Protocol Title	Examining Validity and Sensitivity of Pressure-Mediated Reflection Spectroscopy as a Measure of Fruit and Vegetable Consumption in a Diverse Community Sample (Aim 2 – Randomized Controlled Trial Juice Intervention)
Clinical Trials ID Number	NCT04056624
Working Name(s)	The Veggie Meter® Study Aim 2
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Protocol Date & Version	05/21/21; Version 2

Protocol Revision History

Revision #	Version Date	Summary of Changes	Consent Change?
1	05/21/21	Protocol restructured to suit Aim 2; procedure details removed (will be put into an MOP for later submission); two additional intervention sites added; revised number of participants to enroll due to revised power calculation; revised trial duration; revised carotenoid mass provided per treatment group; revised juice brand	Yes

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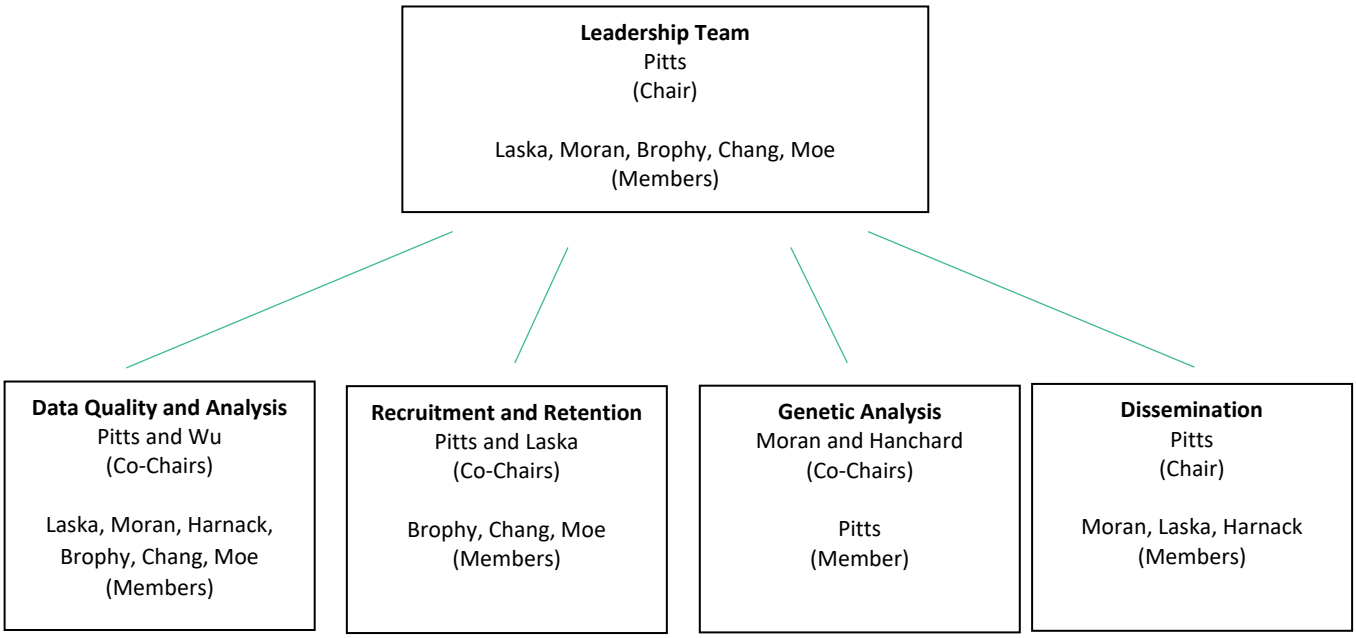
Study Team Structure

Our team includes experts in public health nutrition, nutrition epidemiology, health disparities, biostatistics, and dietary assessment methodology. We have arranged our study team into one leadership team and four working teams. Each team includes one or two leads (chair or co-chairs). Below is a graphic of the organization of the teams and a description of each team's charge and tasks.



The Leadership Team is charged with leading the study and overseeing the study teams. The Leadership Team will communicate and coordinate with the NIH/Funding Institute, Institutional Review Boards (IRB) at both East Carolina University and other universities and will be charged with interim and final reporting.

The Data Quality and Analysis Team is charged with ensuring data are collected thoroughly and managed safely and securely according to federal regulations as specified by the East Carolina University (ECU) IRB. This team is also charged with analyzing all project data for interim reports and peer-reviewed papers and presentations. The duties of the team include overseeing the training of data collectors with regard to data quality, coordinating data storage and management, performing quality control checks on a regular basis, and analyzing all data for reporting.

The Recruitment and Retention Team is charged with recruiting study participants, monitoring the study for timely recruitment, and ensuring that recruitment of study participants is in line with federal regulations and IRB specifications. The team will be tasked with solving problems related to recruitment of Aim 2 participants as well as retention of Aim 2 participants to ensure continued participation.

The Genetic Analysis Team is charged with completion of genetic analyses for Aim 2.

The Dissemination Team is charged with ensuring that data and study reports are disseminated to stakeholders in a timely manner and will oversee creation of interim progress reports, final reports, technical reports, and papers and presentations for both academic and community-based audiences.

These teams will meet on a monthly, bi-monthly or weekly basis, depending upon the needs of each stage of data collection and analysis. All co-investigators and consultants will participate in regularly scheduled project calls and will be updated on project activities via weekly emails and meeting minutes. The ECU Study Coordinator will send out meeting agendas and reminders for all meetings and will take minutes and distribute minutes post-meeting.

Team Member Qualifications, Role, and Responsibilities

East Carolina University

Stephanie Jilcott Pitts, PhD, is the Principal Investigator (PI) and will lead all aspects of the study. Dr. Pitts will interact with the Chairs of the working teams on a regular basis. She will oversee protocol development, hire and train staff, coordinate with the Institutional Review Board, Funding Agency, and all other administrative tasks. She will work closely with the Study Coordinator to ensure that study milestones are reached in a timely manner.

Qiang Wu, PhD, Professor in the ECU Department of Biostatistics, has worked with Dr. Pitts for over five years and has experience with public health nutrition data analysis. Dr. Wu will oversee data collection efforts, assist with ensuring data quality, and will assist with data analysis as needed. Dr. Wu will co-chair the Data Quality and Analysis Team.

ECU Study Coordinator, Patty Brophy, will lead study administrative activities such as Institutional Review Board review and approval, train and monitor data collectors, and will communicate regularly with the study team leadership on progress of data collection and analysis. Ms. Brophy will serve on the Data Quality and Analysis and Recruitment and Retention Teams.

University of Minnesota

Dr. Melissa Laska will serve as site PI. Together with site Study Coordinator, Stacey Moe, Dr. Laska will ensure that study participants are recruited in a timely and effective manner. She and Ms. Moe will communicate regularly with the larger team regarding recruitment efforts and success for the University of Minnesota (UMN) site. Dr. Laska will serve on the Data Quality and Analysis and Dissemination Teams as a member and will co-chair the Recruitment and Retention Team with Dr. Pitts. Dr. Lisa Harnack, an expert in dietary assessment methodology, will provide senior leadership to the project and will serve on the Data Quality and Analysis and Dissemination Teams.

