# Skin and plasma carotenoid response to a provided intervention diet high in vegetables and fruit: uptake and depletion kinetics[1–5]

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## ABSTRACT

**Background:** Objective biomarkers are needed to assess adherence to vegetable and fruit intervention trials. Blood carotenoids are considered the best biomarker of vegetable and fruit intake, but collecting blood is invasive and the analyses are relatively expensive for population studies. Resonance Raman spectroscopy (RRS) is an innovative method for assessing carotenoids in skin noninvasively.

**Objective:** Our objective was to compare blood carotenoid concentrations with skin carotenoid assessments by RRS during a controlled feeding intervention.

**Design:** Twenty-nine participants consumed low-carotenoid diets (6 wk, phases 1 and 3), a provided diet containing 6-cup equivalents (1046 g/d) of vegetables and fruit (8 wk, phase 2), and usual diet (final 8 wk, phase 4).

**Results:** At baseline, skin and plasma total carotenoid values were correlated ($r = 0.61$, $P < 0.001$). Skin and plasma carotenoid values decreased ($P < 0.001$) 36% and 30%, respectively, from baseline to the end of phase 1 and then increased ($P < 0.001$) by >200% at the end of phase 2. Plasma carotenoids returned to baseline concentrations by the middle of phase 3 and skin carotenoid concentrations by the middle of phase 4. Skin carotenoid status predicted plasma values by using a mixed linear model including all time points ($r = 0.72$, $P < 0.001$), which indicates that changes in skin carotenoid status closely follow changes in plasma across a broad range of intakes. At the individual level, skin carotenoids predicted plasma values ($r = 0.70$, $P < 0.001$) over all time points.

**Conclusion:** Skin carotenoid status assessed by resonance Raman spectroscopy is a noninvasive, objective biomarker of changes in vegetable and fruit intake. This trial was registered at clinicaltrials.gov as NCT01403844. *Am J Clin Nutr* 2014;100:930–7.

## INTRODUCTION

Observational studies suggest an inverse relation between intakes of vegetables and fruit (V/F) and cardiovascular disease (1–3), type 2 diabetes (4), some cancers (5), and all-cause mortality (6). The 2010 *Dietary Guidelines for Americans* recommends increased consumption of V/F, with specific guidelines for dark-green and red and orange vegetables (7). Adherence to recommendations is low; 11% of the population meets the recommendations for vegetables, 20% for fruit, and <7% for dark-green and orange vegetables (8). Accordingly, many behavioral interventions have sought to increase V/F intake (9, 10). Change in intake of V/F is difficult to determine because of limitations of available methods to evaluate complex intake behaviors of V/F consumption (11, 12).

Subjective methods such as dietary recalls and food-frequency questionnaires are commonly used to measure compliance with interventions designed to increase V/F intake because of their lower logistical barriers, cost, and subject burden. Bias and measurement error are inherent in self-report methods (13, 14), including bias introduced by the intervention itself (15, 16). By measuring a valid biomarker in conjunction with self-reported V/F in at least a subsample of individuals, researchers can choose to use statistical methods that may reduce the magnitude of the error (12, 17). Carotenoid compounds are attractive biomarkers of V/F consumption because humans cannot synthesize them, and they are readily deposited into body tissues. The blood carotenoid concentration is considered to be the best biological marker of V/F intake (18) but is invasive and expensive. Skin carotenoid status assessed by resonance Raman spectroscopy (RRS) has emerged as a promising new biomarker of V/F intake in both children and adults and was recently reviewed by Mayne et al (19). We have shown a wide distribution of skin carotenoid concentrations (20–22), high reproducibility over 6 mo in the absence of intervention (21, 23), and validity compared with blood concentrations (21, 24), skin biopsy (21, 25), and reported V/F intake (20, 21)—all necessary characteristics for a biomarker of nutritional status and food intake. Rapid, noninvasive, optical assessment of skin carotenoid status holds great promise as a powerful assessment tool for both observational and interventional

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research. However, the ability of the method to track skin carotenoid changes in response to increased V/F consumption has not yet been investigated. Therefore, our primary goal was to compare changes in skin carotenoid status with changes in plasma carotenoid concentrations during a controlled feeding intervention. Our hypotheses were that skin and plasma carotenoids would be positively correlated and responsive to varying levels of carotenoid intakes from foods.

