years). Jahns had exclusion criteria for BMI (exclude if BMI >30) whereas there were no exclusion criteria for BMI in this or Prince and Frisoli's study. Jahns' study had a total of 29 participants, which is slightly lower than the final study population (n=39) of this study; Prince and Frisoli had a study population of 5 participants. These studies reported skin carotenoid status responded quickly and significantly to increased dietary consumption of carotenoids and may appear in the skin in "as little as two weeks post intervention" as measured by RRS (13), which is similar to what is reported in this study for SCS measured by pressure-mediated RS.

The results of the secondary analysis show that the lowest tertile group at baseline had a greater change in mean SCS to other periods than the Median and High tertile groups. These results support the findings from a pilot study, with 3 participants, conducted by Ermakov et al. in which a participant with high baseline levels of skin carotenoids experienced a moderate, non-significant increase in SCS levels after consuming two 3 ounce servings of carrot juice each day for 30 days, and the other 2 participants experienced a much greater apparent increase (36). Unlike this study, Ermakov's paper does not specify whether the carrots were cooked or raw before juicing, which can affect carotenoid bioavailability (28), and it also does not specify whether the apparent increase of the SCS of the 2 participants was significant or not. In contrast to Ermakov's study, this study had the ability to report some significant differences due to a larger number of subjects. The results of this study also support the findings of Darvin et al. in which a participant with low initial levels of skin carotenoids increased levels by two-fold over a three-month period of increased fruit and vegetable consumption (14). Similarly, three

participants in this study doubled their SCS values from baseline to post-intervention over the 47-day period; these participants had low baseline SCS. Both pieces of evidence suggest that supplementation may have a greater effect on individuals with a low baseline SCS, but we are not able to prove this since the overall group effect in our study was not significant (Table 4).

In both the primary and secondary analyses, the covariates of baseline BMI and baseline carotenoid load were not significant predictors of the mean change in SCS over time. This result partially contradicts the findings of Rock et al. who report that high BMI is predictive of lower carotenoid status (29); while BMI may have an effect on overall carotenoid status, our results indicate that BMI did not have an effect on the mean change in SCS over time in this group of participants. The insignificance of baseline carotenoid load, or amount of carotenoids in the diet at baseline, in relation to mean change of SCS over time suggests that participant diet at baseline may not have a significant effect on how SCS improves over the time of an intervention. Diets are fluid and can change rather quickly in response to seasonal availability of foods, the stress level of an individual, and socioeconomic status among other factors (14). Additionally, carotenoid bioavailability differs greatly across different types of foods. The carotenoid values reported in nutrition databases are estimates that are averaged from values reported in various sources, so the values may not be truly valid for all foods. These reasons may explain why baseline carotenoid load is not a significant predictor of mean change in SCS over time. Baseline SCS and baseline tertile group, which were covariates in the primary and secondary analyses, respectively, were significant predictors of mean change in SCS over time. These results support the findings of Ermakov et al. (36) and Darvin et al. (14) that skin carotenoid status at baseline may affect the amount or rate of change in skin carotenoid status over time.

It is worthwhile to point out that the variation, or standard deviations, calculated for SCS group means were high. This is likely a result of the wide-ranging variability that exists both

within and between individuals, as reported in several studies using RRS (6, 9, 13). Jahns et al. found that skin carotenoid levels varied ~7-fold at baseline between participants as measured with RRS (13). Ermakov et al. reports that SCS as measured with pressure-mediated RS has a "wide variation in multiple populations" but describes a lower within-person variability by comparing a subject with a high average score to a subject with a low average score (19). The purpose of his comparison was to test the repeatability of SCS over 50 separate scans at a single timepoint. The variability of the participant with the high average score, 460, was 3.4% and the variability of the participant with the low average score, 200, was 4.1% showing that the standard deviation increases for lower scores. The within-person variability observed in this study is similar to that of Ermakov et al., in that participants with lower baseline scores had a higher variability, 10.6%, than participants with higher baseline scores, 6.6%. Ermakov took 50 scans per subject in comparison to the 9 scans herein. The variability observed in this study is also similar to the within-person variability of 6.8% observed in a large and ethnically diverse study population (n=479) where SCS was measured by pressure-mediated RS (37). Overall, the within-person variability in this study ranged from 3.6% to 20%. The variability observed between participants in this study may be due to the several genetic, physiological, and/or behavioral factors (smoking, excessive drinking, etc.) also reported to have an effect on SCS (14, 28, 29, 38, 39).

Study Strengths

This study had excellent participant retention. From start to finish, this study lasted a total of 47 days. A total of 5 subjects withdrew for reasons unrelated to the study itself. The organization of study activities, participant compensation, and consistent communication with participants throughout the study may have contributed to our retention rate of 87.5% of participants (40).

Participants were given equal amounts (180.00 grams up to + 0.99 grams) of the prepared sweet potato and unsalted butter (5 grams up to +0.50 grams) in each serving during weeks 2 and 3 of the study (intervention period). Jewel sweet potatoes were purchased from the same lot which reduced variability in carotenoid concentration per serving, as did the preparation process in which all sweet potatoes to be served to a group were blended to achieve a homogenous mixture.

Study Limitations

Though 40 individuals were recruited and data from 39 were statistically analyzed, the high inter- and intra-individual variability necessitated a much larger study population to detect significant differences. Based on the data from this study, approximately 148 subjects with a range of baseline skin carotenoid scores would be needed to detect a significant change in SCS from baseline values to the post-intervention period.

Though infrequent, three subjects had to participate on days that were different from the proposed study timeline two times throughout the study due to personal schedule changes. Steps should have been taken by the investigators to limit rescheduling of study appointments, as it was an accommodation that should not have been made. It would have been beneficial to obtain a commitment in writing from participants at the start of the study when they were made privy to the study timeline in order to limit rescheduling and maintain consistency of measurements.

On visit 13 of Wave 3, the calibration tool for the Veggie Meter was found to be damaged which caused all readings to be drastically lower than previous visits. The scores for Wave 3 participants at that visit were thrown out before statistical analysis. The calibration tool was possibly damaged by contact with bleach, which would have changed the color of the tool. When a new calibration tool was tested, participant scores fell within levels that appropriately aligned with previous scores.

Another limitation of this study was the method for quantifying carotenoid load in participants' diets. Dietary data was collected through the use of record-assisted 24-hour recalls, which can, and often do, result in recall bias (41). Food records were then analyzed by the NDSR database, which approximates nutrient totals per serving based on results from analytical studies. As a result, carotenoid loads calculated for both the participant food records and the sweet potato snack were not exact amounts; a wide range of carotenoids exist in each fruit and vegetable, so finding an exact amount of carotenoids is impossible without analyzing each one individually.

The generalizability of the results is limited in a few ways. First, the study took place during the COVID-19 pandemic; the participants were students, staff, or faculty members at Indiana University that were living and working in Bloomington at the time. Much of the university's population had returned to their home state or were working remotely from their homes. As a result, the sampling pool was limited. The study demographics also limit the generalizability of the results. The percentage of participants that identified as "Black" (5.1%) or "White" (69.2%) were similar to the percentage of "Black" (5.2%) and "White" (73.8%) degree-seeking students at Indiana University's Bloomington campus. However, the percentage of individuals who identified as "Asian" (23.1%) or "Hispanic" (12.8%) in the study were higher than "Asian" (7.8%) or "Hispanic" (8.1%) degree-seeking students at Indiana University's Bloomington campus. Those in the study that identified as "Other" (2.6%) were less than those that identify as "Other" (5.0%) at Indiana University Bloomington (42). The study demographics are also quite different from national percentages of degree-seeking students (42). Therefore, results of this study may not be generalizable beyond the study population, however achieving a more racially and ethnically study pool may be helpful in future studies to determine unique differences related to these characteristics.

Recommendations for Future Studies

It would be of interest in similar future studies to make an a priori comparison of SCS values around day 33-36 of the study to baseline SCS values to pursue a more concrete understanding of the time-course of skin carotenoid accumulation in response to an intervention. Future studies can also increase generalizability by recruiting more subjects to improve the ability to detect significant differences and subjects that reflect demographics in the area for which they are providing data.

We will be analyzing the plasma that was collected at three separate timepoints through the study in a future paper. The results, if correlated to skin carotenoid values, may provide greater validation of the use of pressure-mediated RS in similar studies as well as a comparison of the trajectory of blood versus skin accumulation of carotenoids.

CONCLUSION

This study aimed to determine if and when skin carotenoid values would increase both during and after a dietary intervention. It was observed that mean skin carotenoid values slightly increased in the intervention period and significantly increased in the post-intervention period of our study compared to baseline values. While we cannot conclude a concrete time-course to skin carotenoid accumulation following an intervention adding supplement to the usual diet, observations of the data suggest that 2 to 3 weeks post-intervention may be a period of interest when measuring the efficacy of such an intervention.

The findings of this study confirm and support the findings of many publications available in the current literature. First, skin carotenoid values, on average, increase in response to consumption of 180 g of baked and mashed sweet potatoes three times a week for two weeks. Second, skin carotenoid values in our study show high variability, just as they do in study populations that have been reported in the current literature. This suggests that when the diet is supplemented, smaller effects can be expected and therefore a larger participant number is needed. Future studies should recruit more participants to capture the ability to detect a small effect. Third, individuals with low levels of skin carotenoids at baseline may have more room for improvement of carotenoid status over the course of an intervention than those who have a higher baseline level. Finally, accumulation of carotenoids in the skin is more latent than the immediate changes in blood that are reported in the literature. The data from this study suggest that it is beneficial to wait 14-20 days past this type of intervention to measure SCS and determine the success of this type of intervention. Future studies should be designed for participant final measurements to be made approximately 2 to 3 weeks following the start of a similar dietary intervention.

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APPENDICES

APPENDIX A

Operational definitions

Limitations

Assumptions

Delimitations

Statement of the Problem

Independent Variables

Dependent Variables

Research Hypothesis

Operational Definitions

Invasive: An invasive carotenoid measurement is one that requires collection of tissue or blood samples through skin punches or blood draws, respectively, which may be painful for subjects to experience. Once collected, the samples can be used for *in vitro* analysis typically performed by HPLC.

Non-invasive: A non-invasive carotenoid measurement is one that does not require collection of tissue or blood samples for *in vitro* analysis. Rather, non-invasive measurements are done *in vivo* and should cause little burden to subjects.

Raman resonance spectroscopy (RRS): A type of spectroscopy that detects the vibrational energy that is characteristic of a molecule. It does so by emitting light with the same frequency that excites the molecule in the electromagnetic spectrum. Carotenoids are particularly excitable in the blue-green range of the visible spectrum, in which wavelengths are 420-560 nm. When the light energy interacts with the molecule, it vibrates and causes a shift (Raman scattering) in the frequency which correlates with the vibrational energy of the molecule, leading to its identification. More detail about this technology is described in Hata et al. (43).

Reflectance spectroscopy (RS): A type of spectroscopy in which light is emitted at the same frequency that is characteristic for the absorption of the molecule of interest. In this case, carotenoid absorption has a characteristic frequency between 420-560 nm. The light is subsequently backscattered and provides information about the carotenoid concentration of the particular object (4). RS devices are typically smaller and more portable than RRS devices.

Limitations

The following limitations will apply to this study:

1. Values reported for carotenoid concentration in the food items used for the supplementary diet are estimated; subjects may not all be receiving the exact same amount of carotenoids.

2. Self-reported data (24-hour recall) may introduce recall bias.

3. Beta-carotene in blood samples has a short half-life and degrades rapidly when exposed to light and heat.

4. Convenience sampling in SPH may introduce sampling bias.

5. Sample size will be limited due to COVID-19.

6. There is a high inter-individual variability in blood and skin carotenoids which will make it more difficult to detect differences (8).

7. Subjects may alter their usual diet and consume more or less red/orange vegetables upon learning of their skin carotenoid score.

Assumptions

The following assumptions will apply to this study:

1. All subjects are consuming recommended levels of iron, zinc, and vitamin E; abnormal levels of these nutrients can interfere with carotenoid metabolism (2).