Baylor College of Medicine

Dr. Nancy Moran will serve as site PI. Dr. Moran will ensure that study participants are recruited in a timely and effective manner. She and her team will communicate regularly with the larger team regarding recruitment efforts and success for the Baylor College of Medicine (BCM) site. Dr. Moran's research focuses on the complex nutritional and physiologic factors that impact pharmacokinetic and pharmacodynamic responses to dietary carotenoids. She and Dr. Neil Hanchard will lead genetic analyses for Aim 2. Dr. Moran will serve on the Data Quality and Analysis and Genetic Analysis Teams. Dr. Hanchard is a clinical geneticist with research training in quantitative human genetics and will serve on the Genetic Analysis Team.

Objective

The purpose of Aim 2 is to define the RS-assessed skin carotenoid response (RS device sensitivity) compared to plasma carotenoid response during a 6-week randomized controlled trial intervention of a carotenoid-containing juice among 160 participants across four racial/ethnic groups. We hypothesize that there will be RS-assessed skin carotenoid increases after 6 weeks of daily consumption of carotenoid-containing juice for all racial groups, but the magnitude of RS-assessed skin carotenoid responses may differ between groups.

Experimental Questions

1. What is the skin carotenoid response to controlled carotenoid intake in a diverse cohort?
2. What are significant covariates related to the skin carotenoid response?

Background

Significance

A diet rich in fruits and vegetables (F&V) is associated with lower risk of nutrition-related chronic diseases and all-cause mortality.[1-5] Despite these benefits, the US population under-consumes F&V,[6] with particularly low intake in rural and disadvantaged populations.[7-9] Low F&V intake results in higher rates of nutrition-related chronic disease among disadvantaged populations when compared with more advantaged populations.[10,11] Detecting and addressing inadequate F&V intake in these populations is needed to reduce such disparities. Yet, there is still no commonly used predictive, objective measure of F&V intake for surveillance or determination of policy or intervention effectiveness. The current objective gold standard marker of F&V intake is blood carotenoid concentration[12] — an expensive, time-intensive, and invasive measurement. Traditional methods of self-reported dietary assessment are imprecise and have diminished utility in rural and disadvantaged populations due to low literacy, numeracy, and internet connectivity.[13] In the past decade, skin carotenoid status assessed by non-invasive resonance Raman spectroscopy (RRS) has emerged as a promising biomarker of F&V intake.[14-16] Reflection spectroscopy (RS) is an improvement over RRS, offering stronger signals, faster data acquisition, and greater portability in a commercially available device (Veggie Meter®, Longevity Link Corporation).[17,18] However, a hurdle impedes deployment of RS for widespread use: to date, nearly all of the non-invasive skin carotenoid validation has been conducted in non-Hispanic Whites, primarily by RRS. Therefore, it is critical to evaluate RS in racially and ethnically diverse populations.

Biomarker validation across racial/ethnic groups could be confounded by self-reported ancestry, particularly in admixed populations. Race/ethnicity is a strong predictor of circulating carotenoids[19] with recent reports showing plasma carotenoid phenotypes to be partially heritable[20] and genetic variation explaining a significant portion of inter-individual heterogeneity in plasma carotenoids.[21] Thus, to validate RS for diverse populations, genetically confirmed ancestry is important to provide meaningful insights into the transferability of specific diet-biomarker relationships across racial/ethnic population groups. Furthermore, defining the specific genetic determinants of skin carotenoid responses to dietary intake will provide a fundamental mechanistic understanding which can be applied across populations.

Improving F&V intake could be the one dietary change likely to result in the most notable improvements in population health and reduction in chronic disease. Consumption of F&V is associated with lower rates of a wide variety of chronic diseases including cancer,[22] cardiovascular disease,[23,24] diabetes,[25] obesity,[1,2,26] and all-cause mortality.[3,27] The first two recommendations from the Dietary Guidelines for Americans 2015-2020 specify F&V consumption as two main components of a healthy dietary pattern, indicating the importance of a diet rich in F&V for promotion of health and prevention of disease.[28] Accordingly, F&V are a major component

measured by the Healthy Eating Index, a tool used to assess Dietary Guideline adherence, with four of the nine "adequacy" components relating to F&V.[29,30]

This study focuses on racially diverse populations, including those that consume fewer F&V and carry a disproportionate burden of diet-related diseases. In general, the US population does not consume recommended amounts of F&V, with rural and disadvantaged populations[7-9] in particular under-consuming F&V and experiencing a disproportionate burden of diet-related chronic diseases.[10,11] Thus, examining and addressing the low consumption of F&V, particularly in disadvantaged populations, represents an important opportunity to prevent and reduce chronic disease disparities.

Our lack of non-invasive, reliable, objective measures of dietary F&V intake to assess the success of dietary interventions in diverse populations is one of the most pressing problems our field currently faces. National public health institutions and agencies recommend a wide range of community-based programmatic strategies and policy actions to increase access to and consumption of F&V to reduce obesity and chronic disease.[31,32] However, we lack necessary objective, non-invasive, valid markers of F&V consumption needed to monitor the success of these strategies in diverse community samples. Self-reported diet has many weaknesses that include social desirability bias and systematic bias by weight, resulting in attenuated diet-disease relationships,[33] and are also difficult (and often financially prohibitive) to administer among low-literacy populations and in areas with limited internet access. Furthermore, the correlation coefficients between energy intake assessed by doubly labeled water vs. food frequency questionnaires or 24-hour recall were 21% and 26%, respectively, indicating a clear need for objective measures of food intake.[34]

Validating the predictive ability of skin carotenoid measures for F&V consumption of diverse populations could be transformational for public health nutrition studies. Objective measurement of F&V consumption, as opposed to self-reports, will allow for more accurate assessment of diet-disease relationships and more accurate evaluation of community-based programs and policies to improve public health and reduce health disparities. The developers of the RS Device have recently published a feasibility paper collaborating with researchers in Utah, Japan, California and Texas; however, this paper included assessment of associations between plasma and RS-measured skin carotenoids in a small (n = 54) Utah based sample.[18] With the commercial availability of RS for skin carotenoid measurement, this study will provide the most comprehensive and careful analysis of its use for different racial/ethnic groups.

Blood carotenoid concentration measurement is currently the objective gold standard biomarker of F&V intake but is difficult to utilize for community monitoring. Carotenoids are tetraterpene hydrocarbons found abundantly in orange, red, yellow, and green F&Vs, and blood carotenoid concentrations increase in response to F&V interventions,[35] making them a robust marker for F&V consumption. Because of the aforementioned problems with self-reported diet, the Institute of Medicine concluded, "Blood concentrations of carotenoids are the best biological markers for consumption of fruits and vegetables."[12] However, blood collection for population based surveillance and community intervention trials is cost-prohibitive, time consuming, labor-intensive, and invasive, requiring a trained phlebotomist, places to store blood, and other logistical challenges for field-based nutrition studies.

