Recommendations for the Use of the Veggie Meter® for Spectroscopy-Based Skin Carotenoid Measurements in the Research Setting

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ABSTRACT

Carotenoids are a class of phytochemical compounds found in a variety of fruits and vegetables (F/V) and, therefore, are commonly used as a biomarker for F/V intake. The Veggie Meter® is a noninvasive research-grade instrument that detects and quantifies carotenoids in the skin. To determine current practices and examine variability among users, a survey was administered to researchers using the device ($n = 19$, response rate = 35.8%) and variation in anatomical site preparation, calibration, number of measurements, measurement site, and documentation was observed. A protocol was developed in partnership with Veggie Meter® users to outline the preparation, calibration, and data collection procedures for using this device for research purposes. Although many protocol conditions will benefit from additional validation, this standardized protocol supports the development of a universal data repository to establish usual observed ranges, with the ultimate goal of examining associations between skin carotenoid scores and diet-related health outcomes. *Curr Dev Nutr* 2021;5:nzab104.

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Abbreviations used: F/V, fruits and vegetables; RRS, Raman resonance spectroscopy; RS, reflection spectroscopy.

Introduction

Carotenoids are bioactive phytochemicals found in a variety of fruits and vegetables (F/V) that cannot be synthesized de novo in humans and, therefore, are only obtained from the diet (1). For this reason, measurement of carotenoid status has gained traction in population-based nutrition research as an objective biomarker for the estimation of F/V intake. After dietary intake of carotenoids, these lipophilic compounds are metabolized, transported by lipoproteins in the bloodstream, and accumulate in various tissues, including blood, skin, and other organs (2). The kinetics of carotenoid distribution and clearance are dependent on a variety of factors, such as age, obesity status, stress, illness, and oxidative damage (3). Existing methods for quantifying tissue and blood carotenoid concentrations are typically invasive or subject to participant error and bias (4–6). Blood samples and dermal, adipose, or muscle biopsy specimens provide an overview of circulating and deposited carotenoid compounds, but using these methods may be painful and/or burdensome to participants (7, 8). In comparison, methods for determining carotenoid and F/V intakes through dietary assessments, such as self-reported dietary recalls and food records, are subjective and inherently biased, leading to inaccurate estimates (9). Emerging evidence supports the use of spectroscopy-based skin carotenoid measurements as a noninvasive objective method for determining skin carotenoid concentrations, indicative of F/V intake (10).

The Veggie Meter® is a spectroscopy-based skin carotenoid measurement device, created by Longevity Link Corporation in 2015, with the purpose of commercializing pressure-mediated reflection spectroscopy (RS) for detecting and quantifying skin carotenoids as a proxy

FIGURE 1 Veggie Meter® instrument and laptop interface.

for F/V intake in humans (11). The Veggie Meter® is a small, portable device that detects skin carotenoid concentrations in ~15–20 s for a single reading, including processing time and display information, or ~90 s to complete 3 individual measurements (**Figure 1**) (12). Validation studies of RS methodology against HPLC of excised human tissue samples found them to be comparable (13, 14), and serum samples were highly correlated with skin carotenoid scores measured using the Veggie Meter® ($R = 0.81$; $P < 0.001$) (15). The Veggie Meter® connects to an interfaced laptop computer to display an individual's "skin carotenoid score" on a histogram, with the x axis illustrating the range of scores from 0 to 800, and the y axis representing the reference population frequency. Skin carotenoid scores are plotted in relation to a reference population, which has been constructed by aggregating a large convenience sample of individuals' scores recorded using the Veggie Meter® and cross calibrating these values with skin carotenoid scores measured using Raman resonance spectroscopy (RRS) (11). The reference distribution was designed to feature a bell-shaped distribution with a slight skew toward higher skin carotenoid scores, such that the halfwidth of the distribution is ~75% of the peak score (**Figure 2**) (13). The histogram may be used to illustrate where a participant's skin carotenoid score compares to the reference population of individuals of all ages, sexes, and race/ethnicities, previously measured using this device (13). Although accumulated data, to date, indicate that the average Veggie Meter® score may vary among populations for a number of dietary and physiological reasons, specific reference histograms for subpopulations or condition-specific populations have yet to be developed. Compilation of measurements from various groups under varying physiological conditions and a range of dietary carotenoid and F/V intakes is needed to develop tailored reference distributions, which may support the interpretation of skin carotenoid score results.