2. All subjects are "normal responders" to beta-carotene (38, 44).

Delimitations

The following delimitations apply to this study:

1. Subjects will be given equal amounts of the carotenoid-rich vegetable and dietary fats for optimal absorption (8, 10, 17) in pre-packaged portions.

2. Subjects' diet will be evaluated for compliance through the use of dietary recall and food record methods (45).

Statement of the Problem

Despite the growing interest in and use of non-invasive biomarkers of fruit and vegetable consumption, no research has been specifically aimed at determining the time it takes for skin carotenoid levels to change significantly from baseline postprandially. Research has indicated that skin accumulation takes longer than accumulation in the blood- which peaks approximately 24-48 hours post-prandially (8, 10, 17, 21)- and assert that skin carotenoid values reflect long-term fruit and vegetable consumption habits. However, there is inconsistency between studies concerning carotenoid accumulation in the skin, and there is a lack of consensus on average time to noticeable accumulation as reports range from 2 days to 12 weeks. Establishing an approximate time-course to significant change in carotenoid skin levels will aid nutrition scientists and epidemiologists in determining when to collect data post-intervention in order to find appreciable results.

Independent Variable

One independent variable will be evaluated in this study:

1. Consumption of red/orange FV at average recommended weekly level based on the subject's caloric intake.

Dependent Variables

Two dependent variables will be evaluated in this study:

1. Skin carotenoid measurement/score (SCS) (unit: RS intensities)
2. Time to significant change in SCS from baseline (unit: hours/days)

Research Hypotheses

1. There will be a significant difference in mean skin carotenoid measurements from baseline values during consumption of red/orange vegetables at recommended weekly levels for two-weeks.
2. There will be a significant difference in mean skin carotenoid measurements from baseline values after consumption of red/orange vegetables at recommended weekly levels for two-weeks.

Null Hypotheses

1. Skin carotenoid measurement during intervention $H_0: \mu_{during} = \mu_{baseline}$
2. Skin carotenoid measurement after intervention $H_0: \mu_{after} = \mu_{baseline}$

Alternative Hypotheses

1. Skin carotenoid measurement during intervention $H_A: \mu_{during} \neq \mu_{baseline}$
2. Skin carotenoid measurement after intervention $H_A: \mu_{during} \neq \mu_{baseline}$

APPENDIX B

Review of Literature

REVIEW OF LITERATURE

Poor fruit and vegetable status in the U.S.

The majority of U.S. adults and children do not currently meet the average recommended intake levels for fruits and vegetables (FV), which is roughly 1.5-2 cups fruit and 2-3 cups vegetables per day depending on caloric needs (24, 46). This is especially true for red/orange and leafy green vegetables (24). Data from the Behavioral Risk Factor Surveillance System (BRFSS) in 2015 show that only 12% of American adults met the recommendation for fruit consumption and only 9% met the recommendation for vegetable consumption (46). Results from the survey vary across state, sex, ethnicity, socioeconomic status, and age, but nationally, younger adults (ages 18-30) were found to be the age group with the highest proportion that did not meet recommendations for FV consumption (46). FV consumption among youth was reported to be even lower than that of adults; only 9% of high school students in 2015 met the recommended fruit intake and just 2% reported that they ate the recommended amount of vegetables (47).

It is well-documented that FV consumption is negatively correlated with the risk of developing chronic diseases like some cancers, cardiovascular disease, and Type 2 diabetes (1, 3, 5). Research shows that prevention of chronic diseases through the establishment of healthy eating patterns, which promotes adequate consumption of FV, is more effective if started earlier in life, particularly in childhood (47, 48). As a result of poor FV consumption and high rates of chronic diseases in this country, there are many nutrition interventions aimed at increasing overall FV consumption, especially in populations where disease prevention is realizable, like children and young adults.

Carotenoids are antioxidants found in fruits and vegetables

Among the components of FV that may contribute to the prevention of chronic diseases and the promotion of health are a class of compounds known as carotenoids. Carotenoids are lipophilic pigments that are found in all photosynthetic organisms and are responsible for the red-orange-yellow colors of many FV. Green leafy vegetables are also quite rich in carotenoids, but the massive amount of chlorophyll contained in the leaves masks the brighter pigments from showing (3).

Carotenoids are divided into two categories, carotenes and xanthophylls. Hydrocarbon carotenoids like β-carotene, α-carotene, and lycopene are considered carotenes and contribute to the red-orange-yellow pigments of FV. Carotenes are less polar and more hydrophobic than xanthophylls, so they are located in the inner part of the lipid bilayer in cell membranes (3). Xanthophylls, like lutein, zeaxanthin, and β-cryptoxanthin, are more polar and hydrophilic due to the presence of at least one atom of oxygen in their molecular structure (49), so they are typically located within the outer cell membranes (3). They contribute to the orange and yellow pigments of FV. Carotenoids can and do exist as numerous isomers, some of which are more biologically active in humans than others, but most are naturally found in the trans configuration as it is the most stable configuration (49). The presence of carotenoids in cell membranes bolsters the resistance of membranes to reactive oxygen species (ROS) (3).

In fact, all carotenoids possess antioxidant functions, for which they are well-known, because they are potent quenchers of singlet oxygen ($^1O_2$) and peroxyl radicals (5). Most of the antioxidant function of carotenoids has been observed in the skin, as β-carotene and lycopene represent the largest group of cutaneous antioxidants (14). Their antioxidant potential is due in part to the repeating isoprene structure, containing conjugated double C=C backbones which is characteristic of all carotenoids. This allows the molecule to be used in several quenching cycles

without the loss of structural integrity. The $^1O_2$ quenching rate of cutaneous carotenoids is approximately 30-100 times greater than that of α-tocopherol, or Vitamin E, another ubiquitous antioxidant in the body (50). While $^1O_2$ quenching is considered a unique characteristic of carotenoids as a whole (50), lycopene is the most efficient antioxidant (5). However, when carotenoids and Vitamin E work synergistically, as often happens, the combined quenching is more effective than any one carotenoid alone (2). In addition to their antioxidant potential, β-carotene, α-carotene, and β-cryptoxanthin are referred to as "provitamin A" carotenoids, which means they can be converted into retinol (Vitamin A) after digestion for further use in the body. Vitamin A or a derivative aids in the regulation of gene expression, cell growth and development, red blood cell production, immunity, and vision function (2). Whether directly or indirectly, carotenoids from FV serve important roles in maintaining the health of individuals, and the mechanisms with which they do so are eliciting the attention of nutritionists and epidemiologists in intervention design.

Carotenoid bioavailability

Carotenoids cannot be synthesized *in vivo*, so humans must acquire them from food. The most commonly consumed carotenoids in the U.S. are β-carotene, α-carotene, lycopene, lutein, zeaxanthin, and β-cryptoxanthin (Figure 1) (6, 7). Most of the carotenoids that humans consume come from FV, though some can come from the consumption of egg yolks (49) and other foodstuffs like salmon, lobster, shrimp, saffron, paprika, and annatto (51). Regardless of the source, the bioavailability of carotenoids in the body is determined by a number of conditions (28). In most carotenoid studies, bioavailability is usually considered as the fraction of the ingested nutrient that is available for utilization in physiological processes or for storage in tissue (28, 34, 52). However, some researchers assert that this definition of bioavailability lacks clarity,

as it does not describe whether it is referring to the Vitamin A value of the carotenoid or the amount of specific carotenoid *per se* (52). In this paper, the term “bioavailability” refers to the amount of each specific carotenoid that is absorbed and retained by the body, not its Vitamin A value, unless otherwise described.

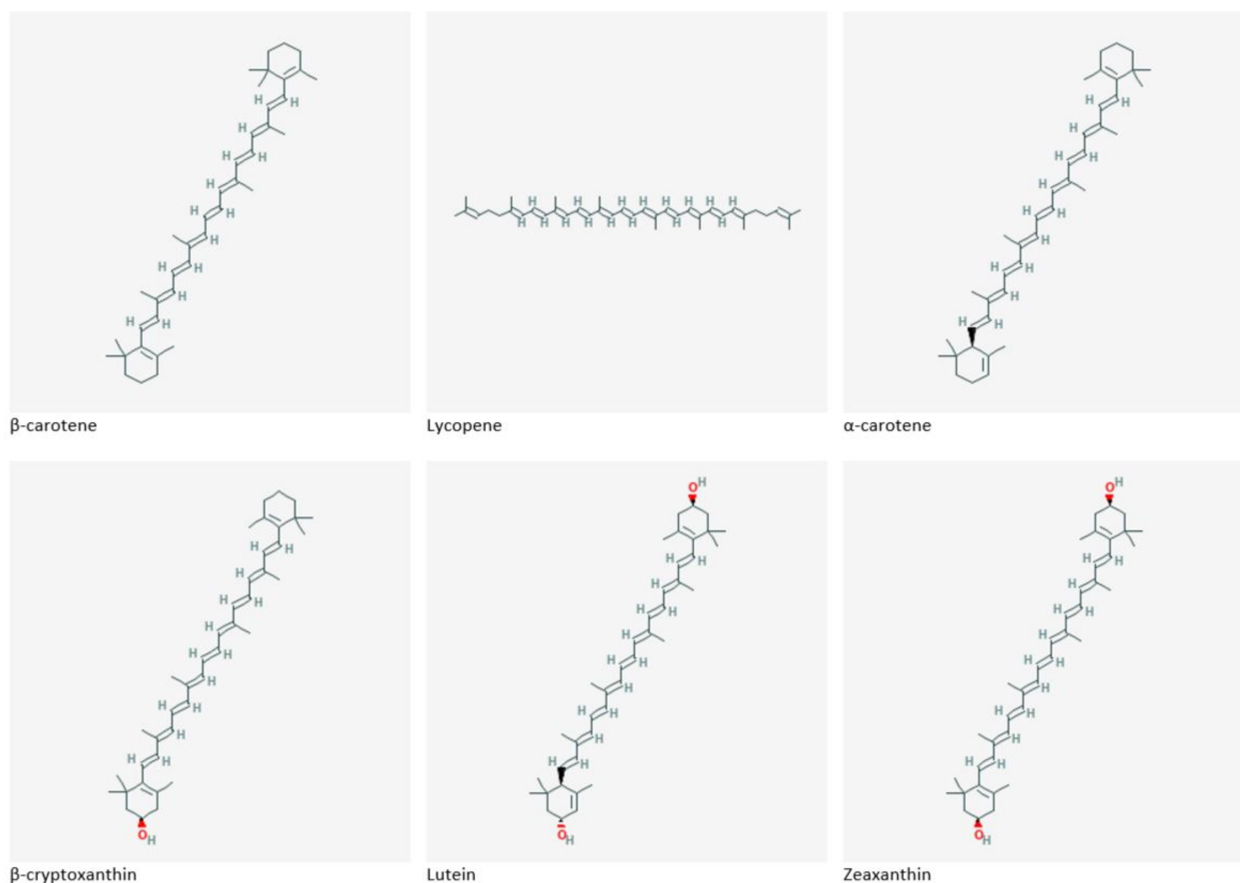

Figure 1. Most commonly consumed carotenoids in U.S. diet *β-carotene, Lycopene and α-carotene are the carotenes and β-cryptoxanthin, Lutein, and Zeaxanthin are the xanthophylls.*

DePee and West (53) describe the main factors affecting carotenoid bioavailability and subsequent conversion to retinol in the mnemonic “SLAMANGHI”: carotenoid species, molecular linkage, amount of carotenoids consumed in a meal, food matrix in which the carotenoid is incorporated, absorption modifiers, genetic factors, host-related factors, and mathematical interactions (28). Carotenoid bioavailability can also be affected by age,

environmental factors like season, and lifestyle factors like smoking, heavy alcohol use, and stress (14). This array of factors is likely responsible for the degree of variability in carotenoid status between and within individuals. For the purposes of this study, only a few factors affecting bioavailability will be discussed.