Carotenoids, themselves, are associated with disease prevention and are worthy of monitoring. In addition to being biomarkers of F&V intake, individual carotenoids have been epidemiologically associated with an inverse risk of a number of chronic conditions. The major carotenoids consumed in the US are α-carotene, β-carotene, lycopene, lutein, zeaxanthin, and β-cryptoxanthin.[12] As an example of the inverse associations of carotenoids with diseases, lutein is associated with better cardiometabolic health,[36] and lycopene is associated with reduced risk of aggressive prostate cancer.[37] Blood carotenoids have been associated with decreased incidence

of breast,[4,38,39] lung,[40] and colorectal cancers,[41] lower cardiovascular disease markers,[42] and decreased mortality.[3,43]

Assessment of skin carotenoid status has emerged as a promising new biomarker of F&V intake.[14,15] A wide distribution of skin carotenoid levels found in the population can be measured with high reproducibility over 6 months (in absence of dietary intervention)[14,44] and with concurrent and convergent validity with plasma carotenoids, skin biopsy carotenoids, and reported F&V intake.[14] These are all necessary characteristics for a biomarker of nutritional status and food intake. Dr. Lisa Jahns recently validated resonance Raman spectroscopy (RRS)-measured skin carotenoids in a feeding study, showing that skin carotenoids rapidly increase in response to a sustained, high F&V diet (providing ~60 mg of carotenoids/d for 8 weeks) and more slowly decrease during depletion phases than plasma carotenoids.[45] Despite the promise of RRS,[14] these devices are not commercially available nor are they portable for field-based public health nutrition research studies.

Pressure-mediated reflection spectroscopy offers an economical, commercially available, sensitive measure of skin carotenoids. Reflection spectroscopy (RS) (Figure 1) is an improved method over RRS for measuring skin carotenoid status.[46] To further explain the difference between RRS (previously published) and RS technology, the most important differences are that Reflection spectroscopy (RS) offers stronger signals, faster data acquisition, greater accuracy, control for melanin in the skin, and most importantly, portability (for field-based nutrition studies) in a commercially available device (Veggie Meter®, Longevity Link Corp.).[17,18,46] RRS devices are not commercially available, and those that are available to researchers via a leasing agreement are not research grade. There are two custom-built RRS devices to our knowledge: One at Yale University and one at the USDA Grand Forks Human Nutrition Research Center. These devices are not portable and are not mass produced. There are other peer-reviewed papers examining “skin yellowness” using an instrument produced by Konica Minolta.[47,48] The instrument works very differently from the RS device we propose to study, and measures "skin yellowness" through the "b" chromaticity coordinate. The Konica Minolta device is not designed for the measurement of carotenoid levels and does not correct for residual blood and melanin levels, so the error for carotenoid measurements is very high. Thus, there is a critical need to test the RS device to assess skin carotenoids in racially and ethnically diverse populations.

Figure 1. Commercially available RS skin carotenoid device (Longevity Link Corp.)

Skin and plasma carotenoid responses to a given carotenoid intake may plausibly differ between racial/ethnic groups. Validation of carotenoids as a biomarker across racial/ethnic groups could be complicated by underlying genetic variation.[21,49-51] Furthermore, differential correlations between self-reported dietary F&V and carotenoid intake and plasma carotenoids may indicate genetic variation in absorption and transport.[52] Ferrucci, et al.[53] found that a single nucleotide polymorphism (SNP) near the β-carotene 15,15’-monooxygenase 1 (*BCMO1*) gene was associated with higher β-carotene and α-carotene and lower lycopene, zeaxanthin, and lutein levels. Findings from NHANES 2003-2006 also suggest that race/ethnicity is a predictor of circulating carotenoids, such that even after controlling for 10 other significant sociodemographic and lifestyle factors, non-Hispanic blacks were found to have 33% greater plasma xanthophylls (oxygenated carotenoids) and 9% greater carotenes (hydrocarbon carotenoids) than non-Hispanic Whites, and Mexican Americans had 57% greater xanthophylls than non-Hispanic Whites.[19] While the study did not control for dietary intake, these findings highlight the need to understand underlying genetic and/or carotenoid intake differences between racial and ethnic groups. Interestingly, genetic studies have revealed the limitations of using self-reported race/ethnicity for understanding biological relationships.[54] In the US, African Americans and

Hispanic Americans often have variably admixed European, African, and Native American ancestry.[55] Furthermore, racial/ethnic populations in different regions of the US differ in their biogeographical ancestry, indicating that the biological relationships discovered for a racial/ethnic group in one region of the US may not replicate in other populations with different ancestry. Thus, to draw the most accurate, translatable conclusions, genetic ancestry informative markers (AIMs) will be determined in this study.

Variation in genes related to lipid and carotenoid metabolism may be critical determinants of skin carotenoid responses to diet. Genetics are emerging as an important predictor of carotenoid responses to diet with recent reports showing that plasma carotenoid concentrations are partially heritable (h^2=0.90 for β-carotene and 0.81 for α-carotene).[20] Furthermore, it has been shown that with ancestral differences come different patterns of genetic variants in metabolism-related genes with a recent example showing that the minor allele of an SNP in *SCARB1*, a gene involved in carotenoid absorption, is more common in African Americans and Hispanics than in Whites, and that this variant is associated with significantly lower (-0.1-0.2 µM) plasma lycopene in these groups.[56] Furthermore, a series of recent studies has shown that genetic variation can explain inter-individual variability in carotenoid absorption from a controlled meal. Specifically, while absorption of lutein, β-carotene, and lycopene showed coefficients of variance (CV) between 70-137%, nearly 70% of response variance could be explained by 25-29 SNP genotypes in 12-16 genes related to lipid and carotenoid metabolism.[57-59] The current study will elucidate the underlying genetic variations responsible for between-ancestral group differences in plasma and skin carotenoids and responses to the controlled dietary intervention. Defining the genetic determinants of skin carotenoid responses to diet will create a fundamental mechanistic understanding which can be applied to predict and interpret skin carotenoid responses of novel populations.

Preliminary Studies

Dr. Lisa Jahns reported the response of skin carotenoid levels (n = 29, each line is a participant) to change in intake of carotenoid-rich foods using the RRS device. Figures 2a and 2b below demonstrate that skin carotenoid status can be used to determine the effectiveness of nutrition interventions focusing on increasing F&V intake. These figures from Jahns et al.[45] establish that skin carotenoid assessment tracks closely with F&V intake.

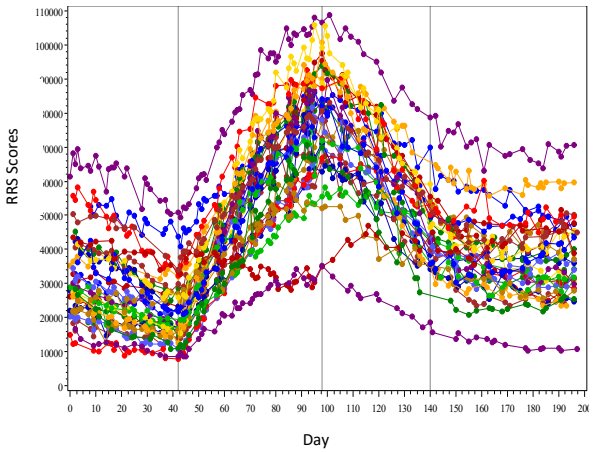


Figure 2a. Day is on the x-axis and RRS scores are on the y-axis. These curves follow F&V depletion/repletion over study phases very closely.

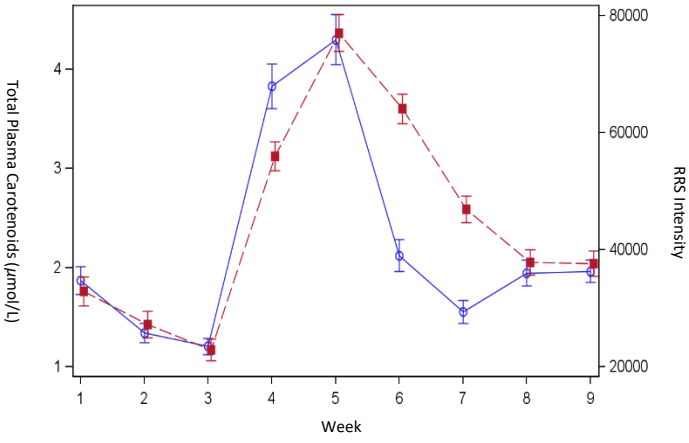


Figure 2b. Demonstrates the change in plasma (blue) and skin (red) carotenoids following the depletion and repletion of F&V intake in the 29 study subjects.