In addition to the RS approach utilized by the Veggie Meter®, other spectroscopy-based skin carotenoid methods include RRS and spectrophotometers, which have also been demonstrated to be valid assessments of F/V intake, with a majority of correlation coefficients >0.40 (10). Although these alternative methods for spectroscopy-based carotenoid detection exist and are used in the research setting, the Veggie Meter® remains an attractive instrument for research use due to its affordability, portability, and responsivity to detect changes in dietary patterns related to F/V consumption (10, 14). For example, RRS requires a spectrally precise LED light source, whereas the Veggie Meter® uses a relatively low-powered white LED for carotenoid excitation. Therefore, the overall cost of the Veggie Meter®, priced at ~$15,000 USD, is relatively inexpensive compared with other spectroscopic devices. Although the light strength and precision of the detection methods differ between RRS and RS, validation studies comparing the 2 methods found comparable skin carotenoid signals ($R = 0.94$; $P < 0.001$) (15). Spectrophotometers used for skin carotenoid estimation detect red and yellow dermatological pigments that fall within the UV range in the color spectra of carotenoid compounds (~350–500 nm) (16). Because various compounds fall within the carotenoid detection UV spectral window, some spectrophotometers may include measurements of additional compounds or chromophores, such as hemoglobin and deoxyhemoglobin, in the absorption measurement, reflecting a higher skin carotenoid score than actually exists (14, 17). Comparatively, the Veggie Meter® applies supradermal pressure at 1 atm (~14.7 PSI) ± 10% to limit blood circulation to the anatomical assessment region, thus preventing other chromophores from interfering with carotenoid detection (13). Furthermore, the Veggie Meter® identifies and corrects for individual melanin concentration by using an algorithmic deconvolution adjustment; therefore, at a group level, melanin was not found to independently correlate with skin carotenoid score (15). Although the Veggie Meter® provides objective measures of skin carotenoid status, if the continued use of the device remains inconsistent among users, there is potential for differences in data interpretation. The need for a protocol outlining the use of the device is critical for minimizing between-user and between-study error.

The aforementioned advantages of the Veggie Meter® support the use of this device in the research setting; however, there is currently no standardized protocol for using the Veggie Meter® to assess skin carotenoids. Thus, the purpose of this study was to determine current practices and examine variability among Veggie Meter® users, ultimately to create and disseminate a standardized protocol for quantifying skin carotenoids in human subjects using the Veggie Meter®. A standardized protocol can support comparability among study data and the creation of a universal data repository to aggregate skin carotenoid scores recorded across all Veggie Meter® devices. Although the Veggie Meter® provides an estimate against a reference population, the device does not account for individual characteristics that may affect skin carotenoid measurements. In order to address the need for population- and condition-specific reference distributions and recommended ranges and trajectories for Veggie Meter® scores, this study aimed to identify protocol-related barriers to Veggie Meter® score aggregation and comparison. It is imperative to provide guidelines for collecting uniform information data for a central repository and as a basis for comparison for any further protocol modifications or deviations indicated by future advances in this field.

Methods

Development of the Current Practices Survey

The Veggie Meter® device is accompanied by an operating procedure (18); however, these instructions are subject to individual interpretation and, therefore, implementation may not be consistent among users.

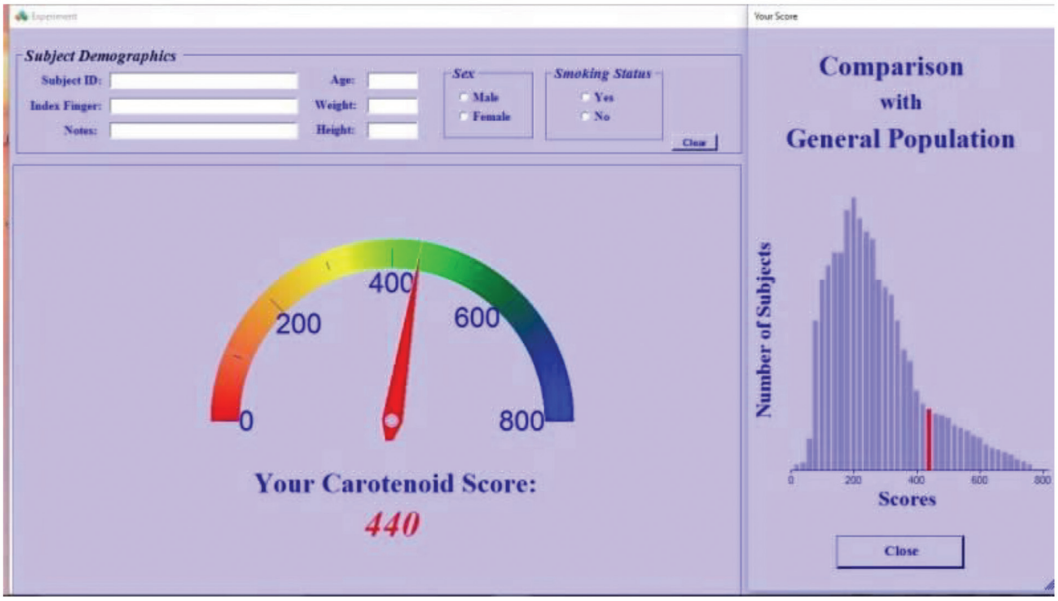

FIGURE 2 Veggie Meter® example output, containing overall skin carotenoid score and comparison to a reference population, indicated on the software output as the general population.