Bioavailability from food vs. purified sources

Just as bioavailability within and between individuals is highly variable, so is carotenoid bioavailability from food and pure supplements (8). In short, carotenoids in food are less bioavailable than those in purified forms, like supplements, beadlets, or oil suspensions (28, 45, 51). This is because carotenoids in food are suspended in the "food matrix", so accessing the molecules for absorption requires either the disruption of that matrix or the facilitation of the matrix into the intestine via emulsification. For example, some carotenoids, namely the xanthophylls, are integrated in protein complexes of the chloroplasts in some FV, while the carotenes are usually suspended in oil droplets of the chromoplasts of certain FV (54). The efficiency with which carotenoids are digested and absorbed by the intestine depends on this initial configuration within the plant, as well as their polarity. Xanthophylls are generally absorbed much better than carotenes due to their heightened polarity. In fact, van het Hof found that the bioavailability of β-carotene (a carotene) from vegetables is just 14%, whereas the bioavailability of lutein (a xanthophyll) from vegetables is 67%, nearly five times greater (45). The polarity of a carotenoid determines their position in the micelles, chylomicrons, and other lipoproteins in which they are integrated during and after the process of digestion. Xanthophylls are typically found in the outer membrane of lipoproteins and can interact much more easily with the digestive enzymes and tissues, whereas carotenes are found in the center of lipoproteins and are more protected from those important interactions (55). Other factors like food preparation

methods and presence of fat during food consumption largely affect how well carotenoids are absorbed in the intestine (10, 54).

Cooking and mechanical or enzymatic disruption of FV during food preparation increases the bioavailability of most carotenoids because it disrupts the complexes and cellular compartments in which the molecules are trapped (28, 51). Micozzi et al. (56) found that cooked carrots increased total plasma carotenoid levels from baseline in a cohort receiving a controlled diet. Both α-carotene and β-carotene levels significantly increased, with α-carotene levels nearly doubling that of β-carotene. They assert that this may be due to the fact that β-carotene is converted more efficiently to retinol than α-carotene in the body, which would result in lower levels circulating in the blood.

Bernhardt and Schlich (54) compared the bioavailability of carotenoids and α-tocopherol in fresh and frozen broccoli and red peppers before and after different cooking methods. The cooking methods employed for the fresh broccoli and fresh red peppers included boiling, steaming, stewing, and pressure steaming. The cooking methods used for the frozen broccoli and frozen red peppers included boiling, steaming, and microwaving. β-carotene content in the fresh broccoli increased significantly from baseline in all cooking methods, whereas the cooking methods actually decreased β-carotene content in the fresh red peppers. The carotenoids in fresh broccoli, and other green vegetables, are incorporated in protein complexes in the chloroplasts, so disruption by the addition of heat is needed to increase bioavailability, which is supported by the results in the study. β-carotene in the frozen broccoli remained relatively unchanged after cooking, showing just a slight decrease. The researchers state the lack of difference in β-carotene content of the frozen broccoli is due in part to the blanching that must take place before freezing; the addition of heat during blanching likely interrupted the food matrix and freed up the carotene,

which can be seen by the high value before cooking. However, β-carotene content in the frozen red peppers decreased significantly after cooking. The significant decreases in β-carotene content of both the fresh and frozen red peppers are due to the fact that carotenes in red/orange FV are suspended in oil droplets of the chromoplasts, which requires less disruption of the food matrix and cellular components for carotenoid release.

One study tested the efficacy of different vegetables in increasing plasma carotenoids (57). Researchers found that cooking the same amount (300 g) of broccoli, peas, and spinach added to prepared meals significantly increased plasma levels of carotenoids but did so at different levels of efficacy. All the vegetables added to the meals significantly increased plasma levels of lutein from baseline; broccoli 1.3-fold, peas 1.3-fold, whole leaf spinach 1.7-fold, and chopped spinach 2.0-fold. Per mg intake, however, lutein was actually more bioavailable from cooked broccoli and peas than from cooked spinach. Additionally, cooked broccoli and peas were more efficient than cooked spinach at increasing plasma levels of β-carotene, as cooked spinach did not result in a significant increase. Similarly, de Pee and West found that bioavailability of β-carotene from orange fruits was much higher than that of leafy greens as orange fruits improved vitamin A status (serum retinol concentrations) in children over 2.0-fold that of leafy greens (34). This is due in part to the differences in food matrix structure found in different types of FV.

As evidenced by the studies above, green and leafy green vegetables generally have lower carotenoid bioavailability than other carotenoid-rich FV because of the chloroplast protein complexes and other cell structures in which carotenoids are trapped (28). Furthermore, the bioavailability of carotenoids compared across different green and leafy green vegetables varies significantly (57). Generally speaking, xanthophylls complexed with proteins in the chloroplasts need more destructive disruption of the food matrix than carotenes suspended in oil droplets of

the chromoplasts (34). The differences in bioavailability of carotenoid species in food is further compounded by the fact that the presence of some carotenoids may affect the absorption of others once ingested (34, 56). Regardless of the mode of preparation, ensuring adequate amounts of fat in a carotenoid-rich meal can help to increase carotenoid bioavailability to the intestine.

Digestion and absorption of carotenoids

Carotenoids contained in FV require at least some fat for optimal absorption into the body because their absorption and transport largely mirrors that of dietary lipids (2, 10, 58). When carotenoids and dietary fats are consumed, they must be emulsified into smaller lipid droplets in order to be absorbed. The presence of fat in the mouth, stomach, and small intestine stimulates the release of lipases and bile salts that aid in the hydrolysis and emulsification process to create mixed micelles. Mixed micelles are able to interact with digestive enzymes, like lipase, at the brush border of the intestinal membrane which allows their contents to be absorbed into the intestinal mucosa. The contents are then incorporated into chylomicrons and transported through the lymph where they are degraded by lipoprotein lipase and subsequently delivered to tissues in the body via very low density lipoproteins (VLDLs) in the blood (2, 3).

Prince and Frisoli (10) assert that essentially no β-carotene can be absorbed in the absence of fat, which highlights the molecule's lipophilic nature. This is true for all carotenoids, but the amount of fat needed for optimal absorption may vary by species. Roodenberg et al. (58) found that α-carotene and β-carotene require very little fat (3-5 g in a meal at minimum; "low-fat meal") for optimal intestinal uptake, whereas lutein esters require much more (36 g in a meal at minimum; "high-fat meal"). This indicates that lutein esters may be more lipophilic than carotenes. However, lutein esters (like cholesterol esters) are absorbed after hydrolysis by esterases, which

creates free lutein. As a result, the absorption of free lutein may require a smaller amount of fat than lutein esters for optimal intestinal uptake (58).

The absorption and metabolism of carotenoids in the body is a much more complicated process than scientists previously thought (52, 59). Though carotenoids follow a similar digestive process as dietary lipids and fat-soluble vitamins, they can be absorbed by intestinal cells either through passive diffusion or facilitated diffusion, with the help of carotenoid-specific binding proteins. In fact, passive uptake of carotenoids is believed to only occur at high, pharmacological doses of the molecule whereas receptor-mediated uptake likely occurs at dietary doses (39). The lipophilicity of the carotenoid in the mixed micelle is positively correlated with their passive uptake by intestinal cells because lipophilic substances more readily penetrate the lipid bilayer of cells (60). It is possible that the bioavailability of carotenoids is affected by their uptake from mixed micelles.

Humans are believed to have a more selective uptake of carotenoids than other mammals because they can absorb carotenoids via facilitated diffusion from their intestines as well (60). This is supported by the presence of scavenger receptor class B type I (SRBI) in the liver and brush border membrane of epithelial cells, among other locations (39, 60). SRBI has a broad ligand specificity but has been known to aid in the epithelial uptake of lutein, zeaxanthin, β-carotene, and lycopene. Furthermore, the expression of SRBI is modulated by the presence of provitamin A carotenoids, which suggests a diet-responsive negative feedback regulation between the two (39). Cluster determinant 36 (CD36), another class of scavenger receptor proteins, may also be involved in the uptake of carotenoids into the mucosal cell, as it has been observed to play a role in the uptake of β-carotene (39). Borel also makes a strong argument that

Niemann-Pick C1-like 1 (NPC1L1) may be involved in carotenoid absorption, as well as Fatty Acid Binding Protein (FABP) and some ABC transporters (39).

Once absorbed into enterocytes, either through passive or facilitated diffusion, carotenoids can take one of two pathways for incorporation into the body. Some carotenoids are converted into retinyl esters and some are packaged into chylomicrons which enter the lymph for secretion into the blood (2, 61). The only carotenoids that can be converted into retinyl esters are the provitamin A carotenoids, namely α-carotene, β-carotene, and β-cryptoxanthin. β-carotene has a higher rate of conversion to Vitamin A than α-carotene and β-cryptoxanthin (2, 52).

The Vitamin A conversion process requires β, β-carotene-15, 15'-monooxygenase 1 (BCMO1). BCMO1 oxidatively cleaves β-carotene into two molecules of all-trans-retinal which can either be further converted into all-trans-retinoic acid or all-trans-retinol (61). It is believed that both α-carotene and β-cryptoxanthin can only yield 1 molecule of Vitamin A in this process (52). BCMO1 is responsible for the symmetrical cleavage of β-carotene in the mucosal cell and liver, but there is another carotene oxygenase that is involved in asymmetrical cleavage in the mitochondria of the enterocyte, β, β-carotene-9', 10'-oxygenase (BCDO2) (39). In order for the carotenoids and retinyl esters to be transported to other tissues after absorption into the enterocytes, they need to be esterified with long-chain fatty acids (LCFA) and incorporated into chylomicrons (2). The esterification process occurs with the help of the enzymes lecithin retinol acyltransferase (LRAT) and acyl-CoA retinol acyltransferase (ARAT) (61). The function of ARAT is due in part to the enzyme diacylglycerol acyltransferase 1 (DGAT 1). LRAT mainly functions when physiological/dietary amounts of Vitamin A are present and DGAT 1 is believed to be more active in the esterification process when pharmacological amounts of Vitamin A are present (2, 61). Cellular retinol binding proteins I and II (CRBPI, CRBPII) also play important

roles in the absorption of retinol and its metabolism intracellularly (2, 61). Once carotenoids are esterified, they are packaged into chylomicrons and enter the lymph system where they will be transported into the blood for delivery to different tissues throughout the body, namely the liver, eyes, and skin (36, 61).

Factors affecting absorption

It is well-documented that there is a high interindividual variability in carotenoid status (38, 39, 55, 62). The source of this variability is multi-faceted and relies on a number of factors including genetics, physiological abnormalities, and lifestyle and environmental factors (29). Carotenoid metabolism is clearly a complex process that requires the proper functioning of many proteins and enzymes. There are a few factors that can cause someone to have a lack of response metabolically to carotenoid intake or supplementation. This phenomenon is often referred to as the "nonresponder concept" in the literature (55). According to Borel et al. (38), a nonresponder to β-carotene is someone who either has no significant increase in blood β-carotene concentration after the intake of a large single dose of β-carotene, or someone who has no significant increase in blood β-carotene concentration after several weeks of ingesting a diet rich in β-carotene. The insignificant response to β-carotene may be due to impaired intestinal uptake, extensive conversion into Vitamin A, or impaired incorporation into chylomicrons (38).

As all steps of carotenoid metabolism require a protein or enzyme, it is likely that a mutation affecting the gene responsible for one of these proteins can result in a lowered response to the carotenoid molecule. It is not uncommon for humans to have a multitude of mutations within their genome. Mutations in the DNA can affect the production of RNA which ultimately interferes with the synthesis and subsequent expression of proteins. Some mutations are

deleterious, and some go unnoticed. Single nucleotide polymorphisms (SNPs), a type of genetic point mutation, have been located in the human genome which results in variance in the population. The gene encoding for BCMO1 has a SNP, rs6564851, close to its position in the genome but it only explains 1.9% of the variability observed in the population (39). Recently, BCO1 SNPs, along with baseline characteristics and diet, have been reported to explain 75.7% of the variability in plasma lycopene response in a population of men undergoing prostatectomies for clinically localized prostate cancer (63). Borel also reports that there are more SNPs affecting the genes encoding for the proteins involved in carotenoid metabolism, like CD36 and SR-BI, so SNPs affecting others may be discovered in the future (39).