Dr. Stephanie Pitts' feasibility study revealed that the RS device reliably and rapidly assessed skin carotenoid status from a diverse population in eastern North Carolina. Before proposing the current large validation study, Drs. Pitts, Laska, Moran, Jahns, Wu, and Bell determined that the RS device could be feasibly deployed for skin carotenoid measures in combination with a conventional dietary recall tool for a targeted, underserved population: customers (N = 466) of 16 corner stores in eastern North Carolina (NC). Customers completed the National Cancer Institute (NCI) F&V Screener[60] and had skin carotenoids assessed using the RS device. The majority of participants were African American (64.6%), and 36.3% reported less than $25,000 annual household income. Mean time to complete three RS readings/person was 1.5 minutes with mean between-reading variation of 6.8% and no differences in variation between African Americans and Whites.[61]

A pilot study of North Carolinians revealed race/ethnicity differences in relationships between skin carotenoids and total F&V intake. In order to further determine if skin carotenoids were associated with dietary F&V and/or carotenoid intake in diverse populations, our team, led by Dr. Pitts, conducted a pilot study among 17 African Americans and 13 Whites in eastern NC.[61] Subjects' skin carotenoids were measured by RS, and they completed the Fred Hutchinson Cancer Research Center Food Frequency Questionnaire (FHCRC FFQ).[62] Over a one month period, 71 participants were screened (44% White, 31% African American, 23% not queried on race because of study ineligibility). Of the total screened, 34 (47%) were eligible, 35 (49%) were ineligible, 2 could not be re-contacted, and 5 eligible participants were not enrolled because target sample size of 30 was met. Surprisingly, while skin carotenoids were highly correlated with reported F&V intake in Whites (Table 1), there was no significant association between skin carotenoid and reported total F&V intake in African Americans.[61] The mean RS score for the corner store population was 234.2 (SD = 86.2) and mean F&V intake was 3.8 (SD = 3.2).[61] The mean carotenoid intake from foods according to the FHCRC FFQ from our pilot study was 16,955 µg and SD = 12,281. However, when total dietary carotenoid intake was estimated from FFQ data (i.e., from all foods), the relationship was significant in African Americans and similar to that of Whites. This may suggest that African Americans in our population consumed more low-carotenoid F&V, attenuating the relationship between total F&V intake and skin carotenoids. In addition, there were high correlations between skin and plasma carotenoids (r = 0.71).[61] This study did not include Hispanics or Asians nor was it powered to quantify differences in skin carotenoid responses to dietary F&V intake (regression coefficients) between racial/ethnic groups. This preliminary data emphasizes the need for studies to validate RS skin carotenoid predictions of F&V intake in diverse groups.

Group	F&V intake	Carotenoids from food	Plasma carotenoids
All	0.48 *0.009*	0.69 *<0.0001*	0.71 *<0.000*
African American	0.12 *0.635*	0.70 *0.002*	0.54 *0.024*
White	0.84 *0.001*	0.75 *0.003*	0.87 *<0.000*

Table 1. Associations (Pearson's correlation coefficient and p-values) between RS-assessed (Veggie Meter®) skin carotenoids and self-reported F&V consumption, carotenoids from foods, and plasma carotenoids.

Using carotenoid pharmacokinetics, we have estimated plasma and tissue half-lives and have revealed key determinants of physiologic responses to carotenoids. Developing skin carotenoid levels as a biomarker of F&V intake requires an understanding of carotenoid pharmacokinetics. Dr. Nancy Moran, a co-investigator, has conducted two NIH-funded studies of the pharmacokinetics of lycopene and phytoene in healthy adult men and women.[63,64] These studies utilized stable isotope labeled carotenoids to trace metabolism, allowing calculation of bioavailability and plasma and tissue carotene half-lives which can, in turn, be used for the rational design of dietary interventions. For instance, the lycopene half-life in slow turnover tissues (such as skin) was estimated to be 12.4 days[64] and 7.9 days for β-carotene.[65] Based on these data and the Plateau Principle, we can estimate steady-state conditions will be achieved in 6 weeks for skin lycopene and 4 weeks for skin β-carotene in response to a dietary intervention.[66] Dr. Moran was involved in the clinical analysis of

plasma and prostate lycopene responses to three different food interventions, finding that tomato soup, juice, and sauce were equally effective in increasing plasma lycopene concentrations in a 3 week intervention and that end-of-study plasma lycopene was predictive of prostate tissue lycopene concentrations.[67] Finally, Dr. Moran's recent analyses of the variables predictive of the change in plasma lycopene and β-carotene in response to a 3 week intervention of 0, 1, or 2 cans of tomato juice (delivering 21 mg lycopene, 1 mg β-carotene/can) showed that while dietary intervention group was the major explanatory variable, baseline plasma lycopene, body mass, and nutrigenetic interactions explained an additional 22% of variance in plasma lycopene response (Figure 3). Genetics, non-intervention β-carotene intake, and diet explained 62% of the variance in plasma lycopene responses to the intervention.[68] These studies were conducted in a primarily non-Hispanic White population highlighting the need for additional research in racially and ethnically diverse populations.

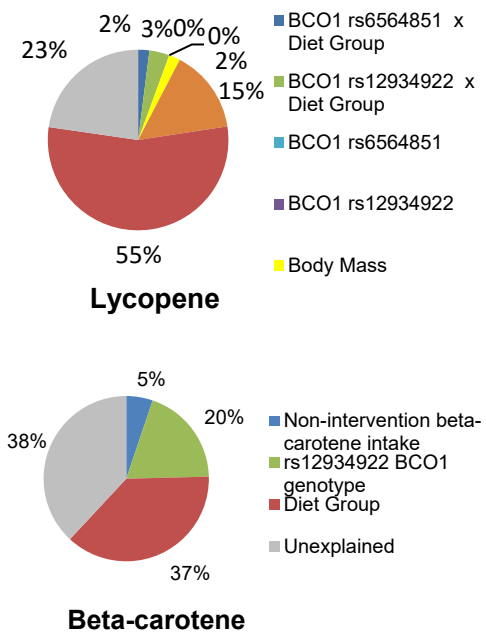


Figure 3. Proportion of variance (R^2) in the change in cholesterol-adjusted plasma lycopene (A) and β-carotene (B) significantly ($P<0.05$) explained by diet, intrinsic variables, and BCO1 SNP genotypes. Determined by multiple linear regression analysis of subjects (N=47) consuming 0, 1, or 2 cans of tomato juice/d for ~3 weeks. (*unpublished data*)

We have found SNPs in the carotenoid metabolizing enzyme, β-carotene oxygenase 1 (BCO1), are associated with plasma and tissue carotenoid responses over a 3-week feeding period. As shown in Figure 3, 5% of the variance in plasma lycopene and 20% of the variance in β-carotene responses were explained by BCO1 SNP genotypes. The BCO1 rs12934922 SNP highlighted here has also emerged in another publication[69] to effect plasma β-carotene responses to a dietary intervention because it impacts the efficiency with which BCO1 enzymatically cleaves β-carotene. While these preliminary data are intriguing, the current study is designed to take a higher dimensional approach to the study of the genetic predictors of physiologic responses to carotenoids by analyzing variants in the nearly 40 genes previously found to be associated with blood and tissue carotenoid responses.[21] This higher dimensional approach will be achieved in collaboration with translational and clinical geneticist, Dr. Neil Hanchard, a study co-investigator.