## SUBJECTS AND METHODS

### Participants

Individuals were recruited by advertisements and flyers posted at the University of North Dakota and throughout the local area. Inclusion criteria included age 18–65 y, a BMI (in kg/m$^2$) between 19 and 30, weight stability (±5 lb, or 2.7 kg) for ≥2 mo, willingness to refrain from the use of nicotine and dietary supplements for the duration of the study, willingness to maintain baseline weight and physical activity levels, and willingness to complete all study requirements. Exclusion criteria included current gastrointestinal disorders, use of medication known to affect carotenoid absorption or storage, current smoking, allergies or intolerances to foods used in the study, or excessive alcohol use. Interested individuals were directed to an online questionnaire and, if qualified, were invited to attend an informational meeting. After written informed consent was provided, height and weight were measured to confirm eligibility. The participants were then scheduled for a baseline appointment. Seventy-seven individuals attended informational meetings. Thirty-nine individuals chose not to participate or were not qualified; the reasons included a BMI that was too high, schedule conflict, dietary or supplement use conflict, and lack of interest. Of the remaining 38 individuals who enrolled in the study, 9 withdrew because of a schedule conflict ($n$ = 5), noncompliance during the experimental diet phase ($n$ = 3), or no reason given ($n$ = 1). Individuals who withdrew did not differ from those who finished by BMI ($P$ = 0.39), age ($P$ = 0.22), or sex ($P$ = 0.60). The data presented here are from 29 participants who completed the study.

### Study design

The study design was a 28-wk single-arm, experimental feeding intervention. The protocol consisted of 4 phases (**Figure 1**). The participants were asked to follow 3 diet treatments: 6-wk depletion diets during which participants followed a low-carotenoid diet prescription (phases 1 and 3) and a provided high-V/F experimental diet for 8 wk (phase 2). The participants were then instructed to return to their usual diet for the final 8 wk (phase 4). The sample size was based on the primary outcome variable: change in skin carotenoid concentrations. Using variability estimates from previously published studies (21, 26) and repeated-measures ANOVA, we estimated that 26 participants would provide 90% power to detect a 20% increase in skin carotenoids from the end of the depletion phase to the end of the experimental diet phase, assuming a CV of 30% and $\alpha$ = 0.05. To account for an estimated 35% attrition, our enrollment goal was 40 participants.

Skin carotenoid status was measured by RRS ≥2 times/wk during phases 1, 3, and 4 and 5 times/wk during phase 2. Fasting blood samples were collected 9 times during the study (at baseline and at the mid- and endpoints of each phase by a trained phlebotomist) and stored at −80°C before HPLC analysis. Usual intake of carotenoids was estimated from self-reported intakes by using the online version of the National Cancer Institute's Diet History Questionnaire (DHQII*Web) (27–29). The participants were asked to report their usual food intake over the past month at baseline and at the end of phases 1, 3, and 4. The study protocol was approved by the Institutional Review Board of the University of North Dakota on 1 June 2011 and conducted between June 2011 and July 2012. The study was completed in 2 groups that began July 2011 and January 2012, respectively.

### Intervention diet

During the depletion phases, participants were instructed to avoid colorful V/F and foods with $\beta$-carotene as an ingredient and were provided a list of high-carotenoid V/F to avoid. This list included red bell peppers; tomatoes; tomato products such as pizza, spaghetti sauce, and ketchup; dark-green vegetables; orange and yellow vegetables such as carrots, corn, and sweet