After a comprehensive review of the literature in early 2020, the novelty of the device was apparent by the limited number of publications (12, 15, 19–25). In the existing literature and supported by anecdotal evidence, inconsistencies among researchers were observed and procedural details pertaining to the Veggie Meter® were not reported in detail. Therefore, to determine the current methodologies among researchers using the Veggie Meter® for research purposes, a survey developed at the University of California, Davis in partnership with the San Francisco Department of Public Health was distributed to Veggie Meter® users and members of the International Carotenoid Database Group (veggiemeter@ucdavis.edu). This survey did not meet the criteria to be considered human subjects research and, therefore, no Institutional Review Board action was required. The survey questions were derived from the Veggie Meter® operating procedure by identifying the areas that either were ambiguous or open for interpretation and asking for clarification or explanation through open-ended response questions. The final survey consisted of 21 questions, with 17 questions directly related to the experience of users, calibration and set-up technique, measurement information, interpretation of results, and additional documentation details for Veggie Meter® use. The remaining 4 questions pertained to the research site–specific Institutional Review Board protocol verbiage and researcher contact information.

Distribution of the Current Practices Survey

On 7 April, 2020 the survey was distributed via the Veggie Meter® listserv (veggiemeter@ucdavis.edu), a previously established listserv to aggregate communication among nutrition scientists using the Veggie Meter®. The survey was distributed online (www.surveymonkey.com) using a modified tailored Dillman approach (26). If the individual on the listserv was not familiar with the operating procedures for the Veggie Meter®, that person was advised to distribute the survey to a more experienced user. Consequently, the exact number of survey recipients may extend beyond those included in the listserv. The survey was open for 2 wk and closed on 21 April, 2020. Responses were compiled and data cleaning was performed on the open-ended questions by identifying emergent themes and categorizing responses on areas of convergence. Descriptive analysis of the categorical variables was summarized by count and frequency (%) in May of 2020.

Development of the standardized protocol

A standardized protocol was developed based on current literature, input from the Veggie Meter® creators, and results from the Current Practices Survey regarding recommendations to reduce user discrepancies. Because the recommendations stated in the standardized protocol were derived based upon these various sources, some of the current practices reported by Veggie Meter® users may not agree with the suggestions in the protocol for collecting skin carotenoid scores for research purposes.

The draft protocol was sent to the Veggie Meter® listserv for feedback. Members of the listserv were asked to review the protocol and complete a questionnaire regarding willingness to adhere to particular aspects of the protocol in future research efforts. Users were asked to elaborate on any areas where they were not willing to adhere to aspects of the protocol.

Results

Survey results

The results from the survey supported the need to develop universal recommendations for the use of the Veggie Meter® in the research setting owing to a majority of users implementing differing methodologies. Listserv members are from a variety of research backgrounds and work with racially/ethnically diverse populations and populations of varying ages, including infants and toddlers, children, and adults. **Table 1**

TABLE 1 Selected questions and responses from the Current Practices Survey to assess the operating methodologies currently implemented by Veggie Meter® users

Questions from the Current Practices Survey	*n* (%)
How experienced are you using your Veggie Meter®?	
0–500 measurements	7 (36.8)
500–1000 measurements	9 (47.4)
>1000 measurements	3 (15.8)
Have you managed to calibrate the Veggie Meter® before each (and every) hour of use using the white and dark calibration sticks?	
Always	8 (42.1)
Most of the time	5 (26.3)
Sometimes	4 (21.1)
Never	2 (10.5)
Which hand do you always use to take measurements?	
Dominant	2 (10.5)
Nondominant	5 (26.3)
Right	5 (26.3)
Left	4 (21.1)
I don't always use the same hand	3 (15.8)
What finger do you always use to take measurements?	
Thumb	0
Index	12 (63.2)
Middle	3 (15.8)
Ring	3 (15.8)
Pinky/little	0
I don't always use the same finger	1 (5.3)
How do you prepare the fingers to prepare for a scan? Select all that apply	
Wash hands (soap and water)	5 (26.3)
Use an alcohol-based solution	12 (63.2)
Other	7 (36.8)
How do you interpret (or plan to interpret) the Veggie Meter® results?	
Relative to the distribution of scores in the current study population	3 (15.8)
Relative to an absolute reference result or score (same value across populations/studies)	2 (10.5)
Relative to a prior value for the same individual	11 (57.9)
Relative to a result associated with a particular diet or outcome in a peer-reviewed publication	2 (10.5)
Other	1 (5.3)
Do you record environmental conditions such as temperature, humidity, etc.?	
Always	0
Most of the time	0
Sometimes	2 (10.5)
Never	17 (89.5)
Do you keep track of which instrument is doing which measurement? (Only for users who indicated that they had >1 device.)	
Always	5 (31.3)
Most of the time	2 (12.5)
Sometimes	1 (6.3)
Never	3 (18.8)

includes the operational- and procedural-related questions from the Current Practices Survey, along with the participant responses. Among participants who completed the survey ($n = 19$), 63% of Veggie Meter® users were considered experienced users, defined as performing >500 skin carotenoid measurements using the device.