Meinke et al.[70] studied 30 adults (15 in the intervention group and 15 in the placebo control group) and used a dose of carotenoids (1.5 mg) most similar to the dose we are proposing. Based upon the data presented in Meinke et al. Figure 4, the standard deviation is 0.23 for 6-week relative skin carotenoid change. Additionally, a shortened duration (6 week vs. 8 weeks) increases the likelihood of higher intervention adherence. Intervention duration and power calculations for Aim 2 are based off of Meinke et al.

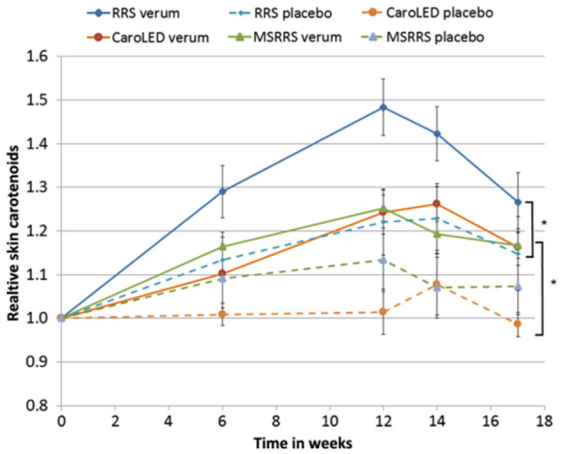


Figure 4: Mean and SEM of skin carotenoids relative to initial values of the placebo and verum groups measured by the two SRRS and the RRS systems.

Study Design & Methods

Rationale/Strategy

To determine the sensitivity of the RS device for measuring change in fruit and vegetable (F&V) consumption in response to community-based nutrition interventions, we need to determine the longitudinal response to a defined F&V intervention. We want to learn if change in RS-measured skin carotenoids can be used to sensitively define F&V consumption changes across a range of consumption values. We would also like to determine if the RS device sensitivity differs by racial/ethnic group. Aim 2 will gauge what an increase in F&V intake would look like in terms of RS-assessed skin carotenoids in an ethnically and racially diverse population.

Study Setting

Participants will be recruited, enrolled, and measured from within the three intervention sites: 1) Greenville, North Carolina - East Carolina University (ECU), 2) Minneapolis, Minnesota - University of Minnesota (UMN), and 3) Houston, Texas - Baylor College of Medicine (BCM). At ECU, participant visits will take place at the East Carolina Diabetes and Obesity Institute facilities and the Department of Public Health Research (ECDOI) offices, located in the East Carolina Heart Institute. At UMN, participant visits will take place at the Epidemiology Clinical Research Center (ECRC) which is a part of the Division of Epidemiology and Community Health in the School of Public Health. At BCM, participant visits will take place at the Children's Nutrition Research Center Metabolic Research Unit (CNRC MRU).

Study Population, Eligibility, and Screening

Participants (n=160) will be recruited from the three intervention sites: ECU (n=60 participants), UMN (n=40 participants), and BCM (n=60 participants). As with Aim 1, participants will be recruited across four diverse groups: non-Hispanic African American/Black, Asian, non-Hispanic White, or Hispanic/Latinx. Though our power calculation found that a sample size of n=156 was needed, we chose to round up in order to easily and evenly divide participants by our three intervention sites. It is our intent to enroll approximately equal numbers of participants in each racial/ethnic group.

Potential participants will be screened for eligibility initially by phone. Eligibility criteria for Aim 2 are identical to those for Aim 1, but participants must also be willing and able to consume the juice to which they are randomized and maintain a fingernail length of ≤ 2 cm on the right index finger throughout the duration of the study. The full list of eligibility criteria are as follows:

- Self-identify as one of the racial/ethnic groups of focus (non-Hispanic African American/Black, Asian, non-Hispanic White, or Hispanic/Latinx)
- Able to speak, read, and understand English
- Have a BMI 18.5 – 34.9 kg/m^2
- Between 18 and 65 years of age (inclusive)
- Healthy (no chronic disease) as determined by screener
- Not taking lipid-altering medication
- Not taking a carotenoid-containing supplement that contains > 2mg carotenoids
- Non-pregnant, at least 6 weeks post-partum, non-lactating, not planning to become pregnant in the next 2 months
- Weight stable, not currently dieting, not planning to follow a special diet
- Not allergic to any fruit or vegetable
- Right index fingernail must be ≤ 2cm at time of each study visit
- Willing and able to drink either juice (V8 Orange Carrot or Mott's Apple Juice) daily for 6 weeks

Final eligibility will be determined/verified during each participant's baseline study visit if/when BMI is 18.5 – 34.9 kg/m^2, fingernail length is ≤ 2 cm, and the participant is willing/able to consume either juice. A juice taste-test will be conducted with each participant to determine their willingness and/or ability to consume both types of juice.

If participants are taking any supplements (not including a supplement containing carotenoids > 2mg), they will not be asked to stop taking these supplements, rather they will be asked to document their supplement use for the past month on the DHQ-III which will be administered during the baseline study visit and again before the week 3 and week 6 study visits (at home). If participants are not taking supplements, they will be asked to abstain from taking supplements for the duration of the study. Participants will also be asked to maintain their usual diet and level of exercise for the duration of the study.

Participant Ineligibility Post-Screening

If a participant is found ineligible at the baseline study visit, they will receive a $15 gift card for their time. If a participant is found ineligible any time after the baseline visit (i.e., some data have been collected), they will receive a $30 gift card for their time.

If a participant becomes ill just prior to their baseline study visit, the scheduled visit will be cancelled, and the participant (if interested) will be added to a wait list to be potentially rescheduled in approximately two months. If a participant becomes ill before the week 3 or week 6 study visit, they will be deemed ineligible at that time and provided with a $30 gift card (as stated above). The participant (if interested) will be added to a wait list to potentially begin the study over again approximately two months later.

Recruitment and Enrollment

Recruitment methods will include contacting Aim 1 participants from ECU and UMN who expressed interest in participating in Aim 2; distributing or posting flyers on listservs, at community locations, and via word of mouth as described for Aim 1; and new, additional methods including distributing flyers and advertisements via university newsletters, social media, and email.

To ensure enrollment targets are achieved and to tailor recruitment efforts to ensure planned recruitment is successful, weekly reports will be run on the racial/ethnic breakdown and male/female breakdown of recruited and enrolled participants. Reports will be then be reviewed bi-weekly. Mean BMI by race/ethnicity will also be reviewed to ensure there is a balance in weight status among all groups.