In addition to genetic variation, some individuals have physiological disorders that interfere with nutrient absorption and metabolism. Any disease that results in fat malabsorption or fat maldigestion can impact the bioavailability of carotenoids in the body since their incorporation into the intestine requires bile and lipases (28).

Even if an individual does not have a genetic mutation or malabsorption issues affecting their carotenoid status, there are still other factors that may explain high interindividual variability. Rock et al. found that there are several determinants of health associated with serum carotenoid concentration (29). They looked at demographic (age, sex, ethnicity), dietary (supplement use, FV consumption), and lifestyle (BMI, smoking, alcohol use) factors to determine if any of these had a significant impact on carotenoid status. In addition to FV consumption (14), serum cholesterol concentrations are predictive of higher carotenoid status and alcohol use, smoking, and high BMI are predictive of lower carotenoid status (29). This is partially because carotenoids are particularly susceptible to the oxidation resulting from smoking and alcohol use and partially because alcohol has been known to interfere with the conversion of β-carotene to Vitamin A

(28). Ultimately, however, they found that the factors they observed could only explain a small amount of the variable carotenoid status in the population (29). Even season can affect carotenoid concentration as higher concentrations were observed in the summer and autumn compared to spring and winter during a year-long study (14). This variation is presumed to mirror the availability of FV, which usually increases during the growing season, and whether the subject was sick or ill, which typically occurs in the winter and spring months (14). Darvin et al. also found that even stress in the workplace can have a negative effect on an individual's carotenoid status (14). The literature clearly confirms that there are a multitude of factors affecting someone's carotenoid status which must be considered when assessing variability in a population.

Preferential accumulation of carotenoids

Once carotenoids are absorbed by the intestine and packaged into chylomicrons, they can be transported to and accumulate in various locations in the body. Humans absorb all carotenoids in an undefined fashion, so blood concentrations of carotenoids are complex in composition and can, of course, be variable between individuals (64, 65). The carotenoids with the highest concentrations in blood, tissues, and organs are the same as those most common in the human diet: α-carotene, β-carotene, lycopene, lutein, zeaxanthin, and β-cryptoxanthin (60, 64, 65). However, carotenoids are not dispersed and distributed uniformly after absorption; they appear in different organs in variable amounts (12). Carotenoids accumulate in a number of organs and tissues, but show the highest concentrations in the adrenal glands, liver, reproductive organs, and adipose tissue (64). There is also noticeable carotenoid accumulation in the retina and epidermis (64).

The accumulation of carotenoids is a non-random process, so preferential accumulation of different carotenoid species is observed in different tissues and organs. For example, Schmitz et al. (65) found that the liver, kidneys, and lungs have a higher concentration of β-carotene and lycopene than other carotenoids. They also found that the liver had the highest concentration of total carotenoids, followed by the kidneys, and then the lungs. There is also preferential uptake of lutein and zeaxanthin in the macular region of the retina (12, 66) as well as preferential uptake of lycopene and β-carotene in the epithelium of the skin (6). The preferential accumulation of various carotenoid species in different tissues and organs facilitates the unique functions they serve to those tissues and organs in the body (65). For example, lycopene, the most effective quencher of $^1O_2$, is found at higher levels in tissues that are subjected to more oxidative damage (i.e. lungs, kidneys, epidermis, and liver). Likewise, lutein and zeaxanthin perform important functions in the eye which contribute to optimal eye health (66). The absorption spectra of lutein and zeaxanthin allow them to protect the eye from damaging UV light and they can also act as antioxidants against free radicals in the macula (6).

Carotenoids also accumulate in the outermost layer of skin which is the layer exposed to the environment, or the stratum corneum. The highest carotenoid concentrations in the stratum corneum are typically observed at a depth of 4-8 µm (4). Skin depth and carotenoid concentration are negatively correlated; the deeper the layer of skin, the lower the concentration of carotenoids (64). Carotenoids exhibit their antioxidant potential in the stratum corneum by serving as a source of photoprotection against harmful UV rays (16). Research suggests that carotenoids are deposited unevenly in the skin through two different mechanisms. One way is through facilitated diffusion from blood, lymph, or adipose tissue by way of the same proteins used in the process of intestinal absorption, SR-BI and CD 36 (6). The second way is via

secretion from sweat and/or sebaceous glands located in the skin (4). Therefore, areas of the epidermis with a higher concentration of sweat glands also have a higher concentration of carotenoids (9, 67). These areas include the back, forehead, and the palm of the hands (6, 9). Carotenoids also tend to accumulate in body sites that have a high lipid to protein ratio which includes the sole of the foot in addition to the aforementioned forehead and palm of the hands (6). Of the carotenoids in the skin, β-carotene and lycopene appear in the highest concentrations. This not only reflects their heightened hydrophobicity and lipid solubility, but also the photoprotection function they express in skin, which is particularly evident in lycopene (6).

Regardless of the variability of carotenoid concentration in various organs and tissues, the relative concentration of species almost always reflects the concentration in the blood (12). Likewise, carotenoid concentrations in the blood almost always reflect dietary intake (65). Blood concentrations reflect recent dietary intake, whereas concentrations in other tissues and organs are believed to reflect long-term dietary habits (64). Carotenoids are mainly transported in the blood by low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL), as well as high-density lipoprotein (HDL) to a lesser extent (65). The hydrocarbon carotenoids tend to be selectively taken up by LDL, whereas oxygenated xanthophylls are relatively evenly distributed between LDL and HDL (64). It is generally accepted across the literature that individuals with high circulating levels of carotenoids will also exhibit high tissue levels of carotenoids (64). This is due in part to the density of lipoprotein receptors found in various tissues. Kaplan et al. assert that the organs and tissues with the greatest number of LDL receptors and highest rates of lipoprotein uptake will generally have higher concentrations of carotenoids (12). Therefore, the high concentration of carotenoids in the adrenal glands is likely due to the fact that the tissue exhibits high LDL uptake (64). Similarly, the high concentration of carotenoids in the liver may

be due to the fact that it is not only a site for lipoprotein degradation, but the primary location of lipoprotein synthesis in the body as well (65). Blood, tissues, and organs are all areas from which specimens have been collected when assessing the carotenoid status of an individual. The method and site of data collection simply depends on the objectives of the study or intervention.

Carotenoids as a biomarker of fruit and vegetable status

Self-reported data are commonly used to assess compliance to interventions, but they lack the reliability and objectivity of biomarkers (18). As a result, biomarkers are sought after as a "gold standard" of quantifying and validating nutrient intake in nutrition interventions, among other things (41). FV consumption comprises the majority of both carotenoid and vitamin A intake in the United States (2). Because blood carotenoid concentrations generally reflect dietary intake, carotenoid status can serve as a useful biomarker of fruit and vegetable consumption (44).

Biomarker: Blood carotenoid concentration

Carotenoid status has historically been measured by skin or blood biopsies which are both invasive and expensive specimens to collect and analyze (18). Blood samples are the "gold standard" of biomarkers used to assess carotenoid concentration and thus FV status in an individual. Once a blood or skin biopsy is taken, it must be stored rapidly in the proper temperature (in most studies the storage temperature is at -80° C in an ultra-low freezer) to ensure the carotenoids do not get damaged by light or heat, as they are apt to do. If the samples are stored correctly, they can then be analyzed by high-performance liquid chromatography (HPLC) either combined with mass spectrometry (MS) or UV-vis absorption detection. The most commonly used HPLC method for carotenoid separation and identification combines HPLC with UV-vis absorption detection (68).

Venous blood samples are collected by venipuncture. When samples are ready for analysis, they are mixed with a solvent that is compatible with the solvent used in the first phase of HPLC. This solution is then injected into the system. The preparation of solution involves multiple steps which can introduce errors into the system. As a result, it is vital that the system is standardized with the incorporation of an internal standard (69). There are two separate phases of HPLC, the first is the mobile phase (the solvent) and the second is the stationary phase (the column). The mobile phase of HPLC is the solvent that flows through the system and carries the compounds that are to be separated, or the analytes. The solvent(s) chosen for the mobile phase of HPLC is/are determined largely by the polarity of the analytes and should be degassed to minimize baseline "noise" (69). The mobile phase can run via isocratic analysis or gradient analysis. In isocratic analysis, the overall solvent composition of the mobile phase remains unchanged during the run (69). Gradient analysis, on the other hand, employs two or more solvents in the mobile phase that differ greatly in polarity and which are programmed to change during the process of elution (69). Once the analytes are transported through the mobile phase, they enter the stationary phase of HPLC in which the analytes are separated by adsorption. Each carotenoid interacts differently with the particles used in the stationary phase due to their differing polarities, and therefore flow through the column at different rates.

There are two main types of HPLC, normal phase and reversed phase. Normal phase HPLC (NPLC) is most commonly used for the separation of isomers. In NPLC the mobile phase is non-polar, and the stationary phase is polar (69). RPLC is better at separating non-polar carotenoids because the mobile phase is polar and the stationary phase is non-polar, so the polar carotenoids elute first and non-polar carotenoids elute slower (68, 69). As a result, RPLC leads to enhanced resolution of non-polar carotenoids, as compared to NPLC, and the two most common columns

used for their separation are suited for RPLC (68). In RPLC, the mobile phase should be a polar solvent or mixture of solvents. Craft (69) suggests that the mobile phase consist of a weak organic solvent with a low viscosity, like acetonitrile or methanol, which are 5.8 and 5.1 on the polarity index, respectively (70). These types of solvents allow for use of a wide range of solvent modifiers, which can help to improve carotenoid solubility and recovery. Water, which is 10.2 on the polarity index, should be used sparingly as a solvent modifier since carotenoids have low solubility in media that are too polar (69, 70).

The most commonly used column for the RPLC separation of carotenoids is a C_{18} column (69). These columns contain silica particles that have been modified by adding 18 carbons to each particle, thus making them non-polar. Non-polar carotenoid analytes (the carotenes) adsorb to the modified silica particles in the column and elute more slowly than their polar counterparts (the xanthophylls). The distance they travel in the stationary phase due to their interaction with the column packing leads to their separation and subsequent identification on the chromatogram (71). The ambient temperature in which the system runs can actually affect the separation occurring in the column. It is generally accepted that an ambient temperature of 20°C is optimal for maximum selectivity (68).

The process of HPLC is reliable and has been used for many years, not just with blood samples but skin samples, too. One of the main drawbacks of using this process is the expense. Additionally, extraction from blood samples is a more invasive approach, due to the blood draw procedure and the time needed for the blood draw, but it is also impractical for large intervention studies, especially those focused on increasing FV consumption in children (36). Further, HPLC analysis usually requires a large amount of samples and it is not suitable for assessing the kinetics of carotenoids in the skin (4, 43).

Despite the drawbacks of using blood as a biomarker of FV consumption, it is a highly validated method for assessing changes in response to supplementation with FV or purified forms of carotenoids. Some studies have supplied participants with a continuous dose of supplementation throughout the length of the study either with carotenoid-rich food sources or with purified forms of carotenoids and observed carotenoid saturation in the blood (15, 33, 34, 44, 56). Other studies supplied just a single dose of purified carotenoids and observed the depletion kinetics of the molecule in the blood (17, 21). Regardless of the design chosen, these studies support the correlation between dietary intake and blood accumulation of carotenoids.

Results from continuous dose studies are less variable than single-dose studies and have been performed with both carotenoid-rich foods, purified supplements, or a mixture of both (56). Yeum et al. demonstrated that plasma concentrations of most carotenoids will increase in a relatively short amount of time if fruits and vegetables are made more available in the diet (15). The supplementation in this study occurred during three study periods with each period consisting of 15 days and separated from subsequent periods by 6 weeks of self-selected dietary intake which allowed participants to return to baseline blood carotenoid concentrations. During the 15-day study periods, participants were served a large amount (10 servings/day) of commonly eaten FV in the U.S. each day as part of a mixed diet. The plasma concentrations of carotenoids dramatically increased in most participants by day 5 following supplementation, despite the diversity of the study population. Findings from Bowen et al. also support the notion that an increase in daily FV consumption (to 5-6 servings/day) will result in an increased serum carotenoid concentration (44). They found this to be true even if the participant was considered a "low-responder" to β-carotene as previously described (38).