Discrepancies were observed in the following operational and procedural steps.

Device set-up and calibration.

Differences regarding the set-up and calibration of the Veggie Meter® using the dark and light reference materials were recorded, along with a low number of responders documenting the date, time, and location of the calibrations (Table 1). Per manufacturer instructions, calibration of the device is recommended before use and every hour thereafter. Improper or infrequent calibration may result in inaccurate skin carotenoid measurements. On occasion, an error in calibration may occur when the expected calibration display does not match the actual display. Many users reported simply repeating the calibration until the expected display was achieved, and some users take the additional step of cleaning the device before repeating the calibration step. An additional question regarding concerns about device set-up and calibration resulted in requests for proper instructions on what to do when the calibration display does not match, as well as an explanation for the importance of repeated calibration.

Measurement preparation and anatomical site selection.

Variation in the selection of the anatomical site was observed, such that users differed in the use of the dominant compared with the nondominant hand and in digit preference for the measurement location (Table 1). In addition, site preparation before the measurement was

inconsistent: the use of alcohol-based solutions such as a presoaked wipe or hand sanitizer, washing hands using soap and water, using multiple cleaning methods, using whatever method is convenient at the research location, or not having participants wash the anatomical site before performing the measurement were all reported (Table 1).

Data collection and interpretation.

The number of individual skin carotenoid measurements was generally consistent among users, with a majority of researchers ($n = 16$; 84%) performing triplicate measurements per participant and using the mean of the 3 readings, using the "Average of 3 Scans" mode or conducting 3 separate measurements and averaging manually, to reduce intraindividual variability. However, differences in the interpretation of skin carotenoid scores were identified, such that 58% of users interpret Veggie Meter® results relative to the baseline value for the same individual, 16% interpret results relative to the current study population, 11% relative to the absolute reference score, another 11% relative to a previously reported value associated with a specific diet or outcome, and 1 user stated that interpretation is dependent on the particular study (Table 1).

Documentation.

Although the manufacturer indicates that the Veggie Meter® is highly sensitive to excessive heat, cold, or bright light owing to the potential for optical interference with the LED, 89% of users did not document environmental conditions, such as temperature or humidity (Table 1) (11). This documentation of environmental conditions is not currently stated in the most recent version of the manufacturer manual (18); however, when using the device outside of a controlled setting, environmental conditions may affect findings. In addition, with 32% of users having >1 Veggie Meter®, proper documentation of which device is being used is important in order to limit inconsistencies and interinstrument variability; of the users who indicated they had multiple devices, only 31% recorded which instrument was being used for each study participant.

User-reported acceptability of the standardized protocol

To address the inconsistencies observed in the survey results and make future collected data amenable to intersite analysis, a standardized protocol was developed to support the use of the Veggie Meter® in the research setting. Detailed explanations corresponding with the recommendations in the standardized protocol are provided in the subsequent discussion. This standardized protocol outlines the operating procedure for use of this device as a research-grade instrument to ensure researchers using the Veggie Meter® are following steps for consistency, repeatability, and generalizability. The standardized protocol can be followed verbatim whenever the Veggie Meter® is being used to collect research data that can be compared with data from other research locations. The standardized protocol can be found in **Supplemental Data Appendix A**.

To determine the likelihood that the standardized protocol will be implemented in the research setting, current Veggie Meter® users were asked to review the protocol recommendations and indicate whether they would be willing to implement the protocol steps in future research efforts (**Table 2**). Responses were received from Veggie Meter® users ($n = 21$, response rate = 39.6%) and the qualitative feedback was used to modify the final draft of the standardized protocol.

Discussion

Justification for standardized protocol recommendations

The survey results indicated a need for the development of a standardized protocol for use of the Veggie Meter® to improve the generalizability and applicability of skin carotenoid scores recorded using this device. Moreover, the results from the Current Practices Survey suggested that methods currently in use may introduce additional variability into skin carotenoid score data. The discrepancies identified in the calibration and set-up technique, anatomical site selection and preparation, measurement methods, interpretation of results, and documentation processes yield insight into the importance of developing and disseminating a standardized protocol for the Veggie Meter®.

Device set-up and calibration.