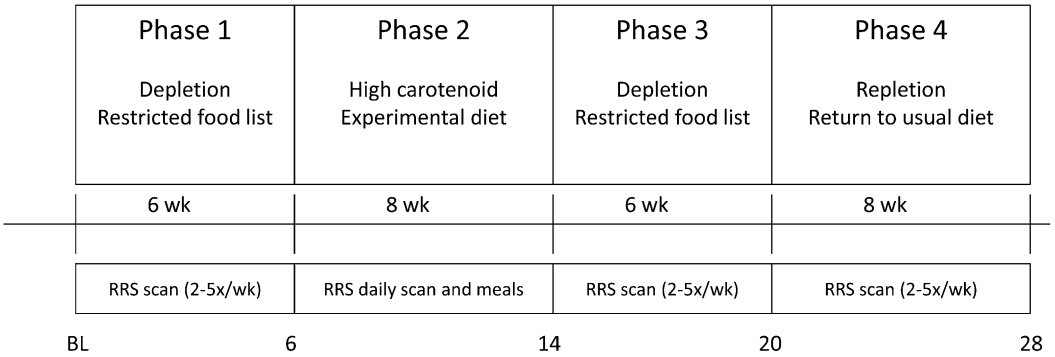


**FIGURE 1.** The study design consisted of 4 phases, with 3 diet treatments: a 6-wk depletion diet during which individuals ($n$ = 29) were asked to follow a diet prescription avoiding high-carotenoid vegetables and fruit (phase 1), an 8-wk provided diet high in vegetables and fruit (phase 2), and a second 6-wk depletion diet (phase 3). Participants then resumed their usual diet for the final 8 wk (phase 4). Blood was drawn 9 times: at BL and at the mid- and endpoints of each phase (3, 6, 10, 14, 17, 20, 24, and 28 wk). RRS scans were performed 2 to 5 times/wk during phases 1, 3, and 4 and 5 times/wk during phase 2. BL, baseline; RRS, resonance Raman spectroscopy.

potatoes; and orange, red, or yellow fruit or juices such as peaches and melons. Participants were allowed limited servings of foods such as egg yolks, corn-based ready-to-eat cereal, and iceberg lettuce daily. After 1 wk into each depletion phase, the participants completed 3-d diet records, which were reviewed by a research dietitian and analyzed with the USDA National Nutrient Database for Standard Reference, release 23 (30), by using a customized in-house nutrient analysis program to ensure an understanding of and compliance with the dietary restrictions.

The experimental diet consisted of a 7-d rotating menu based on the example 2000-calorie food pattern menu found at ChooseMyPlate.gov (31). The diet was designed to provide 62 mg mixed carotenoids (range 59–65/d) from an average of 6-cup equivalents (1046 g) V/F daily [vegetables: 3.0–4.5-cup equivalents (450–705 g); fruit: 1.0–2.5-cup equivalents (360–560 g)]. V/F were served cooked, raw, and as 100% juice. The menu was designed by research dietitians and met the food-based recommendations of the dietary guidelines. The menu was slightly modified to include more high-carotenoid V/F (such as substituting carrots for peas), for suitability for carryout for later consumption, and for acceptability to participants. Energy requirements were estimated for each person by using the equations from Mifflin et al (32) and an activity factor calculated from a 7-d activity log. To account for the different energy needs of the participants, the caloric content of the diet was adjusted by using noncarotenoid-containing foods, such as bread or rice, to meet requirements for weight maintenance. All participants received the same amount of calculated carotenoids. Details of the experimental diet can be found in **Table 1**.

On each weekday of phase 2, the participants consumed breakfast at the Grand Forks Human Nutrition Research Center and took home a cooler containing the rest of the day's food and beverages. All study food and beverages were provided; the participants were allowed to consume their own noncaloric beverages ad libitum and 1 alcoholic drink per day for women and 2 for men. No other food or drinks were allowed. The participants were weighed each day, and the caloric intake was adjusted if the mean of weekly weights increased or decreased by 2%. Food containers were returned for inspection, and the participants completed a compliance questionnaire at each visit.

### Skin carotenoid measurements

Carotenoid status in skin was measured by RRS. The method and instrumentation were developed by members of our group; the device and basic protocol are described in detail elsewhere (21–25). Briefly, the device uses a 488-nm solid-state laser for blue light excitation of the tissue carotenoids. The laser light is directed onto the skin via optical light delivery, and the collection module is placed in contact with the palm of the hand. Light backscattered from the skin is routed via fiber bundle to a spectrograph interfaced with a cooled charge-coupled device detector array. The recorded spectrum is analyzed for resonance Raman response of the skin carotenoids at their carbon double bond (C = C) frequency at 1525 $cm^{-1}$. Because all carotenoid subspecies contribute almost equally to this frequency, RRS intensity can be used as a measure of total carotenoid content in the measured tissue volume (24). At each visit, each individual's palm was scanned 3 times and the average value used in this analysis. Intra-individual variation of the triplicate measurements was 5.2%. The sensitivity of the instrument was checked daily against a calibration standard (sodium nitrate), and individual skin carotenoid RRS intensities were calibration-adjusted.