Participant Treatment Group Randomization

Participants will be randomly assigned to one of three treatment groups – a control group (negligible-dose carotenoids), a moderate-dose carotenoids group, or a high-dose carotenoid group – by intervention site and by race/ethnic group. Dr. Qiang Wu, study biostatistician, will conduct randomization of participants in the following manner:

1. At ECU, Dr. Wu will generate random numbers for each site by race/ethnic group. Each number will represent one of the three treatment groups. Dr. Wu will place temporary stickers over the random numbers so that they are not visible until randomized.
2. Each randomly generated number (and, corresponding treatment group) will be printed on cards and placed in a pile for that specific race/ethnic group. Each pile represents a randomization sequence for that group.
3. Each pile will then be sealed in an envelope and sent to the appropriate intervention site.
4. Each site will receive four sealed envelopes (of randomized cards) – one for each race/ethnic group.

5. Once received at each site, study staff/data collectors should **not** open the envelopes **until** the first participant's visit.
6. At each participant's baseline study visit, data collectors will select the appropriate race/ethnic group envelope and will choose the card at the top of the pile.
7. The treatment group printed on the card will determine the participant's treatment group.
8. The participant's study ID should be written on the card once the treatment group is determined and kept within the participant's study folder.
9. It is important to keep track of each participant's assigned treatment group so that each participant receives the appropriate juice and instructions.
10. Note: Randomization for each participant will occur in the same way. The randomly assigned cards within each envelope should not be shuffled at any time after opening the envelope, and data collectors should always choose from the same place (e.g., top of the pile).

Data Collection and Study Visits

Over the course of the six-week trial, participants will complete three study visits at baseline, week 3, and week 6. During the baseline visit, participants will complete the informed consent process before beginning any study-related activities.

All data collection measures and methods outlined in Aim 1 will be repeated at all three Aim 2 study visits. These measures include height (baseline-only), weight and body fat percentage, 10-hour fasting blood draw, RS-assessed skin carotenoid measurements (via the Veggie Meter® device), skin melanin measurements (via the Konica Minolta CM-700d Spectrophotometer), and a food frequency questionnaire (the National Cancer Institute's web-based Diet History Questionnaire III (DHQ-III)). Demographic questions (such as birthdate, age, sex, and race) will be asked at baseline only. Demographic questions with the potential for responses that could change over the course of the study (such as education, marital status, and income) will be asked at all three time points (baseline, week 3, and week 6). Sun exposure and tobacco use questions will be asked at each visit as well to track any changes to these behaviors.

Blood collected at the study visit will be sent to three sites for analysis and storage:

1. Eurofins Craft Technologies in North Carolina for carotenoid, glucose, and cholesterol analysis.
2. Baylor College of Medicine for genetic analyses to determine participant genetic ancestry and how variations in DNA can impact skin and blood carotenoid concentrations. Genetic results will not be provided to participants as they are research grade only. Whole genome sequencing will not occur.
3. East Carolina University for storage/banking of specimens for future research.

Juice Intervention

Juice was selected as a proxy for F&V consumption because it is practical to administer to a large group, can be purchased in a single lot for characterization, and is well tolerated by participants (Lisa Jahns, personal communication). The juice will be purchased from Campbell's in large lots (rather than small batches) to minimize potential variability in carotenoid content across all juices.

Providing a controlled juice product in lieu of a controlled diet affords greater control and ability to assess compliance (by providing a juice log to participants). Based on a detailed analysis of various potential fruit and vegetable juices conducted by Dr. Nancy Moran, it was determined that the V8 Orange Carrot juice provides sufficient carotenoid mass to change skin carotenoid scores over a 6-week study duration. Thus, it was decided that participants in the two carotenoid-dose treatment groups will consume V8 Orange Carrot juice. Each 6 fl. oz of V8 Orange Carrot juice contains 6.1 mg total carotenoids (2.11 mg α-carotene, 3.97 mg β-carotene, 0.01

mg β-cryptoxanthin, 0.00 mg lycopene, and 0.01 mg lutein). Participants in the control group will consume Mott's Apple Juice (no carotenoids detected per Dr. Moran's analysis).

Participants randomized to the moderate-dose treatment group will be asked to consume 6 fl. oz of V8 Orange Carrot juice each day. Participants randomized to the high-dose group will be asked to consume 12 fl. oz of V8 Orange Carrot juice (providing 12 mg total carotenoids/12 fl. oz) each day. A review article of studies to increase F&V intake found that in healthy adult populations, increases in fruit and vegetable intake ranged from about +0.1 to +1.4 serving/day,[71] demonstrating the wide range of effects of such interventions and indicating that the Orange Carrot juice provides a dose of vegetables that would fall within this range.

After completing baseline measures and randomization, participants will receive the first three weeks' worth of Mott's Apple Juice (control) or V8 Orange Carrot Juice (moderate- or high-dose carotenoid-containing juice). Participants in the control group will receive Mott's Apple Juice in 64 fl. oz bottles while those in the moderate- or high-dose groups will receive V8 Orange Carrot Juice in 46 fl. oz bottles. Participants will also receive a pack of disposable, graduated 16 fl. oz cups to measure out the allotted daily amount. All participants will be instructed to drink their allotted amount of juice every day for 6 weeks with fat- or oil-containing meals (as carotenoids are absorbed more efficiently with lipids). The control group will be instructed to drink 6 fl. oz of apple juice/day (containing no carotenoids); the moderate-dose carotenoids group will be instructed to drink 6 fl. oz of orange carrot juice/day (containing 6 mg carotenoids/6 fl. oz); and the high-dose carotenoid group will be instructed to drink 12 fl. oz of orange carrot juice/day (containing 12 mg carotenoids/12 fl. oz). Participants will receive the same amount of juice again at the week 3 visit. In the event that a participant's body weight has increased by greater than 5% at the 3 week visit (from baseline), dietary education materials will be provided.

Juice Intervention and Study Measurements Timeline

Intervention Group	Baseline	Week 3 and Week 6
Control Mott's Apple Juice (6 fl. oz per day)	• Obtain Consent • Collect variable measures: height, weight, skin carotenoids, skin melanin, and DHQ-III; demographics, sun exposure, and tobacco use • 10 hour fasting blood draw • Schedule week 3 and week 6 visits • Provide participant with juice, instructions, pack of graduated cups, juice log/calendar (for weeks 1 – 3) • Study incentive ($50)	• Collect variable measures: weight, skin carotenoids, skin melanin, and DHQ-III (to be completed the day before visit); demographics, sun exposure, and tobacco use • 10 hour fasting blood draw • Collect completed juice log/calendar (for weeks 1 – 3) • Provide participant with juice and juice log/calendar (for weeks 4 – 6), additional cups if needed • Study incentive ($100 at week 3 and $150 at week 6)
Moderate-Dose V8 Orange Carrot Juice (6 fl. oz per day)		
High-Dose V8 Orange Carrot Juice (12 fl. oz per day)		

Participant Incentive

All participants will receive $300 across the 6-week study: $50 at the baseline visit, $100 at the week 3 visit, and $150 upon completion of the final measures at week 6 visit. Participants at ECU and BCM will receive incentives through university issued Greenphire ClinCards. Participants at UMN will receive incentives as Target gift cards.

Intervention Adherence

One of the main challenges of the Aim 2 juice trial intervention is ensuring intervention adherence. To reduce risk of non-adherence, participants will be provided with an instruction sheet (see MOP Appendices) as well as a hard copy juice log (see MOP Appendices) on which we will ask them to mark the amount(s) of juice consumed each day. If all juice is not consumed, we will inquire why during the following study visit. Possible reasons include not liking the taste, spills, allergic reaction, someone else drinking the juice, and other. We will use this to measure dose and adjust for dose in our statistical analyses. Participants will also be reminded to maintain current diet and exercise habits. We will send all participants emails and allow them to opt in to receive text messages and/or phone calls (ensuring participant confidentiality) as periodic weekly reminders to drink the juice. The V8 Orange Carrot juice provides 60 kcal/6 fl. oz (or 120 kcal/12 fl. oz), and the control Mott's Apple Juice provides 90 kcal/6 fl. oz. We will provide an educational handout on the additional energy provided by the juices and dietary strategies to adjust caloric intake to remain isoenergetic.