Calibration of the Veggie Meter® using the dark and white reference blanks should be conducted before performing measurements. The calibration graphs should be compared to the reference graphs to confirm that calibration of the device was successful. If the graphs are not consistent with the reference images, calibration should be repeated. Calibration of the Veggie Meter® should occur at least every 2 h after continuous use. Nevertheless, more frequent calibration at the 1-h time lapse is recommended for research purposes (11). The Veggie Meter® should be recalibrated if the device is elevated from a flat surface or transported to a different data collection site within the 2-h time period.

Measurement preparation and anatomical site selection.

Before conducting skin carotenoid measurements, it is advantageous to describe the operational procedure of the Veggie Meter® to participants to avoid apprehension due to the frequent assumption that the measurement will be painful (15, 19, 24). When working with toddlers, children, or adolescents, it is recommended to both verbally explain and demonstrate the process of measuring skin carotenoids using the Veggie Meter® to ensure the participants feel comfortable during data collection procedures (19). In infants and toddlers, digit size will play a role in accuracy of measurements; therefore, before working with infants, toddlers, or children, contact the manufacturers for instructions and device modifications.

Emerging evidence has demonstrated variability in skin carotenoid scores due to differences in anatomical site selection (27). To reduce the inconsistencies due to differences in hand and digit selection, the nondominant digitus medicinalis, commonly known as the ring finger, should be used when conducting skin carotenoid measurements with the Veggie Meter®. Because the dominant hand has increased vasculature and musculature resulting from more frequent use, the nondominant hand recommendation was selected to ensure minimal deoxyhemoglobin and oxyhemoglobin chromophore interference (28). The digitus medicinalis or ring finger is preferred owing to the decreased callusing compared with the index finger, thereby reducing skin thickness to allow for increased light penetration to the subdermal detection region (29). Therefore, to minimize potential error due to vasculature and skin thickness, the nondominant ring finger is the preferred measurement site. Additional research is required to determine individual variability using different digits under varying amounts of carotenoid intake.

In addition to the physiological differences driving the selection of the nondominant ring finger, observations at the community level,

TABLE 2 Responses from Veggie Meter® users about implementing the recommendations in the standardized protocol in future research efforts

Veggie Meter user feedback on standardized protocol	*n* (%)
Would you consider implementing the proposed recommendation of allowing a 15-minute acclimation period if the Veggie Meter® is introduced to a new environment, especially one with substantial changes in temperature, lighting, or relative humidity?	
Yes, I will implement	17 (81.0)
No, I will not implement	0
I am not sure if I will implement	4 (19.0)
Qualitative responses[1]	
Time constraint	2
Need to see the data for why this is important and how much of an effect it would have on the outcome	2
Would you consider implementing the proposed recommendation of calibrating the Veggie Meter® every 1 hour of operation or if the device is relocated or moved prior to the 1-hour time interval?	
Yes, I will implement	18 (85.7)
No, I will not implement	2 (9.5)
I am not sure if I will implement	1 (4.8)
Qualitative responses[1]	
Time constraint	2
Need to see the data for why this is important and how much of an effect it would have on the outcome	1
Would you consider implementing the proposed recommendation of using the triplicate (three-scan) mode on the Veggie Meter® to record skin carotenoid scores?	
Yes, I will implement	16 (76.2)
No, I will not implement	1 (4.8)
I am not sure if I will implement	4 (19.0)
Qualitative responses[1]	
Remain consistent during ongoing study	1
Work with young children (2–5 y)	1
Time constraint, particularly with large sample sizes	2
Need to see the data for why this is important and how much of an effect it would have on the outcome	1
Would you consider implementing the proposed recommendation of recording the following individual characteristics: age, sex, BMI, smoking status, supplement use, and diagnosed chronic diseases?	
Yes, I will implement	12 (57.1)
No, I will not implement	1 (4.8)
I am not sure if I will implement	8 (38.0)
Qualitative responses[1]	
Collecting disease status is outside the scope of Institutional Review Board approved protocols	2
Collecting personal information in a field-based setting may be inappropriate	2
Work with young children (2–5 y)	3
Depends on purpose of the study—such information may not be needed	2
Would you consider implementing the proposed recommendation of using the nondominant, ring finger to record skin carotenoid scores using the Veggie Meter®?	
Yes, I will implement	14 (66.7)
No, I will not implement	3 (14.3)
I am not sure if I will implement	4 (19.0)
Qualitative responses[1]	
Remain consistent during ongoing study	2
Additional data is needed on finger variation	4
Not consistent with information provided in the user manual	1
Would you consider implementing the proposed recommendation of recording environmental conditions, such as temperature and relative humidity when using the Veggie Meter®?	
Yes, I will implement	9 (42.9)
No, I will not implement	3 (14.2)
I am not sure if I will implement	9 (42.9)
Qualitative responses[1]	
Time constraint	2
Not consistent with information provided in the user manual	2
Unsure how to collect environmental conditions	7
Need to see the data for why this is important and how much of an effect it would have on the outcome	1