### Plasma carotenoid analysis

The analysis of plasma carotenoids was based on the method of Thurnham et al (33), in which $\alpha$-tocopherol acetate is used as an internal standard. HPLC standards for lutein, $\alpha$-carotene, and lycopene were purchased from Southcot Inc, and standards for $\beta$-cryptoxanthin, $\beta$-carotene, and $\alpha$-tocopherol acetate were purchased from Sigma-Aldrich Co. All standards were diluted in organic solvent with the concentrations verified spectrophotometrically by using published extinction coefficients. Single-use aliquots of the standards were stored at −80°C. The carotenoids were separated and quantitated by using a Shimadzu Prominence system with a photodiode array detector followed inline by a fluorescence detector. A 5-$\mu$m Spherisorb ODS2 (4.6 mm × 250 mm) reversed-phase column (Waters Corp) with an isocratic solvent system (3.5 mL/min) consisting of acetonitrile/methanol/chloroform (47/47/6 by volume) was used. All carotenoids were detected by using $A_{450\ nm}$. The internal standard was detected fluorometrically (292 nm excitation/330 nm emission). The intersample variation from samples prepared in parallel was <6%.

### Statistical analysis

Data are reported as means ± SEs. A linear mixed-effects model with subject as a random effect was used to estimate the rate of increase or decrease in RRS-measured skin concentrations during each phase of the study. The model, which used all RRS intensity measurements on every subject, allowed for different slopes during each phase; quadratic terms were included to test whether RRS intensities plateaued during any phase. The GLIMMIX procedure in SAS (SAS Institute Inc) was used to fit the mixed-effects model.

Repeated-measures ANOVA was used to test for changes over phases of the study in blood carotenoid concentrations and in RRS intensities. For comparison purposes, only the RRS intensities measured on the day of each blood draw were used. Tukey contrasts were used for post hoc comparisons of means. Pearson correlations were used to test whether there was a cross-sectional correlation between total carotenoids and RSS intensity at baseline and at the time of blood draw. Pearson correlation coefficients were calculated between total carotenoid concentration and RRS intensity. Maximum likelihood estimation was used to calculate the overall correlation between total plasma carotenoid concentrations and RRS intensity by using the 9 pairs of measurements on all subjects. The method used (34) provides estimates of both the within-individual correlation and the between-individual correlation across the course of the study and correctly accounts for the multiple measurements on each subject. In addition, the relation between total plasma carotenoid concentration and RRS intensity was modeled by using a random coefficients model. This model is appropriate when there are multiple observations from independent subjects and the regression model for each subject can be assumed to be a random deviation from the overall population regression model (35). The MIXED procedure in SAS was used for the repeated-measures

**TABLE 1**
Seven-day rotating menu provided to participants ($n$ = 29) during phase 2: high-carotenoid experimental diet (8 wk)