Statistical Considerations

Power Calculation

Power calculations for Aim 2 are based off of Meinke et al.[70] which studied 30 adults (15 in the intervention group and 15 in the placebo control group) and used a dose of carotenoids (1.5 mg) most similar to the dose we are proposing. Based upon the data presented in Meinke et al. Figure 4, the standard deviation is 0.23 for 6-week relative skin carotenoid change. A sample size of 41 per group will have 90% power to detect 6-week relative skin carotenoids change of 1.13 vs. 1.28 when a one-sided two-sample t-test was used with a significance level of 0.05. With 20% attrition, we will need to enroll 52 per group, in each of three groups, for a total sample of 156.

Statistical Analysis

We hypothesize there will be RS-assessed skin carotenoid increases after 6 weeks of daily consumption of carotenoid-containing juice. To examine this hypothesis, we will compute changes in RS-assessed skin carotenoids from baseline to 3 weeks, from baseline to 6 weeks, and from 3 weeks to 6 weeks. Each level of change in RS-assessed skin carotenoids (as a response) will be analyzed using a general linear model to test the effects of carotenoid dose, melanin levels, and genetically determined ancestry, and dose x melanin and dose x genetically determined ancestry interactions. We will test baseline skin carotenoid level, baseline total plasma carotenoid level, baseline carotenoid intake and non-intervention carotenoid intake during the study, sex, age, skin hemoglobin index, blood total cholesterol, BMI status, body fat, season, and changes in self-reported total F&V intake in the model as potential covariates. If baseline confounding arises when the covariates differ significantly among the three intervention groups, a propensity score method will be adopted to balance the groups and control for the confounding. Similar analyses will be conducted when changes in plasma carotenoid are the response.

Plasma carotenoid analysis will be measured as described for Aim 1. We will also measure vitamin A status in the plasma as this can influence carotenoid absorption.

Interpretation

Upon successful completion of Aim 2, we will be able to translate RS intensity increases to an increase in F&V-derived carotenoid consumption. This will establish sensitivity of the RS device and enable better interpretation of findings from nutrition evaluation and surveillance studies using the RS device as an outcome measure.

Protection of Human Subjects

Aim 2 includes data from human subjects achieved by recruiting, enrolling, and collecting data from participants from North Carolina (East Carolina University, Greenville, NC), Minnesota (University of Minnesota, Minneapolis, MN), and Texas (Baylor College of Medicine, Houston, TX). Carotenoid analyses will occur at Eurofins Craft Technologies in Wilson, NC, and genetic analyses will occur at the USDA-ARS Children's Nutrition Research Center (CNRC) at Baylor College of Medicine in Houston, Texas. For Aim 2, we will conduct a 6-week randomized trial of a carotenoid-containing juice intervention among 156 racially and ethnically diverse participants in NC, MN, and TX. All study data will be stored in password-protected spreadsheets on password-protected computers and/or in locked filing cabinets.

Potential Risks

The primary risk for participants is risk of confidentiality breach. We will take all possible precautions to minimize this risk. Although there will be a link between a participant's data and their study-specific identification number, this link will be kept secure in password-protected computers available only to researchers directly involved in the study.

In addition, there is a risk of weight gain for Aim 2 participants who will be provided with Orange Carrot or apple juice. The Orange Carrot juice provides 60 kcal/6 fl. oz, and the apple juice provides 90 kcal/6 fl. oz. We will provide an educational handout on the additional energy provided by the juices and dietary strategies to adjust caloric intake as to remain isocaloric. If we see a >5% increase in body weight at the week 3 timepoint, staff will provide additional dietary education to the participant(s).

Upon study completion, we will share de-identified data with the scientific community, including investigators with appropriate credentials who wish to study relationships not proposed by investigators affiliated with the current project. The final dataset will not contain identifiers. However, to safeguard participants from potential breach of confidentiality/privacy, we will create a data-sharing agreement that ensures that 1) those with whom the data are shared will use it only for research purposes and will not identify any individual participant; 2) the data will be secured on password protected and appropriate computer technology; and 3) data will be destroyed or returned upon completion of data analysis.

Adequacy of Protection Against Risks

Study investigators and IRB-approved project staff will have access to individually identifiable data. If data are sent to investigators at other research sites, we will use ECU Institutional Review Board protocols to maintain confidentiality and protect identifiable information. All data will be stored in password-protected spreadsheets on password-protected computers and/or in locked filing cabinets. Study records will be kept a minimum of three years upon study completion.

Samples and data to be shared with researchers at the Baylor College of Medicine (for genetic analyses) and Eurofins Craft Technologies (for carotenoid analysis) will be stripped of any potential personal identifiers. DNA samples will be maintained in secured and alarm-protected labs at Baylor College of Medicine. Information on SNPs genotyped on the microarray have minimal established clinical utility at this time and individual-level

genotype results will not be returned. The SNPs on the array are commonly observed throughout world-wide populations, therefore, no unique genetic information will be generated, and there is consequently little to no risk for stigmatization, isolation or discrimination. The samples will be from independent/unrelated individuals; there is thus no risk for identifying non-paternity or undisclosed family relationships. The statistical analyses will not be able to reveal potential incest. Although analyses will evaluate general genetic ancestry, existing constitutional statutes ensure that there is thus no associated risk for discrimination. Analyses will focus on aggregate data that will be shared with the public and researchers through the usual dissemination mechanisms, including publications and presentations at scientific meetings.

To ensure high quality data management, the Research Electronic Data Capture (REDCap) system will be used. REDCap is a secure, HIPAA-compliant, web-based application for building and managing research databases. REDCap provides automated export procedures for data downloads to Excel and common statistical packages including SPSS, SAS, Stata, and R, a built-in project calendar, a scheduling module, and reporting tools (http://www.ecu.edu/itcs/help/redcap). Dr. Pitts has been trained in the use of REDCap and our team will utilize the following features to maximize data quality and management:

- The online survey building and administration tool, used by research teams in NC and MN
- The export function, to export data into secure Excel and SAS datasets in NC and MN
- The project calendar, used to monitor enrollment of each of the racial/ethnic sub-groups
- The reporting tool, used to generate weekly reports during the recruitment and enrollment period

The ECU Department of Public Health will house the data for this multi-site study and will be responsible for programming REDCap data collection forms. Illogical or out-of-range values will be denoted, and prompts for confirmation of data entry will appear. REDCap will allow for rapid display of descriptive statistics to aid in identifying potential data entry errors. Potential data entry errors and other problems will be resolved through examining original forms and contacting the research personnel who collected the data. Only Drs. Wu and Pitts will have administrative rights to make changes to the data and all changes will be tracked through the REDCap audit trail. The data entered into the REDCap database is stored on ECU servers which are backed up every 24 hours. Data will be exported via REDCap to Excel and to SAS datasets. A random sample of 10% of observations will be selected and will be verified between the exported Excel and SAS dataset and the original database. Descriptive statistics and plots will be used to examine the data for any additional problems not caught in the initial quality checks within REDCap. During recruitment and enrollment, summary descriptive statistics will be provided to the Principal Investigator and Co-Investigators to ensure accuracy of data entry and to provide early opportunities for clarification of questions and correction of errors.