(*Continued*)

TABLE 2 (*Continued*)

Veggie Meter user feedback on standardized protocol	*n* (%)
Would you consider implementing the proposed recommendation of having participants wash hands with soap and warm water prior to recording skin carotenoid scores? If hand washing resources are unavailable, would you use a premoistened alcohol prep pad or hand wipes as an alternative?	
Yes, I will implement	19 (90.4)
No, I will not implement	1 (4.8)
I am not sure if I will implement	1 (4.8)
Qualitative responses[1]	
Some individuals may be sensitive to alcohol-based cleaners	1
Hand washing station not available in field setting	1

[1]Emerging themes from qualitative responses and number of respondents corresponding to each theme.

particularly regarding the use of the Veggie Meter® in the school setting, were also considered. It was evident in the residual staining on the thumb and index finger of the dominant hand that children regularly consumed highly pigmented foods or had remnant splotches from colored markers or paint, which may subsequently affect skin carotenoid measurements owing to these pigments being incorrectly identified within the carotenoid detection spectral range (30). Although the Veggie Meter® is equipped with a spectral deconvolution algorithm to correct for melanin and other chromophore interference, supracutaneous pigmentation may not be accurately detected or corrected for in this process.

Before performing skin carotenoid measurements, it is recommended that participants wash hands using soap and warm water to ameliorate potential confounding residue (31); however, because the Veggie Meter® may be used in settings where access to proper handwashing equipment is not available, a premoistened alcohol prep pad or hand wipes may be used instead. Hand sanitizer is not recommended, because it may not remove pigment or interfering debris. In addition, to ensure minimal lens interference, the surface of the contact lens should be cleaned using an optical cloth after every participant (23).

Data collection and interpretation.

Triplicate measures should be conducted to increase the reliability of skin carotenoid scores. The triplicate measurement feature of the Veggie Meter® (also known as "Average of Three Scans" mode) averages the scores across 3 consecutive measurements and reports the mean value. The digit should be retracted from the device as indicated by the measurement software between respective measurements to ensure reperfusion occurs (23). The triplicate mode is equivalent to measuring skin carotenoid scores 3 separate times, writing down the score each time, summing them up, and dividing by 3; however, the triplicate mode computes the mean automatically and reports a single average skin carotenoid score for the participant. The triplicate mode increases the accuracy and reproducibility of skin carotenoid detection by 2-fold owing to the light excitation disk sampling slightly different measurement sites, which minimizes tissue irregularities due to repeated blood reperfusion to the measurement site and accounts for sweat gland ducts or papillary ridges, which may affect carotenoid detection. Some users indicate using the single measurement mode to obtain 3 unique values for an individual, in order to determine intraindividual variation. This is a method that can be considered if a researcher is interested in the respective values that comprise the mean. Regardless of whether the triplicate measurement mode or repeating the single measurement mode 3 separate times is used, it is recommended to collect 3 measurements to generate a mean skin carotenoid score to account for tissue inhomogeneities. The single measurement mode may be used in situations with limited time allotted for data collection; however, for research purposes, the triplicate measurement should be used exclusively when possible.

Rarely, the Veggie Meter® will be unable to compute an individual's skin carotenoid score and an error message due to a nondetectable or 0 measurement will appear in the display window. If this occurs, confirm the finger has no contaminants, reposition the finger in the center of the contact lens, and repeat the measurement process. The output may display a skin carotenoid score on the second attempt; however, in some cases the error message may persist, in which case there will be no quantitative skin carotenoid value computed. Therefore, the researcher should consider removing this participant from the study, deeming them ineligible for inclusion. It is not recommended to attempt to record an individual's skin carotenoid score using a different finger, because this may affect the reproducibility of measurements.

Because the Veggie Meter® computes skin carotenoid scores immediately, researchers have the option of communicating the results to participants or blinding participants to their scores. Recommendations may vary based on study design. If conducting a randomized controlled trial or intervention study aimed at increasing skin carotenoid scores over time, it is not recommended to share results, because this may influence dietary behaviors. If the research study focuses on dietary tracking or monitoring, chronic disease prevention, or nutrition education effectiveness, sharing the results with the participants may create a visual representation as a strategy to promote and maintain positive behavior change.

Researchers should carefully consider what to share, and how to share skin carotenoid score results, with study participants. Depending on the study design, the results may bias the behaviors of the participant, or may cause the participant to experience stress or concern about their own score. If the participant expresses stress, the researcher can emphasize that scores, at this point, cannot be compared between individuals and are the result of many different personal considerations, such as age, sex, BMI, alcohol consumption, smoking status, and dietary factors. It should be conveyed that a low skin carotenoid score does not necessarily equate with poor health or risk of chronic disease.