| | Day 1 | | Day 2 | | Day 3 | | Day 4 | | Day 5 | | Day 6 | | Day 7 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Food | Amount | Food | Amount | Food | Amount | Food | Amount | Food | Amount | Food | Amount | Food | Amount |
| | | *g* | | *g* | | *g* | | *g* | | *g* | | *g* | | *g* |
| Breakfast | | | | | | | | | | | | | | |
| | Skim milk | 240 | Vegetable juice | 200 | Skim milk | 240 | Strawberry banana juice | 240 | Skim milk | 240 | Skim milk | 240 | Skim milk | 240 |
| | Raspberry juice | 240 | Burrito | | Oat cereal | 50 | Scrambled eggs | 50 | Peach mango juice | 240 | Raspberry juice | 200 | Whole-grain waffles | 100 |
| | Oat cereal | 55 | Flour tortilla | 45 | Whole-wheat bread | 35 | Whole-wheat English muffin | 75 | Bran cereal | 60 | French toast | 120 | Spread | 10 |
| | Peaches | 120 | Scrambled eggs | 50 | | | Spread | 5 | Whole-wheat bread | 35 | Spread | 5 | Syrup | 30 |
| | | | Black beans | 50 | Spread | 5 | Blackberry jam | 15 | Spread | 5 | Syrup | 25 | Apricots | 150 |
| | | | Salsa | 40 | Mandarin oranges | 150 | | | Blackberry jam | 15 | | | | |
| | | | Red grapefruit | 240 | | | | | | | | | | |
| Lunch | | | | | | | | | | | | | | |
| | Taco salad | | Skim milk | 240 | Skim milk | 120 | Skim milk | 240 | Vegetable juice | 240 | Skim milk | 240 | Skim milk | 240 |
| | Taco meat | 80 | Sandwich | | Vegetable juice | 200 | Bean soup | | Sandwich | | Vegetarian chili | | Vegetable juice | 180 |
| | Romaine | 60 | Whole-grain hoagie bun | 90 | Sandwich | | Navy beans | 60 | Whole-grain hoagie bun | 90 | Chili beans | 150 | Wrap | |
| | Tomatoes | 60 | Mustard | 10 | Rye bread | 70 | Tomato soup | 300 | Mustard | 10 | Celery | 30 | Flour tortilla | 45 |
| | Black beans | 75 | Roast beef | 60 | Tuna | 60 | Crackers | 35 | Mayonnaise | 15 | Salsa | 150 | Turkey | 90 |
| | Tortilla chips | 35 | Mozzarella cheese | 50 | Mayonnaise | 15 | Spread | 5 | Turkey | 90 | Potato wedges | 140 | Baby spinach | 10 |
| | Cheddar cheese | 30 | Romaine | 10 | Celery | 15 | Baby carrots | 60 | Romaine | 10 | Cheddar cheese | 30 | Tomatoes | 60 |
| | Ranch dressing | 30 | Tomatoes | 60 | Romaine | 10 | | | Tomatoes | 60 | Cantaloupe | 240 | Ranch dressing | 30 |
| | Salsa | 60 | Sweet potato fries | 120 | Tomatoes | 60 | | | Watermelon | 150 | | | Baby carrots | 60 |
| | | | Ketchup | 30 | Baby carrots | 60 | | | | | | | Watermelon | 150 |
| | | | | | Peaches | 240 | | | | | | | | |
| Supper | | | | | | | | | | | | | | |
| | Skim milk | 240 | Skim milk | 240 | Chicken breast | 90 | Baked rotini | | Skim milk | 240 | Skim milk | 240 | Stir fry | |
| | Spinach lasagna | | Baked salmon | | Baked sweet potato | 200 | Ground beef | 60 | Sirloin steak | 120 | Vegetable juice | 240 | Broccoli | 120 |
| | Mafalda | 90 | Quinoa | 120 | Broccoli | 160 | Rotini | 90 | Beef broth | 15 | Garden salad | | Red bell pepper | 40 |
| | Italian sauce | 180 | Italian seasoning | 2 | Wheat roll | 40 | Italian sauce | 180 | Baked sweet potato | 165 | Romaine | 60 | Carrots | 60 |
| | Spinach | 90 | Spread | 10 | Spread | 15 | Parmesan | 20 | Broccoli | 80 | Tomatoes | 60 | Water chestnuts | 30 |
| | Ricotta cheese | 90 | Salmon | 120 | | | Spinach salad | | Wheat roll | 40 | Cucumbers | 60 | Tofu | 120 |
| | Mozzarella cheese | 30 | Lemon juice | 10 | | | Baby spinach | 35 | Spread | 10 | Ham | 30 | Tsao sauce | 40 |
| | Wheat roll | 40 | Squash soup | 200 | | | Carrots | 40 | | | Cheddar cheese | 30 | Brown rice | 100 |
| | Spread | 5 | Almonds | 15 | | | Mandarin oranges | 120 | | | Catalina dressing | 35 | Spread | 10 |

*(Continued)*

**TABLE 1** (*Continued*)

| | Day 1 | | Day 2 | | Day 3 | | Day 4 | | Day 5 | | Day 6 | | Day 7 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Food | Amount | Food | Amount | Food | Amount | Food | Amount | Food | Amount | Food | Amount | Food | Amount |
| | | | | | | | Walnuts | 30 | | | Wheat roll | 40 | Pumpkin custard | 205 |
| | | | | | | | Creamy Italian dressing | 30 | | | Spread | 5 | | |
| | | | | | | | | | | | Tropical fruit | 120 | | |
| Snack | | | | | | | | | | | | | | |
| | Watermelon | 150 | Cantaloupe | 240 | Dried apricots | 85 | Lemon yogurt | 200 | Berry yogurt | 200 | Whole-wheat crackers | 25 | Strawberry yogurt | 200 |
| | Almonds | 15 | | | Pina colada yogurt | 200 | | | | | Hummus | 55 | | |

models and for the maximum likelihood estimation. All statistical analyses were done by using SAS version 9.3.