It is clear that FV contain variable amounts of carotenoids, which can lead to variable amounts being absorbed and subsequently accumulating in the blood. Some FV are considered carotenoid-rich, while others are relatively low in carotenoids. Pezdirc et al. conducted a study to determine how diets supplemented with high-carotenoid FV and low-carotenoid FV affect plasma concentrations and skin yellowness in study participants (33). Thirty women were enrolled in this 4-week study and received a weekly box of FV containing either high-carotenoid FV (HCFV) or low-carotenoid FV (LCFV). FV were considered "high-carotenoid" if they contained >50 μg of β-carotene per 100 g serving and "low-carotenoid" if they contained <50 μg of β-carotene per 100 g serving. Participants in the LCFV group were given a list of foods high in β-carotene and were asked to avoid eating them during the study in order to maintain the integrity of the intervention. As expected, the HCFV group had higher plasma concentrations of α-carotene, β-carotene, lutein, and total carotenoids following the 4-week supplementation period when compared to the LCFV group. These results indicate that the types of FV consumed in a study are important if the goal is to increase plasma carotenoids (33). Results from dePee et al. also support the idea that some FV are more effective at increasing serum carotenoids than others (34).

In addition to the increase in blood carotenoids observed after supplementation with food, similar, more impactful results have been observed after supplementation with purified forms of carotenoids. The greater impact of purified carotenoids on plasma carotenoids compared to food was observed in several studies. Micozzi et al. conducted a study to determine serial changes of plasma carotenoids when 30 men were given defined doses of carotenoids for 6 weeks either from carotenoid-rich vegetables (broccoli, tomato juice, or carrots) or purified forms of β-carotene (56). The purified tablets contained either 12 mg β-carotene or 30 mg β-carotene and

increased plasma β-carotene concentrations dramatically after the 6-week period. The average maximum increase was greatest in the group receiving 30 mg/day, followed by the group receiving 12 mg/day, and finally the group receiving carrots. The sharp increase in β-carotene levels persisted for the entirety of the 6-week supplementation and would have eventually reached a steady state if supplementation continued (56). Likewise, Törrönen et al. conducted a similar study to determine how vegetables/vegetable juice and purified β-carotene capsules affected serum levels of carotenoids (72). The study started with a 10-day depletion period in which participants consumed a self-selected diet that was low in carotenoids. After the washout, participants were divided into three groups, one of which received a 12 mg capsule of purified β-carotene per day for 6 weeks. The groups consuming vegetables also consumed 12 mg β-carotene per day, but it was either in the form of raw carrots or carrot juice. As expected, the group receiving the purified capsules experienced much greater increases in serum carotenoids than those supplemented with vegetables. However, they noticed that there was a wide variation in serum response to β-carotene supplementation regardless of the source (72).

Interindividual variability can be a limitation to observing statistical significance in a study, but a few studies have found that supplementation with purified carotenoids can still increase blood concentrations despite those differences. Dimitrov et al. conducted a plasma kinetics study in which 34 participants were divided into three groups receiving purified β-carotene supplements for various lengths of time and at different doses (ranging from 15-150 mg) (21). The three dosage groups included those receiving just a single dose of β-carotene, those receiving a 3-day loading dose, and those receiving a continuous dose for 30 days. The group receiving a single dose (either 15, 30, 45, 90, or 150 mg) was instructed to omit foods rich in β-carotene from their diet and given a list of foods to avoid. The individuals in the group had their blood collected

every three hours for the first 24 hours of the study and analysis shows that the peak response to the supplementation was between 6-24 hours after the single dose. There was wide variability in response from this group. After the first 24 hours, samples were collected at 48-, 72-, 96-, 120-, and 144-hours post-dosage until baseline plasma levels were reached. The loading dose (30, 45, 90, or 150 mg for three consecutive days) and daily dose (45 mg/day for 30 days) groups had their blood sampled daily, but steady-state plasma levels were the desired end point for collection rather than returning to baseline levels. The study found that loading doses can be effective at reaching higher plasma β-carotene levels and that single doses resulted in more variability than other groups. This study also found that response to supplementation is widely variable between individuals and that different individuals may need different doses of β-carotene for obtaining the same plasma response as each other (21). Results from the year-long study by Nierenberg et al. also support the idea that there is high variability in the plasma response of subjects receiving supplementation with β-carotene capsules (62).

Though interindividual variability is inevitable, individuals who respond to supplementation tend to do so in a certain window of time post-dosage. The point of time at which their blood levels peak after supplementation is considered the time course of peak accumulation in the blood. Canfield et al. studied the plasma response to oral doses of β-carotene (0, 15, or 30 mg) in school children over a 48-hour period (17). The children were divided into three groups, one of which received a 15 mg capsule of purified β-carotene, one which received two 15 mg capsules (for 30 mg β-carotene total), and one which served as the control group (0 mg of β-carotene). The capsules were administered with a cup of chocolate drink that contained 8.4 g of fat after which a meal was consumed approximately one hour later. Blood samples were withdrawn on two different schedules; one schedule withdrew blood 2-, 4-, 8-, and 24-hours post-dosage and the

other schedule withdrew blood 3-, 6-, 9-, and 48-hours post-dosage. The responses for both schedules were combined and the data show that the average maximum increase in blood concentrations for both dosages occurred at 24 hours. However, increases in plasma concentrations appeared earlier in the individuals receiving the larger dose. Approximately 1/3 of the participants experienced their maximum plasma change at or before 9-12 hours, and approximately 2/3 of the participants experienced their peak change at either 24- or 48-hours post-dosage. The suggested 24-hour time course of blood carotenoid accumulation is consistent with findings from previous studies (17, 21, 73).

Biomarker: Skin carotenoid status

Skin carotenoid levels are directly correlated with fruit and vegetable intake (74). Meinke et al. (9) showed that supplementing the diet with purified carotenoid extracts increases levels in the skin just as it does in the blood. They found that while the increase occurred faster in the blood, the reservoir function of the skin was superior. With the obvious drawbacks of collecting biopsies, the literature promotes the use of non-invasive measurement techniques to assess skin carotenoid status. Non-invasive techniques like Raman resonance spectroscopy (RRS) and reflectance spectroscopy (RS) have been in use since the late 1990s. In most studies using these techniques, the forehead and palms are the preferred sites for taking measurements since they are easily accessible and contain a high concentration of carotenoids. Sites with minimal melanin content, like the palm of the hand, heel of the foot, or tip of the finger are also preferred since other skin chromophores may obscure carotenoid readings (19, 36).

Both forms of spectroscopy employ excitation of skin pigments at particular wavelengths in order to measure dermal carotenoid levels in individuals. Neither process is invasive nor

expensive, and both allow for the rapid assessment of skin carotenoids. These factors make each of the spectroscopy methods an appealing alternative for an objective measure of FV consumption in large intervention studies.

There are over 10,000 RRS tools currently in use at labs across the country and abroad and many of them are being used specifically in the field of nutrition science and epidemiology (19). RRS uses either a laser or focused light source to excite carotenoids in the blue-green range of the visible spectrum in which wavelengths range from 450-565 nm (4). However, carotenoids are not the only chromophores that exist in human skin, as melanin and both types of hemoglobin also contribute to skin color and have absorption wavelengths that tend to overlap that of carotenoids (19, 75). As a result, there is only a small window for the spectroscopic detection of dermal carotenoids, without much overlap from other chromophores, in the wavelength range of 460-520 nm (19).

In RRS, the frequencies at which the light or laser is emitted can be changed to target specific carotenoids. For example, Meinke et al. applied two different excitation wavelengths to the skin in their 2010 study. The blue light emitted at 488 nm was effective at exciting all carotenoid substances observed (β-carotene, lycopene, and lutein), while the excitation of lycopene was particularly prevalent at the wavelength 514 nm (9). The light from the spectrometer interacts with the characteristic C=C backbone of the carotenoids which leads to the excitation of the molecule (19). This subsequently produces a Raman light scattering effect that can be measured and quantified.

Jahns et al. conducted a 28-week experimental feeding intervention to compare changes in skin carotenoid status with changes in plasma carotenoids (13). The intervention consisted of four

phases and three diet treatments. Phases 1 and 3 both involved 6-week depletion diets in which participants were given a low-carotenoid diet to follow and a list of carotenoid-rich foods to avoid. Phase 2 involved an 8-week intervention diet high in FV. In phase 4, the final phase, participants were asked to return to a self-selected diet. Skin carotenoids were measured by RRS ≥2 times/week for phases 1, 3, and 4 and 5 times/week during phase 2. Data show RRS intensities decreased by 37% in the first depletion phase (phase 1) increased by 264% during the intervention phase (phase 2) and decreased in phase 3 (the second depletion phase) by 43%. The researchers also found that plasma and skin carotenoids were correlated at baseline and throughout the study. They suggest that skin carotenoid status responds quickly to increased carotenoid intake and may appear in skin as little as two weeks post intervention (13). Results from Massenti et al. also suggest that skin carotenoid status, measured with RRS, can increase with regular intake of FV (76). Furthermore, supplementation of a normal diet with natural extracts containing high amounts of carotenoids can also significantly increase skin carotenoids as measured by RRS (9).

Instead of conducting a dietary intervention like the studies mentioned above, Rerksuppaphol et al. used FFQs to assess FV intake of individuals and compare how self-reported intake correlates with skin carotenoid status (74). They categorized individuals into groups of low, moderate, and high intake of FV and found that the mean skin carotenoid status of low intake was significantly lower than moderate or high FV intake. These results, and those found in Mayne et al. (18), further support the correlation between self-reported intake and skin carotenoid status as measured by RRS.

Clearly, the use of RRS to measure carotenoid status has been highly validated since its inception. There is a strong correlation between skin carotenoid values and blood carotenoid

values, measured by RRS and HPLC respectively, despite the high inter- and intra-individual variability (6, 9, 13). The obvious merits of RRS in non-invasive carotenoid assessment include its degree of validation and its ability to differentiate between different carotenoids (75).

Despite the advantages of using this type of measurement, RRS tools tend to be rather bulky and are sensitive to vibration, which can become an issue while transporting the machine to study sites (75). Because of these drawbacks, the use of reflectance spectroscopy (RS) has emerged as a useful alternative for the optical detection of carotenoids, especially when it comes to remote data collection.

RS uses the same basic concept as RRS, but the frequency at which light is emitted is fixed and does not require nearly as much energy as RRS (19). A couple methodologies preceded the pressure-mediated RS methodology that is widespread and most commonly used today. Ermakov et al. (36) describe a diffusive light transport model that, while finding a significant response in the skin and serum after the supplementation of β-carotene, was ultimately problematic in its detection of carotenoid chromophores. They also describe another reflection-based methodology that was implemented in the cutaneous detection of carotenoids by using the tri-stimulus b*-value (which detects yellow to blue color saturation in an object) (16, 36). However, the scattering and absorption of the carotenoids was obscured by the scattering and absorption of other skin chromophores.

In response to these unsuccessful early uses of RS to measure dermal carotenoids, Ermakov et al. (36) proposed the use of pressure-mediated reflection spectroscopy instead and developed an instrument that implemented this methodology. Pressure-mediated RS still derives skin carotenoid measures via reflectivity, but it does so with the addition of applied topical pressure

on the site of measurement (typically the fingertip). The finger is placed in direct contact with the lens and is gently covered by a spring-loaded clamp which applies approximately 1 atm of pressure to the finger. The pressure temporarily pushes away the blood in the finger so that the hemoglobin does not interfere with carotenoid measurements. Once the fingertip is enclosed in the window, a light delivery/collection module projects a "spectrally broad" white light onto the skin (36). When the light hits the skin, it is absorbed and scattered, and that data is sent to an accompanying software in a laptop connected to the implement. The software then calculates a skin carotenoid score (SCS) which is a composite score of all carotenoids measured and can range from 0-900. The current implement is capable of measuring all carotenoids that absorb in the 480 nm range, which includes α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene (36).