TABLE 3 Sample document for recording information when using the Veggie Meter® for research purposes, with a sample entry provided

Veggie Meter® data collection record					
Date	**Location**	**Environmental condition**	**Initial calibration**	**Additional recalibration times**	**Notes**
01/01/2021	Elementary School	78°F; overcast and humid (60–70%)	09:00	09:55, 10:52, 11:48	Device was moved to 3 classrooms; measurements at 11:48 occurred outside; Device II was used

Internet connections may pose connectivity issues or make the device more vulnerable to harmful software viruses. It is recommended to back up the data on a USB drive and transfer them to a project-specific password-encrypted file. For this reason, confidential or identifiable participant information should not be directly inputted into the Veggie Meter software. It is advised to assign subject identification codes to ensure participant anonymity, in accordance with the guidelines enforced by the respective Institutional Review Boards.

Documentation.

Skin carotenoid scores may be influenced by a variety of intrapersonal factors. To account for individual characteristics that may alter skin carotenoid measurements, the following data should be collected whenever possible: age, sex, BMI, smoking status, supplement use, and diagnosed chronic diseases.

The Veggie Meter® is an optoelectronic device, making it highly sensitive to changes in temperature and light; therefore, it is imperative to record environmental conditions to examine whether the results were confounded by environmental exposure (**Table 3**). This is particularly of interest when working in community settings, outdoors, at a hospital, or in any other environment where exposure to mechanical shock from sudden movement, excessive heat, or bright lights may occur. In addition to the environmental conditions, researchers with >1 Veggie Meter® should record which device is used to collect the skin carotenoid scores, because this will minimize potential errors arriving from interdevice variability. Although this is a recommendation from the manufacturers, future research is needed to investigate the influence of different environmental conditions on Veggie Meter® output.

Previous research has indicated possible seasonal differences in the intake of carotenoid-rich foods, therefore, documenting the date the device was used should be factored into data analysis to account for potential unintended changes in skin carotenoid scores due to seasonality (3, 10, 32). In addition, individual health status, such as BMI, acute and chronic illnesses, supplementation, or long-term medication intake, may affect skin carotenoid scores (23, 33, 34). If a participant experiences drastic weight loss or is diagnosed with a chronic disease during the study, it may be important to document these changes because they may alter skin carotenoid scores.

Veggie Meter® users expressed areas of concern regarding the additional time and resources required to document individual characteristics and environmental conditions; however, because these are important requirements for the development of a universal skin carotenoid data repository, it is recommended to collect consistent information among Veggie Meter® users whenever possible.

Future applications

Effective methods for measuring F/V consumption, such as the rapid and noninvasive carotenoid detection method using the Veggie Meter®, have the capacity to assess public health and nutrition education interventions; assist physicians, registered dietitians, and other health professionals with monitoring the health status of patients; and assist in research as an objective biomarker for F/V intake (10, 20, 21, 24, 35, 36). The Veggie Meter® has been used to measure skin carotenoids in ethnically diverse toddlers, children, and adult populations in both clinical and nonclinical settings (12, 15, 19–24, 37, 38). Future research efforts should focus on conducting a systematic review of the studies using the Veggie Meter® to determine if the differences in published methods for skin carotenoid data acquisition affect the reported outcomes (**Table 4**).

Recommendations to increase the validity and generalizability of the Veggie Meter® in the research setting include continued usage and validation of the RS method across the life span, in individuals of various BMI classifications, and in populations of diverse races and ethnicities. Introducing this technology into the clinical setting in primary health care facilities and nonclinical sectors, such as the public school systems and other community environments, has the potential to support preventative health services and health interventions aimed at improving health outcomes in children and adults.

With the proposed integration of the Veggie Meter® into various health, education, and community settings, expanded utilization of the device will facilitate an increase in the number of unique Veggie Meter® measurements, thereby broadening the diversity and generalizability of the skin carotenoid data repository. Implementing the standardized protocol for the Veggie Meter® is fundamental to the development of a universal skin carotenoid data repository to establish recommended ranges of skin carotenoid concentration values at the population level. With the previously identified differences in skin carotenoid scores based on individual characteristics, the proposed repository will incorporate these factors to determine an estimated range of skin carotenoid scores based on these factors. Therefore, it is recommended that researchers report consistent nonidentifiable individual-level characteristics to be incorporated into the database and to facilitate comparisons among studies when using the Veggie Meter®. With the emergence of new literature, Veggie Meter® users will be contacted using the established listserv to determine if the recommendations presented in this protocol remain the most appropriate for the research setting.