Potential Benefits of the Research to Human Subjects and Others

There is no direct benefit for participants or others; however, there is the benefit to society for learning whether the RS-Device (Veggie Meter®) can be used as a valid and sensitive indicator of fruit and vegetable consumption among racially and ethnically diverse participants. If shown to be valid and sensitive, the Veggie Meter® can be deployed in many settings to determine effectiveness of community-based public health nutrition interventions and policies, ultimately to improve dietary practices and reduce risk of nutrition-related chronic disease.

Data and Specimen Banking

A small amount of blood collected for each participant will be stored temporarily at each of the three intervention sites (ECU, UMN, and BCM) while data collection occurs. After data collection is complete, this small amount of blood will be sent and stored/banked indefinitely at ECU to be used for future analysis/research. Participant information and privacy will be protected by using a unique ID to label

participant samples and other information. This ID will be kept securely and separately from other participant identifying information. Participants will be given the choice of opting to bank their samples or not on the consent form.

Safety Considerations

Data and Safety Monitoring Plan

This goal of Aim 2 is to examine sensitivity of RS-assessed skin carotenoid status as a marker of fruit and vegetable consumption in a racially and ethnically diverse sample of individuals. We will use data from Aim 2 (along with data from Aim 1) to provide predictive algorithms for interpretation.

The intervention and measurement protocols associated with this proposed study pose minimal risk to participants. Because of the low risk status, the Data Safety Monitoring Plan (DSMP) for this proposed study focuses on close monitoring by the Principal Investigator as well as the Safety Officer. We will promptly report excessive Adverse Events (AE) as well as any Serious Adverse Events (SAE) to the IRB at East Carolina University and to NHLBI by following the necessary protocols established by those institutions. Safety reports will also be created for the Safety Officer as part of the DSMP and will reflect the following: 1) recruitment and retention rates, 2) compliance for juice intervention subjects, 3) Adverse Events, and 4) Serious Adverse Events and other situations that might be of safety concern. Safety reports will be compiled by the Study Coordinator and sent to the Principal Investigator and Safety Officer. The frequency of data review for this study will be dependent on the type of data and is summarized in the following table.

Data Type	Frequency of Review
Participant Recruitment Rate	Monthly during recruitment of participants.
Participant Retention Rate	Monthly during the juice intervention beginning with the week 3 data collection period.
Juice Intervention Compliance	Monthly during the 6-week intervention.
Safety Report & Adverse Events	Every 6 months (safety report) and within 72 hours of being notified of an event (AE).

- Participant Recruitment Rate: Reflects the description of recruitment efforts and demographic characteristics (sex and race) for Aim 2. This will be especially important to track as we plan to recruit and enroll approximately equal numbers of participants in each racial/ethnic group for Aim 2.

- Participant Retention Rate: Reflects the summary of retention efforts and participant numbers by wave of participants for Aim 2. This will be reported monthly for Aim 2.

- Intervention Compliance: Description of text messages, phone calls, and completed juice logs at 3- and 6-week follow up. This will be reported monthly during the juice intervention.

Safety Report: AE (injuries) and SAE (if they are severe enough to be classified as an SAE) will be documented and reported in a safety report format and provided every 6 months. All protocols for reporting SAE to the necessary institutions within the appropriate timeframe will be followed. In addition, AE will be documented within 72 hours of being notified of the event and shared with the Safety Officer to determine if they qualify as an SAE.

Qualifications and Responsibilities of the Safety Officer

The Safety Officer for this study is Dr. David N. Collier, MD, PhD, FAAP, Department of Pediatrics and Center for Health Disparities Director: Pediatric Healthy Weight Research and Treatment Center and Associate Director of the Integrative Health Sciences Facility Core NCSU Center for Human Health and the Environment at ECU. Dr. Collier will address any questions with clinical implications. He has previously served as Safety Officer on a study of similar nature and design, so he is the ideal person to serve in this role. As the Safety Officer, Dr. Collier will review the reports sent by the Study Coordinator (at the frequency described in the table above) and will determine whether there is need for a corrective action that should be communicated to the study Principal Investigator, the ECU IRB, and NHLBI. This review by Dr. Collier will ensure adherence to safety protocols and achievement of recruitment and retention rates commensurate with our aims.

Measurement and Reporting of Adverse Events

We do not expect any AE as a result of participating in this study. However, if any AE occur study staff will report them using the AE section within the ECU IRB website. The Safety Officer will review and evaluate AE within 72 hours of being notified of the event by study staff. Any study-related SAE will be reported to NHLBI within the timeline set forth by the institute. A safety report containing AE, SAE, and their rates of occurrence will be generated every 6 months and reviewed by the Safety Officer at that time.

- Measurement and reporting of participant compliance to treatment protocol: Once participants who have been randomized to the intervention treatment group have started the juice intervention, compliance will be reviewed monthly by the Principal Investigator and Safety Officer. If compliance in the juice intervention becomes an issue, we will re-evaluate our compliance plan to improve compliance.
- There is a modest risk of weight gain for Aim 2 participants who will be provided with carotenoid-containing or apple juice. The juices provide 60 and 90 kcal/6 fl. oz, respectively. We will provide education on the additional energy provided and dietary strategies to adjust their caloric intake as to remain isocaloric. If we see a >5% increase in body weight at the week 3 timepoint, we will provide additional dietary education to the subjects.
- Measurement of retention status: For the Aim 2 juice intervention, we will collect measures similar to Aim 1 at baseline, 3 weeks, and 6 weeks after the participants are randomized to a control group (negligible-dose carotenoids), a moderate-dose carotenoids group, or a high-dose carotenoid group. Our goal is to retain at least 85% of our initial sample, though we have powered our study to accommodate a more conservative retention rate (80%). We will review the rate of subject accrual monthly during the initial enrollment period and dropout rates on a quarterly basis. We will make regular contact with participants to encourage continued participation.
- Stopping rules: In this minimal risk study to determine the sensitivity of the RS device (the Veggie Meter®), it is very unlikely that an event would occur that would require stopping the trial. However, as previously described, we will monitor injury rates of all participants. The Safety Officer, together with the study investigators, will alert the IRB at East Carolina University and NHLBI if a larger than reasonably expected injury rate should occur among participants in the intervention condition.

COVID-19 Safety Plan

Research staff at each of the intervention sites will follow their home University's approved/required COVID-19 screening plan. Participant COVID-19 screening will occur via phone the evening before the participant's study visit and again upon arrival to the visit the following day. This process will occur at each of the three participant visits.

In addition to participant COVID-19 screening questions, each site has and will follow site-specific requirements for study/clinic/lab spaces. Common requirements across all three sites includes:

- Staff will be required to wear various levels of personal protective equipment (PPE) such as masks, gloves, and face masks
- Participants will be required to wear face masks
- Staff and participants should maintain a distance of 6 ft whenever possible during the study visit
- Staff will clean common surfaces (e.g., tables and chairs, pens/pencils/stylus, light switches, doorknobs/handles) and equipment (e.g., the Veggie Meter® device, the Konica Minolta device, scale/height board, TANITA scale, iPads/computers, computer mouse/touchpads) before and after each participant's visit.

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