## RESULTS

The participants were mostly women (69%) and non-Hispanic white (96%) with a mean (±SE) age of 32.1 ± 2.5 y and BMI of 23.6 ± 0.6. At baseline, the average total carotenoid content calculated from self-reported diets was 11.5 ± 1.2 mg total carotenoids/d. During phases 1 and 3, the calculated intakes were 1.4 ± 0.2 and 1.5 ± 0.4 mg total carotenoids/d, respectively. The intakes during phases 1 and 3 were not significantly different ($P = 0.77$). At the end of phase 4, intake returned to 9.0 ± 1.0 mg/d (not different from baseline, $P = 0.11$). Although the study was conducted by using 2 groups starting in different seasons of the year, no significant differences in skin or plasma carotenoid concentrations were found between the groups at baseline ($P = 0.78$), at the end of phase 2 ($P = 0.41$), or for the increase from phase 1 to phase 2 ($P = 0.47$). One individual's plasma concentrations did not increase during phase 2. Whereas we suspected that dietary noncompliance was the most likely explanation, we felt that it was most appropriate to include these data in our final analyses.

RRS-measured skin carotenoid intensities measured in 29 individuals throughout all phases of the study are shown in **Figure 2**. At the start of the study, individual levels varied ~7-fold (40% CV), and this large variation generally persisted after the first depletion phase. Individual increases during the experimental diet (phase 2) appeared to occur with comparable slopes. Starting from individually varying levels at baseline, RRS intensities declined by 37% during the first depletion (phase 1), increased by 264% during the feeding phase (phase 2), and then decreased by 43% during the subsequent depletion phase (phase 3). No plateau was observed by the last day of phase 2. The rate of decrease in RRS-measured levels in the second depletion phase (phase 3) was less ($P < 0.05$) than the rate of increase during the feeding phase (phase 2). In the natural repletion phase (phase 4), individual levels continued to decrease, reaching a minimum value at week 25, and then increased slightly. Final RRS intensities exhibited the same large variation (31% CV) as the baseline values. The kinetics of the HPLC-determined plasma carotenoid concentrations and RRS-measured skin carotenoid concentrations are shown in **Figure 3**, A and B, respectively. Both skin and plasma carotenoid values decreased ($P < 0.001$) between baseline and the end of phase 1 and increased ($P < 0.001$) from the end of phase 1 through the end of phase 2. Plasma carotenoid concentrations returned to baseline by the middle of phase 3, whereas skin concentrations returned to baseline by the middle of phase 4, ie, a delay of >1 mo compared with plasma. Finally, we examined correlations between carotenoid skin concentrations and plasma concentrations. At baseline, the cross-sectional correlation was $r = 0.61$ ($P < 0.001$). With the exception of one participant, each individual's skin concentrations and plasma carotenoid concentrations were positively correlated over time (median $r = 0.76$; range: 0.39–0.91). All but 6 individuals' correlations were statistically significant ($P < 0.05$) (data not shown). Per a random coefficients model, RRS intensity predicted ($P < 0.001$) total plasma carotenoids [total plasma carotenoids = 0.18 + 0.045 (RRS intensity for study instrument)]. The overall within-

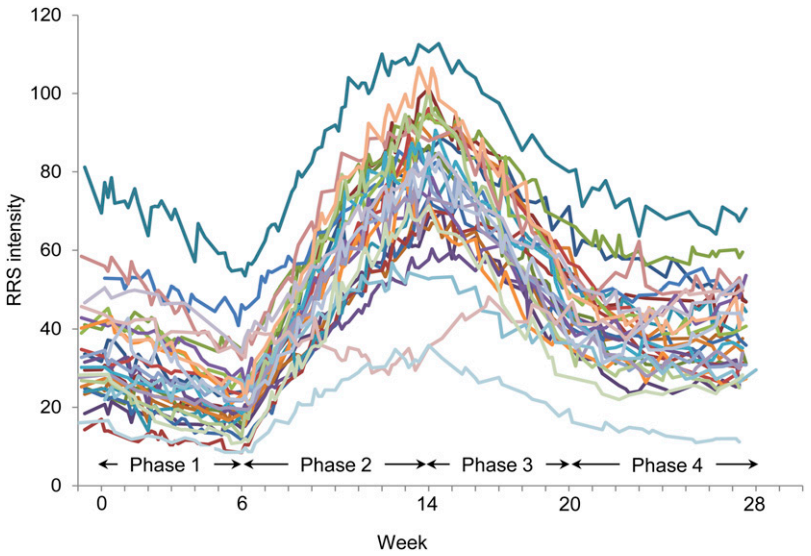


**FIGURE 2.** Individual responses of skin carotenoid status throughout the study ($n$ = 29). RRS, resonance Raman spectroscopy.

individual correlation was 0.70, and the between-individual correlation was 0.72. Both correlations were statistically significant at $P < 0.001$ (**Figure 4**).