While the use of a fixed light source disables the ability to differentiate between specific groups of carotenoids, several studies have demonstrated a significant correlation between baseline measures of serum and skin carotenoids as well as between dietary intake and skin carotenoid scores. Stahl et al. (20) measured the correlation between blood and skin carotenoid concentrations after 12 weeks of supplementation with Betatene, an extract rich in carotenoids, using simple RS methods (not pressure-mediated). The skin areas of interest were measured at the same time points as the blood was extracted and included the forehead, back, back of the hand, palm of the hand, and the volar forearm. They found that serum and skin levels of β-carotene were correlated, with the highest correlation coefficients found in the palm of the hand and the skin of the forehead (20).

Pressure-mediated RS methods are also becoming increasingly validated through several studies that specifically investigate the correlation between dietary intake and skin carotenoid score

(SCS). Ermakov et al. (36) conducted a pilot study assessing the viability of pressure-mediated RS in the measurement of carotenoid uptake after supplementation in three individuals. During the ten days prior to supplementation, when subjects consumed their normal diet, the SCS of the subjects stayed relatively constant. After ten days, the subjects supplemented their normal diet with two three-ounce servings of carrot juice per day. The SCS of two of the subjects quickly increased and doubled within three weeks' time. The third subject had relatively high baseline levels of skin carotenoids, so there was only a moderate, non-significant increase in their levels following supplementation. In the same publication, Ermakov et al. (36) reported a separate uptake study with 90 participants. In this larger study, three of four groups supplemented their regular diet with a two-ounce serving of juice containing β-carotene twice per day for a period of seven weeks. The fourth group, which remained un-supplemented, served as the control. The data show that the average increase in carotenoid scores rose 80% in the supplemented groups, with the highest individual response showing a 200% increase. These two studies validate the use of pressure-mediated RS in assessing changes in SCS.

Pitts et al. (23) also performed two small studies to validate the use of pressure-mediated RS in assessing SCS as it relates to dietary intake. In one study, they examined the association between self-reported FV intake and RS scores. The FV intake was reported through the National Cancer Institute's (NCI) Fruit & Vegetable (F&V) Screener which is a validated tool that measures overall daily intake of FV. The second study examined the associations between FV intake reported through a food frequency questionnaire (FFQ), plasma carotenoids, and RS scores. The first study found no correlation between the NCI F&V Screener responses and RS scores. The researchers suggested that the lack of association might be due to the fact that the questionnaire was not specific to consumption of carotenoid rich FV, but to FV consumption in general. In

contrast, the second study showed a significant correlation between FFQ responses, plasma carotenoid concentrations, and RS scores, after adjusting for race and age. As a result, Pitts et al. suggest that RS is a feasible and reliable tool for the assessment of carotenoid rich FV consumption in a diverse sample of the population.

Ermakov and his team (19) have published further validation for the use of pressure-mediated RS in assessing skin carotenoid status. One particular study evaluated the correlation between RS and total serum carotenoid concentration, as well as the correlation between RS and RRS scores in 54 subjects. The data showed a high correlation between RS and serum carotenoid concentrations, with an R value of 0.81. An even higher correlation was observed between RS and RRS scores, with an R value of 0.94. The paper reports on additional validation studies performed by the team and all results are consistent, indicating a high correlation between RS, RRS, and plasma levels of carotenoids.

A recent systematic review reported that of the non-invasive methods available for measurement of skin carotenoids, the strongest correlations exist between spectroscopy and blood carotenoids but there were statistically significant correlations between spectroscopy and self-reported dietary intake (77). The authors of the review purport that spectroscopy is an objective biomarker of dietary FV intake, but recommend that additional research be conducted before spectroscopy is considered equivalent to plasma or serum carotenoids (77).

Protocols for skin carotenoid studies

Pressure-mediated RS devices are becoming more widely used in light of their viability as effective measurement tools of dermal carotenoids. However, there is a lack of detail in the literature about a standard protocol followed during data collection with pressure-mediated RS.

Ermakov and Gellermann (22) were the first ones to develop a pressure-mediated RS tool, and also led the way in describing an appropriate protocol to follow while using the tool. In fact, Ermakov et al. (19) are the only team to date that describe a comprehensive and formal RS protocol. This protocol was followed during routine public health screenings for children ages 2 to 5 years old in San Francisco starting in 2016 and specifies the exact steps followed to obtain accurate RS readings in the study population. The protocol described in the paper is as follows: "a) cleaning of the contact lens of the device with an eye glasses cleaner, b) checking of each child's finger for the absence of ink, c) cleaning of the fingers with a Purell wipe, d) opening the finger cover to show the absence of any needles, e) instructing each child to place one index finger in the device with the cover closed over the finger, e) ensuring the finger completely covered the contact lens before closing the lid, f) asking the child to count to ten, e) taking the finger off the contact lens once the device's 'scanning in progress' message was complete, f) recording the score that was displayed as an integer between 0 and 800, g) using an alternate finger if the index finger had a cut or scar" (19). This protocol is clear and specifies both the participant's and researcher's role in the data collection process.

Researchers using pressure-mediated RS have the responsibility to contribute details from the study methods they employ to both fortify and expound on the aforementioned protocol. For example, though Pitts et al. (23) do not describe whether they adhered to the protocol described above, they do describe the subject finger measured (index finger) and the number of scans taken (three, using the average of the last two scores in analysis) during the data collection process in their study. It is these detailed descriptions of the RS protocol followed that allow for repeatability, leading to further validation of pressure-mediated RS in the measurement of dermal carotenoids.

In addition to establishing a standard protocol for pressure-mediated RS during data collection, it is also advantageous to determine time points of when to collect data during the study. Since pressure-mediated RS is becoming widely used in nutrition intervention studies, it is likely that baseline data will be collected before supplementation and at time points of interest both during and after the intervention. Researchers usually look for a significant deviation from baseline carotenoid concentrations in nutrition interventions promoting increased intake of FV. The change, if it takes place, is presumed to be in response to the intervention. Jahns et al. assert that, in addition to blood carotenoid concentrations, skin carotenoid measurements are useful for measuring changes in FV intake (13). Many studies have established an approximate time course of blood carotenoid accumulation, particularly Canfield et al. (17) and Dimitrov et al. (21). However, an approximate time course of skin carotenoid accumulation has not been comprehensively established, so there's not a clear-cut time point in which to assess participant adherence during an intervention.

The approximate half-life of β-carotene in the blood is less than 12 days (18). It is widely accepted that peak tissue and skin accumulation appear later than peak blood accumulation (9), but the literature is inconsistent about when to expect the peak accumulation to occur. For example, Jahns et al. (13) and Prince and Frisoli (10) suggested that peak accumulation in the skin may take up to two weeks. However, Mayne et al. (18) proposed that skin carotenoid status reflects intake from the past two months and Stahl et al. (20) suggested peak accumulation occurs within 12 weeks of supplementation. Establishing a comprehensive time course for when to expect significant changes from baseline levels that lead to peak accumulation of carotenoids in the skin will aid nutrition scientists and epidemiologists in study design and protocol, particularly when employing pressure-mediated RS as a form of measurement.

Conclusion

Carotenoids are effective biomarkers of FV intake/status, but they can be affected by many factors including but not limited to the digestion and absorption process, lifestyle factors, dietary behavior, genetics, and food composition and processing. These factors contribute to a high variability in carotenoid status both within and between individuals.

Carotenoids can be measured non-invasively which is especially important for large intervention studies, particularly those that target increased FV consumption in children. The most commonly consumed carotenoids in the U.S. are also those that can be measured by the pressure-mediated RS implement which makes this tool a good option for assessment of dietary FV intake.

Pressure-mediated RS is a validated and legitimate tool for the measure of skin carotenoids. It is portable, inexpensive, and decreases "noise" from other skin chromophores which can obscure the true carotenoid measurements, or "skin carotenoid scores". There is at least one formal protocol described for the use of this tool. But there is no agreement about an approximate time course of peak skin carotenoid accumulation or when to expect a significant change in skin carotenoid score from baseline during or following an intervention. Research shows that skin accumulation correlates with blood accumulation and that blood accumulation correlates with dietary intake. Several studies confirm that peak blood accumulation after single dosing is 6-24 hours and after continuous dosing it is 24-48 hours. Continuous dosing lowers variability between individuals and more accurately represents the dietary behavior of free-living subjects. Since time to skin accumulation lags that of time to blood accumulation, it must take longer than the minimum peak time in the blood of 24 hours after continuous dosing for skin carotenoids to accumulate.

Determining the approximate time course of skin carotenoid accumulation using pressure-mediated RS will contribute to the body of knowledge in the fields of nutrition science and epidemiology. It will establish a point at which RS can be used to assess participant adherence to an intervention. This information can aid researchers in their study design and protocol. It may also help determine whether consumption of the average recommended intake of FV results in detectable differences in skin carotenoid status, another valuable piece of information for intervention studies. Finally, determining a time course may increase the understanding of the kinetic patterns of carotenoids in the skin, which is important if researchers continue using non-invasive measures to assess FV intake.

APPENDIX C

Screening Survey

Informed Consent Document

Background Survey

SCS Data Sheet

Screening Survey, Day 1

Name of prospective participant: ____

Name of interviewer: ___________________________________ Date: ____________

Question	Answer (Y/N)
1. Are you a student, staff, or faculty member at IU?	
2. What is your age?	
3. Do you have psoriasis?	
4. Do you currently use self-tanner or spray-on tanning (within the last two weeks)?	
5. Are you willing to eat baked sweet potato with butter 3 times a week for weeks 2 and 3 of the study? (Note: the meal will be prepared for you)	
6. Do you have a fat malabsorption disorder?	
7. Have you been diagnosed with liver disease?	
8. Are you currently pregnant?	
9. Do you currently smoke or vape tobacco products?	
10. Did you binge drink* on 5 or more days in the past month?	

* "NIAAA defines binge drinking as a pattern of drinking that brings blood alcohol concentration (BAC) levels to 0.08 g/dL. This typically occurs after 4 drinks for women and 5 drinks for men—in about 2 hours." https://niaaa.nih.gov

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For interviewer use only: *If participant answers yes to any question (to which Y/N applies), they are NOT eligible for the study.*

Does participant meet eligibility requirements (Y/N)? ______________________________

- ➔ If *yes*, move forward with Day 1 protocol. If *no*, thank participant for their time and let them know they are not eligible for the study.

INDIANA UNIVERSITY INFORMED CONSENT STATEMENT FOR RESEARCH

Skin Carotenoid Accumulation in Response to a Two-Week Diet Change Promoting Increased Red/Orange Vegetable Consumption

ABOUT THIS RESEARCH

You are being asked to participate in a research study. This consent form will give you information about the study to help you decide whether you want to participate. Please read this form, and ask any questions you have, before agreeing to be in the study.

TAKING PART IN THIS STUDY IS VOLUNTARY

You may choose not to take part in the study or may choose to leave the study at any time. Deciding not to participate, or deciding to leave the study later, will not result in any penalty or loss of benefits to which you are entitled and will not affect your relationship with Indiana University-Bloomington.

WHY IS THIS STUDY BEING DONE?

The purpose of this study is to examine the effect of increased red/orange vegetable consumption on skin and blood carotenoid concentration. This study is being conducted to determine when to measure blood and skin carotenoids after eating a food source of carotenoids.

You were selected as a possible participant because you responded to the flyer, announcement, or email about being a volunteer for this study. The study is being conducted by Dr. Alyce D. Fly and Taylor Erickson from the School of Public Health at IU. It is funded in part by Indiana University.

HOW MANY PEOPLE WILL TAKE PART?

If you agree to participate, you will be one of 100 participants taking part in this study.

WHAT WILL HAPPEN DURING THE STUDY?