TABLE 4 Proposed future validation efforts for use of the Veggie Meter® in the research setting for skin carotenoid detection

Inconsistencies among users	Recommendation in the research setting and rationale	Type of study design needed to verify best practice
Recording of environmental conditions	Because the Veggie Meter is an optoelectronic device, it is sensitive to environmental exposures; therefore, it is recommended to record where the device is being used, especially when in variable conditions, such as outdoor settings	Study to assess discrepancies when using the device indoors compared with outdoors to determine if skin carotenoid values on the same participants are affected by environmental conditions
Measurement site preparation	Participants should wash hands with soap and water before having skin carotenoid measurements performed. If there is limited access to soap and water, an alcohol prep pad or hand wipe may be used to clean the measurement site	Different site preparation techniques should be performed to assess the variability that may be caused owing to residue or pigments that remain on the measurement site, which may interfere with carotenoid detection. These values obtained using different site preparations should be correlated with plasma carotenoids
Measurement site	The nondominant ring finger should be used when possible to minimize potential error due to vasculature, musculature, and skin thickness	Digit variability has been observed (26); however, additional research is required to determine the error between measurement sites as compared with dietary intake and/or plasma carotenoids
Number of measurements	The "Average of 3 Scans" mode or recording skin carotenoid scores 3 times and manually averaging the scores should be performed when possible	Study to assess the reproducibility of results observed using the "Average of 3 Scans" mode and manually averaging the scores to determine whether the same margin of error is recorded
Individual-level data collected and reported	Age, sex, BMI, smoking status, supplement use, and diagnosed chronic disease status should be recorded when possible, because these individual characteristics may affect skin carotenoid status	Efforts to create standardized skin carotenoid score ranges require the documentation of individual-level characteristics. Controlled-feeding studies evaluating which of these characteristics are the most influential with respect to skin carotenoid scores are necessary

Further validation is needed to determine the extent to which differences in the digits measured and site preparation are correlated with plasma carotenoids and dietary carotenoid intake assessed by controlled dietary interventions or rigorous 24-h dietary recalls.

Limitations

It is important to acknowledge potential limitations in the standardized protocol developed for the use of the Veggie Meter® in the research setting. The survey that informed current practices was distributed to known Veggie Meter® users and members of the International Carotenoid Database Group in April of 2020, amid a peak in the Covid-19 pandemic. For this reason, it is possible that some researchers who use the Veggie Meter® for research did not receive or respond to the survey; however, because there were many discrepancies observed among the 19 survey respondents, it is expected that an increase in survey responses would further exacerbate the inconsistencies. The proposed standardized protocol may not address some sources of error.

Because the Veggie Meter® is an emerging technology among researchers, it is important to expedite the adoption of this standardized protocol to inform future practices for using this device in the research setting. It is important to note that some of the information presented in this protocol was extracted from published abstracts, in addition to peer-reviewed manuscripts, which was expected owing to the novel, innovative, and emerging nature of the device as a research-grade instrument. It was difficult to acquire precise methodologies because many articles did not state the specific procedures used when operating the device. Because the Current Practices Survey was conducted in the spring of 2020, it should be acknowledged that an updated review of more recently published literature using the Veggie Meter® was performed, confirming that procedural discrepancies still persisted in more recent literature (39–50). This finding emphasizes the importance of establishing a standardized protocol for the use of the Veggie Meter® in the research setting to ensure consistency among users moving forward, which will allow for comparisons between studies.

Regarding the Veggie Meter®, there are limitations to the use of the device in the research setting that should be recognized. The majority of studies that have used the Veggie Meter® to determine skin carotenoid status have been performed in populations consisting of children and adult participants. Current efforts to evaluate the validity of the Veggie Meter® in infants are ongoing (51). To the authors' knowledge, no

studies have been conducted using the Veggie Meter® in older adult populations. Therefore, owing to the limited data available on the use of the Veggie Meter® in infants and older adults, amendments to the standardized protocol may be appropriate in these populations.

Conclusions

The standardized protocol for use of the Veggie Meter® will provide researchers with comprehensive instructions on how to operate the device in the research setting. Standardization of the procedure will increase the comparability of the results among studies and allow for a more robust database of skin carotenoid measurements to be developed. The recommendations provided in the standardized protocol are based upon the most recent understanding of carotenoid physiology and the method by which the Veggie Meter® measures the carotenoids deposited in the skin. These recommendations can be strengthened by systematic experimentation and evaluation of discrepancies commonly occurring in Veggie Meter® use (Table 4). With the goal of the standardized protocol being to reduce variability among researchers, this protocol neither invalidates nor undermines the significance of data previously collected utilizing the Veggie Meter® with different approaches or methodologies. The use of the standardized protocol will strengthen the field of spectroscopy-based skin carotenoid measurements and as researchers use the standardized protocol for the Veggie Meter®, this will allow the opportunity to create suggested ranges for specific populations, including different stages across the life span, different BMI classifications, and individuals living with chronic diseases.

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