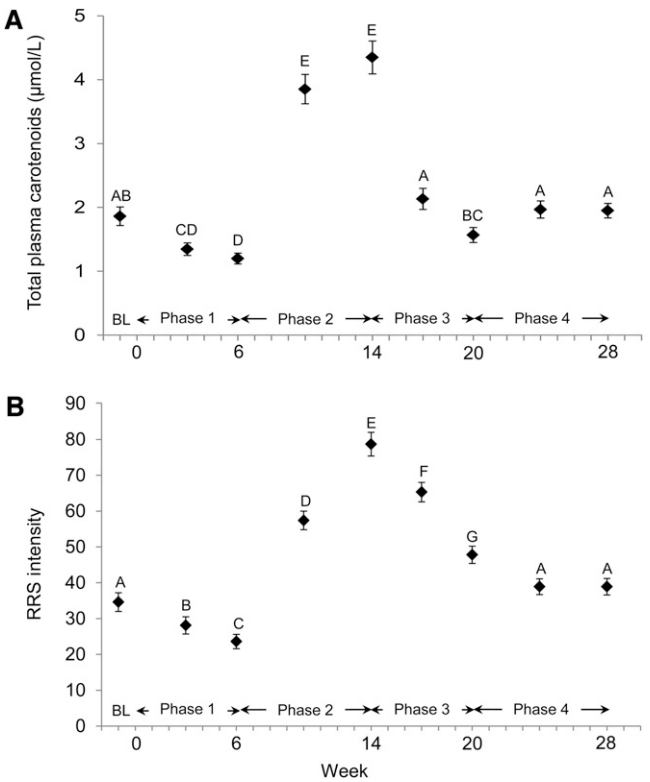


**FIGURE 3**. Mean (±SE) plasma carotenoid concentrations of 29 participants assessed by HPLC at BL and the mid- and endpoints of each phase of the study (A) and RRS intensities at 9 time points (B). Repeated-measures ANOVA, followed by Tukey contrasts for post hoc comparisons of means, was used to test for changes over the different phases of the study in plasma total carotenoid concentrations and RRS intensities. Means with different uppercase letters are significantly different, $P < 0.05$. BL, baseline; RRS, resonance Raman spectroscopy.

## DISCUSSION

In this study we showed that skin carotenoid status measured by RRS is a valid and reliable biomarker of change in skin carotenoid status in response to V/F consumption at amounts recommended by the *Dietary Guidelines for Americans* as implemented by the USDA Food Patterns (7). Plasma and skin carotenoid concentrations were correlated at baseline ($r$ = 0.61, $P < 0.001$) and across the study ($P < 0.001$), which was similar to that observed in our previous studies, which report a coefficient of 0.62 ($P$ = 0.006) for the correlation between RRS-measured skin concentrations and HPLC-measured plasma concentrations in a study of 28 adults (21) and a coefficient of 0.78 ($P < 0.001$) for the correlation between RRS-measured skin concentrations

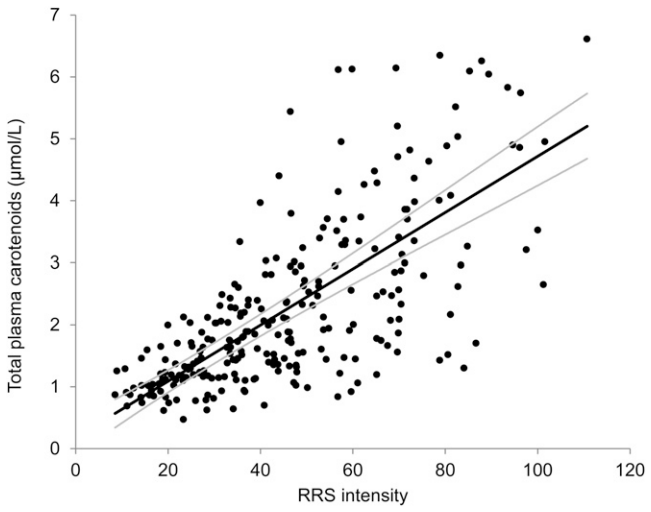


**FIGURE 4.** Total carotenoid concentrations in blood compared with RRS intensities for all subjects ($n$ = 29) at each of the 9 blood drawings. Per a random coefficients model, RRS intensity significantly ($P < 0.001$) predicted total plasma carotenoids [total plasma carotenoids = 0.18 + 0.045 (RRS intensity)]. The light gray lines indicate the 95% CIs around the regression line (solid black line). Per maximum likelihood, the overall within-individual correlation between skin and plasma carotenoids was 0.70, and the between-individual correlation was 0.72 ($P < 0.001$ for both). RRS, resonance Raman spectroscopy.