You will visit the lab 17 times over a period of 7 weeks. You will be emailed reminders.
If you agree to be in the study, you will do the following things:

- Day 1: you will be asked to fill out a brief background survey and a survey about what you regularly eat, have your height and weight measured, have your index finger scanned by the Veggie Meter to determine your skin carotenoid score, and have 10 mL (about 2 teaspoons) of blood drawn via venipuncture. This visit will take about 30-45 minutes.
- Day 3-5: you will have your finger scanned by the Veggie Meter. This will take less than 10 minutes per visit.
- Day 8-19 (Weeks 2 & 3): you will eat a baked sweet potato with butter around lunch time three times each week. Next, you will have your finger scanned by the Veggie Meter. Then you will fill out a food log to record food consumed during the previous day. These visits will take about 30 minutes.
    - On day 19, you will also have a 10 mL (about 2 teaspoons) sample of blood drawn via venipuncture.
- Day 22-43 (Weeks 4-7): you will have your finger scanned by the Veggie Meter twice each week. This will take less than 10 minutes per visit.

- Day 47: you will have your finger scanned by the Veggie Meter and have a 10 mL (about 2 teaspoons) sample of blood drawn via venipuncture. This visit will take about 15 minutes.

WHAT ARE THE RISKS OF TAKING PART IN THE STUDY?

The risks and/or discomforts include a potential loss of confidentiality and mild physical discomfort during venipuncture. Measures will be taken to minimize the risks and/or discomforts that may occur. Electronic data collected from you will be stored on password-protected computers and written data will be stored in a locked drawer in a locked laboratory. Blood will be drawn by certified phlebotomy technicians that were trained on proper techniques for safe and efficient collection of blood.

WHAT ARE THE POTENTIAL BENEFITS OF TAKING PART IN THE STUDY?

You won't benefit from taking part in this study, but we hope to learn things that will help scientists in the future.

HOW WILL MY INFORMATION BE PROTECTED?

Efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. No information which could identify you will be shared in publications about this study. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the study investigator and his/her research associates, the IU Institutional Review Board or its designees, and state or federal agencies who may need to access the research records (as allowed by law).

WILL MY INFORMATION BE USED FOR RESEARCH IN THE FUTURE?

Information or specimens collected from you for this study may be used for future research studies or shared with other researchers for future research. If this happens, information which could identify you will be removed before any information or specimens are shared. Since identifying information will be removed, we will not ask for your additional consent.

WILL I BE PAID FOR PARTICIPATION?

Participants can earn $205 upon completion of the study. Should you withdraw participation prior to completion of the study, you will only be partially compensated as seen on the schedule below.

- Payment 1: Participants are paid $25 after the first week of the study.
- Payment 2: After completing weeks 2 and 3, participants are paid $60.
- Payment 3: After completing weeks 4-7, participants are paid $120.

WILL IT COST ME ANYTHING TO PARTICIPATE?

There is no cost to you for taking part in this study.

WHO SHOULD I CONTACT WITH QUESTIONS OR PROBLEMS?

For questions about the study, contact the researcher, **Taylor Erickson** at **tericks@iu.edu** or **Dr. Alyce Fly** at **afly@indiana.edu**. For questions about your rights as a research participant, to discuss problems, complaints, or concerns about a research study, or to obtain information or to offer input, please contact the IU Human Subjects Office at 800-696-2949 or at irb@iu.edu.

CAN I WITHDRAW FROM THE STUDY?

If you decide to participate in this study, you can change your mind and decide to leave the study at any time in the future. If you decide to withdraw, please email **Taylor Erickson** at **tericks@iu.edu**.

PARTICIPANT'S CONSENT

In consideration of all of the above, I give my consent to participate in this research study. I will be given a copy of this informed consent document to keep for my records. I agree to take part in this study.

Participant's Printed Name:________________________________

Participant's Signature:____**Date**:_____________

Printed Name of Person Obtaining Consent:_____________________________

Signature of Person Obtaining Consent:___________________________**Date**:_____________

Background and Health History Survey, Day 1

WT: ___________ HT: ___________

1. Name: ____ 2. Sex (M/F/Other): ______________

3. Are you Hispanic or Latino/Latina? ___ Yes ___ No

4. What is your race? Check all that apply.

a. ___ White
b. ___ Black or African American
c. ___ Asian
d. ___ Hawaiian or Other Pacific Islander
e. ___ Native American or Alaskan Native
f. ___ Other _______________________

5. Are you vegan or vegetarian? ___Yes, describe ________________________ ___No

6. Do you take any vitamins or supplements?
___ Yes, describe _______________________ ___No

7. If you answered "Yes" to question 6 above, why do you take vitamin(s)/supplement(s)?
____

8. During a typical 7-Day period (a week), how many times on the average do you do the following kinds of exercise for more than 15 minutes during your free time (write on each line the appropriate number). *

Times Per Week

STRENUOUS EXERCISE
(HEART BEATS RAPIDLY) __________
(e.g., running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long-distance bicycling)

MODERATE EXERCISE
(NOT EXHAUSTING) __________
(e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)

MILD EXERCISE
(MINIMAL EFFORT) __________
(e.g., yoga, archery, fishing from riverbank, bowling, horseshoes, golf, snow-mobiling, easy walking)

**From Godin Exercise Leisure-time Questionnaire: Godin G, Shephard RJ. A simple method to assess exercise behavior in the community. Can J Appl Sport Sci 1985;10:141-146.*

Skin Carotenoid Data Sheet
Participant Name: ____ ID: _______________

Dominant Hand? (R or L) ____________ Index Finger Used? (D or ND) ______________

Date	Ink/scars present (Y/N)	Week 1, Day #	SCS
		1	
		1	
		1	
Date	**Ink/scars present (Y/N)**	**Week 1, Day #**	**SCS**
		3	
		3	
		3	
Date	**Ink/scars present (Y/N)**	**Week 1, Day #**	**SCS**
		5	
		5	
		5	
Date	**Ink/scars present (Y/N)**	**Week 2, Day #**	**SCS**
		8	
		8	
		8	
Date	**Ink/scars present (Y/N)**	**Week 2, Day #**	**SCS**
		10	
		10	
		10	
Date	**Ink/scars present (Y/N)**	**Week 2, Day #**	**SCS**
		12	
		12	
		12	
Date	**Ink/scars present (Y/N)**	**Week 3, Day #**	**SCS**
		15	
		15	
		15	
Date	**Ink/scars present (Y/N)**	**Week 3, Day #**	**SCS**
		17	
		17	
		17	
Date	**Ink/scars present (Y/N)**	**Week 3, Day #**	**SCS**
		19	
		19	
		19	
Date	**Ink/scars present (Y/N)**	**Week 4, Day #**	**SCS**
		22	

		22	
		22	
Date	**Ink/scars present (Y/N)**	**Week 4, Day #**	**SCS**
		26	
		26	
		26	
Date	**Ink/scars present (Y/N)**	**Week 5, Day #**	**SCS**
		29	
		29	
		29	
Date	**Ink/scars present (Y/N)**	**Week 5, Day #**	**SCS**
		33	
		33	
		33	
Date	**Ink/scars present (Y/N)**	**Week 6, Day #**	**SCS**
		36	
		36	
		36	
Date	**Ink/scars present (Y/N)**	**Week 6, Day #**	**SCS**
		40	
		40	
		40	
Date	**Ink/scars present (Y/N)**	**Week 7, Day #**	**SCS**
		43	
		43	
		43	
Date	**Ink/scars present (Y/N)**	**Week 7, Day #**	**SCS**
		47	
		47	
		47	

APPENDIX D

NDSR Recipe Analysis for Sweet Potato Snack

University of Minnesota
NDSR
Nutrition Data System for Research

NDSR 2018 Nutrient Totals Report

Project Abbreviation: SPS
Participant ID: 0 Date of Intake: 05/18/2020

Primary Energy Sources

Nutrient	Amount
Energy (kilocalories)	198 kcal
Energy (kilojoules)	828 kj
Total Fat	4.326 g
Total Carbohydrate	37.281 g
Available Carbohydrate	31.341 g
Total Protein	3.660 g
Animal Protein	0.043 g
Vegetable Protein	3.618 g
Gluten	0.000 g
Alcohol	0.000 g
% Calories from Fat	19.160 %
% Calories from Carbohydrate	75.937 %
% Calories from Protein	5.175 %
% Calories from Alcohol	0.000 %

Fat and Cholesterol

Nutrient	Amount
Cholesterol	11 mg
Solid Fats	4.056 g
Total Saturated Fatty Acids (SFA)	2.618 g
Total Monounsaturated Fatty Acids (MUFA)	1.175 g
Total Polyunsaturated Fatty Acids (PUFA)	0.316 g
Total Trans-Fatty Acids (TRANS)	0.164 g
Total Conjugated Linoleic Acid (CLA 18:2)	0.026 g
Omega-3 Fatty Acids	0.023 g
% Calories from SFA	11.611 %
% Calories from MUFA	5.220 %
% Calories from PUFA	1.369 %
Polyunsaturated to Saturated Fat Ratio	0.121
Cholesterol to Saturated Fatty Acid Index	3.182

Fiber

Nutrient	Amount
Total Dietary Fiber	5.940 g
Soluble Dietary Fiber	1.566 g
Insoluble Dietary Fiber	4.374 g
Pectins	1.440 g

Vitamins

Nutrient	Amount
Total Vitamin A Activity (Retinol Equivalents)	3494 mcg
Total Vitamin A Activity (International Units)	34717 IU
Total Vitamin A Activity (Retinol Activity Equivalents)	1764 mcg
Beta-Carotene Equivalents (derived from provitamin A carotenoids)	20763 mcg
Retinol	34 mcg
Vitamin D (calciferol)	0.013 mcg

UNIVERSITY OF MINNESOTA
NDSR
NUTRITION DATA SYSTEM FOR RESEARCH

NDSR 2018 Nutrient Totals Report

Project Abbreviation: SPS
Participant ID: 0 Date of Intake: 05/18/2020

Vitamins

Vitamin D2 (ergocalciferol)	0.003 mcg
Vitamin D3 (cholecalciferol)	0.010 mcg
Vitamin E (International Units)	2.081 IU
Vitamin E (Total Alpha-Tocopherol)	1.394 mg
Natural Alpha-Tocopherol (RRR-alpha-tocopherol or d-alpha-tocopherol)	1.394 mg
Synthetic Alpha-Tocopherol (all rac-alpha-tocopherol or dl-alpha-tocopherol)	0.000 mg
Total Alpha-Tocopherol Equivalents	1.396 mg
Beta-Tocopherol	0.000 mg
Gamma-Tocopherol	0.018 mg
Delta-Tocopherol	0.000 mg
Vitamin K (phylloquinone)	4.490 mcg
Vitamin C (ascorbic acid)	35.280 mg
Thiamin (vitamin B1)	0.193 mg
Riboflavin (vitamin B2)	0.193 mg
Niacin (vitamin B3)	2.679 mg
Niacin Equivalents	3.889 mg
Pantothenic Acid	1.597 mg
Vitamin B-6 (pyridoxine, pyridoxyl, & pyridoxamine)	0.515 mg
Total Folate	11 mcg
Dietary Folate Equivalents	11 mcg
Natural Folate (food folate)	11 mcg
Synthetic Folate (folic acid)	0 mcg
Vitamin B-12 (cobalamin)	0.009 mcg

Carotenoids

Beta-Carotene (provitamin A carotenoid)	20724 mcg
Alpha-Carotene (provitamin A carotenoid)	77 mcg
Beta-Cryptoxanthin (provitamin A carotenoid)	0 mcg
Lutein + Zeaxanthin	0 mcg
Lycopene	0 mcg

Minerals

Calcium	70 mg
Phosphorus	98 mg
Magnesium	49 mg
Iron	1.243 mg
Zinc	0.581 mg
Copper	0.291 mg
Manganese	0.895 mg
Selenium	0.410 mcg
Sodium	65 mg

UNIVERSITY OF MINNESOTA
NDSR
NUTRITION DATA SYSTEM FOR RESEARCH

NDSR 2018 Nutrient Totals Report

Project Abbreviation: SPS
Participant ID: 0 Date of Intake: 05/18/2020

Minerals

Potassium	856 mg

Note: DSAM nutrients are not included in these totals. Nutrient totals may not equal the sum of their parts. (Refer to the NDSR User Manual.)

APPENDIX E

IRB Protocol Approval Summary

KC IRB

Protocol Summary

Protocol Number: 1912399684

Title: SKIN CAROTENOID ACCUMULATION IN RESPONSE TO A TWO-WEEK DIET CHANGE PROMOTING INCREASED RED/ORANGE VEGETABLE CONSUMPTION

Status: Active - Open to Enrollment

Expiration Date:

Last Approval Date:

Investigator: Fly, Alyce D.

Protocol Details

Type: Expedited

Application Date: 05/18/2020

IU or Investigator held IND/IDE?

FDA Application No:

Organizations

Type	Organization
Performing Organization	Indiana University (UA)

Funding Source

Funding Type	Code/Number	Sponsor Name	Sponsor Type	Prime Sponsor Name	Prime Sponsor Type
Unfunded					

Subjects

Subject	Count
Total	100
Students	75

Areas of Research

Code	Description
000001	All Research Areas

Personnel

Person Name	Units		Role	Affiliate	Training Flag
Fly, Alyce D.	BL-APHS	APPLIED HEALTH SCIENCE	PI	IU	Y
Erickson, Taylor	BL-APHS	APPLIED HEALTH SCIENCE	CO-PI	IU	Y

Roles

Protocol

Aggregator

User Name
Erickson, Taylor

Study

Manager / Correspondent

User Name
Fly, Alyce D.

Actions

Description	Comments	Action Date
Expedited Approval	Amendment-001: Approved	05/18/2020
Assigned to Agenda	Amendment-001:	05/18/2020
Submitted to IRB	Amendment-001: Submitted to IRB	05/18/2020
Returned To PI	Amendment-001: removing unneeded document	05/18/2020
Submitted to IRB	Amendment-001: Submitted to IRB	05/13/2020
Amendment Created	Amendment-001: Created	05/13/2020

Actions

Description	Comments	Action Date
Expedited Approval		01/21/2020
Assigned to Agenda		01/21/2020
Submitted to IRB	Submitted to IRB	01/21/2020
Returned To PI	uploading stamped consent	01/21/2020
Submitted to IRB	Submitted to IRB	01/13/2020
Returned To PI		01/13/2020
Submitted to IRB	Submitted to IRB	01/10/2020
Protocol Created	Protocol created	12/16/2019

Questionnaires

Label
A - Level of Review Assessment
B - Lay Summary & Research Design
C - Sites & Collaborations
D - Recruitment Methods
E - Risks, Benefits, Protections
F - Data Safety Monitoring
H - Informed Consent Process
K - HIPAA
M - ClinicalTrials.gov
Conflicts of Interest

Attachments

Description	Attachment Type	Last Updated	Updated By
Dietary History Questionnaire	Data Collection Instrument	12/20/2019 12:02:49	tericks
Screening Survey	Data Collection Instrument	01/13/2020 15:07:39	tericks
Background Survey	Data Collection Instrument	01/09/2020 10:00:53	tericks
Informed Consent Form	Informed Consent Statement	01/13/2020 15:09:46	tericks
Template for participant email	Recruitment Materials	01/13/2020 15:12:13	tericks
Template email to professors	Recruitment Materials	01/13/2020 15:19:15	tericks
Verbal script for classroom visits	Recruitment Materials	01/13/2020 15:33:39	tericks
stamped consent form for use	Informed Consent Statement	01/21/2020 15:18:54	kmumaw
Recruitment Flyer	Recruitment Materials	05/13/2020 15:59:28	tericks

Other Attachments

Description	Last Updated	Updated By
Phlebotomy Certificate for Fly	01/10/2020 13:11:43	tericks
Phlebotomy Certificate for Erickson	01/10/2020 13:12:17	tericks

Other Attachments

Description	Last Updated	Updated By
Study Timeline Handout for Participants	01/10/2020 13:18:50	tericks
Script for reminder email	01/10/2020 14:05:11	tericks

Determinations

Determination	Date Assigned	Date Inactive	Status	Comments
Study meets the criteria for approval defined by the HRPP Policy on IRB Review Process	01/21/2020		A	
Minimal Risk	01/21/2020		A	
Study continues to meet the criteria for approval defined by the HRPP Policy on IRB Review Process.	05/18/2020		A	

IRB APPROVAL

This research project, including all noted attachments, has been reviewed and approved by the Indiana University IRB.

☐ Exempt Category(ies), if applicable:

☒ Expedited Category(ies), if applicable: (2) (4) (7)

Reviewing IRB Committee: IRB-IUB
Level of Review: Expedited

Authorized IRB Signature: ____IRB Approval Date:_________________

Printed Name of IRB Member: ____

Review Comments

Protocol Number:	1912399684		
Principal Investigator:	Fly, Alyce D.		
Title:	SKIN CAROTENOID ACCUMULATION IN RESPONSE TO A TWO-WEEK DIET CHANGE PROMOTING INCREASED RED/ORANGE VEGETABLE CONSUMPTION		
Committee Id:	IRB00000222	**Committee Name:**	IRB-IUB
Schedule Id:	556555	**Schedule Date:**	07/16/2020

Review Comments:

Taylor L. Erickson

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EDUCATION

Indiana University
School of Public Health, Dept. Applied Health Science — Bloomington, IN
MS in Applied Health Science, Major: Nutrition Science — Expected Dec. 2020

Northern Arizona University, Center for Science Teaching and Learning
College of Engineering, Forestry, and Natural Sciences — Flagstaff, AZ
BSED Biology — December 2014

RESEARCH

1. RESEARCH EXPERIENCE

Indiana University- Bloomington — Bloomington, IN
Graduate Student; advisor: Dr. Alyce D. Fly — Aug. 2018-July 2020
Determinants of fruit and vegetable intake in rural elementary students; dermal accumulation of carotenoids using reflectance spectroscopy

Northern Arizona University — Flagstaff, AZ
Research Technician, Ecology Lab, Drs. Peter Fule and Larissa Yocom-Kent — 2012
Climate change response of Ponderosa pine forests on the Colorado Plateau

Undergraduate Research Assistant, Ecological Restoration Institute — Flagstaff, AZ
Understory, tree canopy, and small mammal surveys following wildfires in Ponderosa pine and pinyon-juniper forest types on the Colorado Plateau — 2009-2010

2. RESEARCH POSITIONS

NAU, School of Forestry, Forest Ecology Lab — Flagstaff, AZ
Research Technician — March 2012-Dec. 2013
Data collection and entry
Fuels transect measurements
Understory vegetation sampling
Tree, shrub, and forb identification
Sample processing in wood shop
Tree core dendrochronology

NAU, Ecological Restoration Institute — Flagstaff, AZ
Undergraduate Research Assistant — March 2009- Dec. 2010

3. ABSTRACTS

i. Fly AD, Foland EB, Kenworthy S, **Erickson T**, Kaschalk E, Lyu C, Houchin O, Graves M, Stuckey A, Isaacs O, Simko J, Ansu V and Dickinson S. 2019. Power-Up with Produce Supported Some Changes

in Determinants of Fruit and Vegetable Intake of Rural Elementary Students. Society for Nutrition Education and Behavior. ACCEPTED

ii. Olivia Isaacs, Jamie Simko, Elizabeth Foland, Olivia Houchin, Stephanie Dickinson, **Taylor Erickson**, Priyanka Ramamurthy, Elizabeth Kaschalk, Velarie Ansu, Alyce Fly, Baseline Comparison of Fruit and Vegetable Purchases at Indiana Elementary Schools for Power up with Produce Team Nutrition Intervention Study (P04-140-19), Current Developments in Nutrition, Volume 3, Issue Supplement_1, June 2019, nzz051.P04–140–19, https://doi.org/10.1093/cdn/nzz051.P04-140-19

iii. **Erickson TL,** Dickinson S, Golzarri-Arroyo L, Fly AD. 2020. Effect of Handwashing on Skin Carotenoid Measurements Using Pressure-Mediated Reflectance Spectroscopy. American Society for Nutrition Annual Conference. May 30- June 2, 2020. ACCEPTED.

4. LETTER TO THE EDITOR

i. **Erickson T.** Comment on "Plasma, Urine, and Adipose Tissue Biomarkers of Dietary Intake Differ between Vegetarian and Non-Vegetarian Diet Groups in the Adventist Health Study-2". J Nutr. 2019, doi:10.1093/jn/nxz129.

5. SCHOOL RESEARCH COMPETITION PRESENTATION

i. **Erickson T**, (Fly AD, Foland EB, Kenworthy S, Kaschalk E, Lyu C, Houchin O, Graves M, Stuckey A, Isaacs O, Simko J, Ansu V and Dickinson S). 2019. Power-Up with Produce Supported Some Changes in Determinants of Fruit and Vegetable Intake of Rural Elementary Students. Indiana University School of Public Health Research Day 2019.

SKILLS, TECHNIQUES, CERTIFICATIONS

Phlebotomy Technician, MET Services PLLC, certified 11/2019, certificate #: 11-10-19-123
Clinical Trials Training, PPD, completed 08/2019
Nutrition Data System for Research (NDSR) Software, University of Minnesota, certified 08/2019
SPSS Software; R Software
LabScan XE Spectrophotometer; VeggieMeter Spectrophotometer
Light microscopy and slide preparation
DNA gel electrophoresis
Zoological dissection techniques

PROFESSIONAL EXPERIENCE

1. TEACHING

Indiana University- Bloomington — Bloomington, IN
Teaching Assistant — F 2018-Sp 2020
Guest Lecturer, Lipids Lecture, N320 — Spring 2020
Guest Lecturer, Protein Lecture, N231 — Spring 2019
Guest Lecturer, Bread Lecture, N120 — Fall 2018

Edgewood Junior High School, RBBCSC — Ellettsville, IN
General Science Educator — June 2016-May 2018;

July 2020-present

Lesson design and implementation
Microscopy use and slide preparation tutorials
Curriculum design for general science, environmental science, and honors biology
SPED instructional support; 504 and IEP tracking and implementation
Positive classroom management

Chaparral High School, Scottsdale USD — Scottsdale, AZ
Biological and Environmental Science Educator — Jan. 2015-May 2016
Lesson design and implementation
Zoological and botanical dissection tutorials
Microscopy use and slide preparation tutorials
DNA electrophoresis tutorials
SPED instructional support; 504 and IEP tracking and implementation
Positive classroom management

2. OTHER

NAU, College of Engineering, Forestry, and Natural Science — Flagstaff, AZ
Administrative Assistant — Jan. 2013- June 2014

Darthia Farm — Gouldsboro, ME
Farm Apprentice — July 2011- Oct. 2011

NAU, Residence Life — Flagstaff, AZ
Learning Community Mentor — Aug. 2009- Dec. 2010

AWARDS AND SCHOLARSHIPS

Second Place, Master's Student Poster Division — 2019
Indiana University School of Public Health Research Day

Dr. Anita Aldrich Research Fellowship Award — 2019-2020
Indiana University School of Public Health

Ruth Mary Griswold Scholarship
Indiana University School of Public Health, Dept. Applied Health Science — 2019-2020

PROFESSIONAL ASSOCIATIONS

American Society for Nutrition — Since 2019
Society for Nutrition Education and Behavior — Since 2018
National Association of Biology Teachers — 2015-2016
National Educator Association — 2015-2016; 2020-present

RECENT VOLUNTEER EXPERIENCE

Girls Inc. Monroe County, Summer Camp Volunteer, SS 2019; Board Trustee, 2017-2020; Executive Board Secretary, 2018-2019
Edgewood Junior High School, Track and Field Assistant Coach, Spring 2019, Spring 2018
IU Health Bloomington, G.O.A.L. volunteer, 08/2018-11/2018
Bloomington Animal Shelter, Kennel Assistant, 02/17-08/18