with total HPLC-measured serum in a separate study ($n$ = 104) (24). Our new results indicate that skin concentrations respond quickly to increased carotenoid intakes and that increases in skin carotenoid concentrations may be used to assess adherence to interventions as soon as 2 wk after the intervention. The slope of increase was roughly similar for each individual, regardless of different baseline values, which indicates that RRS-measured skin carotenoid status can be used to detect increases in V/F intake independent of a relatively high or low baseline status.

Plasma carotenoid concentrations returned to baseline after 3 wk. This is consistent with depletion studies showing decreases in blood carotenoids within 2–3 wk (36, 37). However, skin concentrations did not return to baseline until participants had returned to consuming their usual diet for 4 wk. This is consistent with the fact that blood acts as a transport medium for these compounds, whereas skin acts as a storage medium. The rapid detection of increased carotenoids in the stratum corneum during phase 2 indicated a faster transport from blood into the outer tissue than we anticipated, but was consistent with similar skin carotenoid uptake and the decrease in dynamics observed in feeding studies using nutritional supplements (26, 38). Our results support the use of skin status as a biomarker of increased V/F intake with a performance similar to that of blood carotenoids but a longer half-life. RRS offers the additional advantage that frequent measurements can be collected noninvasively compared with venipuncture. However, it is important to not confuse a status biomarker, such as skin or blood carotenoids, with a reference biomarker of absolute intake of either carotenoids or V/F. Blood and skin carotenoid concentrations are clearly useful for measuring changes in intake of V/F.

The wide variation seen in RRS intensities between individuals at baseline remained throughout the study (Figure 2). This may be explained by several factors that influence carotenoid status in addition to diet. We investigated potential determinants of observed skin carotenoid concentrations and discussed the existing literature in a recent review (19). Briefly, we found that season can affect skin carotenoid status. Whereas some seasonal effects may be attributable to diet (eg, increased intake of V/F during summer), variations in ultraviolet light exposure are also common across seasons, with ultraviolet exposure negatively affecting carotenoid status; however, in this particular study, we did not observe seasonal differences. Genetic variation in the uptake and metabolism of some carotenoids may also play a role. Other potential factors that affect skin and/or circulating concentrations of carotenoids include smoking, BMI, and possibly skin pigmentation, although confounding cannot be ruled out with regard to pigmentation (19). In this study, we attempted to limit the variability due to the former 2 factors by only including nonsmokers and people with a BMI $\leq$30, and our participants were nearly all non-Hispanic white, which was consistent with our source population.

This study had several strengths and limitations. This was the only controlled trial designed specifically to investigate skin carotenoid response to a diet intervention. The diet treatment contained cooked and raw V/F and 100% juices, mimicking usual food consumption. In this way, the results provide evidence for the efficacy of the RRS method in free-living interventions.

The limitations of this study included the homogenous sample, which was predominantly female and non-Hispanic white, with a limited range of ages and BMI and living in a single geographically limited area. The large proportion of non-Hispanic whites of European descent was not a goal of the study design but reflects the local population. Consumption of the experimental diet was observed only during breakfast on weekdays. However, both plasma and skin carotenoid changes occurred in expected ways, which indicated compliance. Another limitation was that it was not possible to blind either participants or study personnel to the treatment; however, we do not believe that the relation of RRS and plasma concentrations was biased due to the open intervention design. Future studies are needed to validate skin carotenoid status as a biomarker of adherence among individuals with different levels of skin pigmentation, sun exposure, and age and during weight loss or gain because adipose tissue is a depot for carotenoids. It will also be important to determine whether skin and blood carotenoid concentrations track in the same way during interventions by using higher or lower amounts of V/F than we used in this study. In addition, future studies should investigate changes in carotenoid status when consuming V/F with differing nutrient compositions, because skin carotenoids may increase in the presence of other antioxidants (39).

In conclusion, this study established that skin carotenoid concentrations assessed by RRS are a valid and reliable biomarker for change in V/F intake and carotenoid status. Detailed blood and skin carotenoid changes for all phases of dietary intervention and depletion have been assessed for the first time. RRS-measured skin carotenoid concentrations respond quickly to changes in V/F intake, which makes RRS an ideal tool for both interventional and observational studies of V/F intake.

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