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# Innovative Techniques for Evaluating Behavioral Nutrition Interventions[1–4]

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**ABSTRACT**

Assessing outcomes and the impact from behavioral nutrition interventions has remained challenging because of the lack of methods available beyond traditional nutrition assessment tools and techniques. With the current high global obesity and related chronic disease rates, novel methods to evaluate the impact of behavioral nutrition-based interventions are much needed. The objective of this narrative review is to describe and review the current status of knowledge as it relates to 4 different innovative methods or tools to assess behavioral nutrition interventions. Methods reviewed include *1*) the assessment of stress and stress responsiveness to enhance the evaluation of nutrition interventions, *2*) eye-tracking technology in nutritional interventions, *3*) smartphone biosensors to assess nutrition and health-related outcomes, and *4*) skin carotenoid measurements to assess fruit and vegetable intake. Specifically, the novel use of functional magnetic resonance imaging, by characterizing the brain's responsiveness to an intervention, can help researchers develop programs with greater efficacy. Similarly, if eye-tracking technology can enable researchers to get a better sense as to how participants view materials, the materials may be better tailored to create an optimal impact. The latter 2 techniques reviewed, smartphone biosensors and methods to detect skin carotenoids, can provide the research community with portable, effective, nonbiased ways to assess dietary intake and quality and more in the field. The information gained from using these types of methodologies can improve the efficacy and assessment of behavior-based nutrition interventions. *Adv Nutr* 2017;8:113–25.

**Keywords:** community nutrition interventions, public health, nutrition assessment, program evaluation, brain responsiveness, smartphone, biosensors, eye-tracking, resonance Raman spectroscopy, reflective spectroscopy

## Introduction

Because of the current rates of obesity and related chronic diseases, effective behavior-based nutrition interventions are needed more than ever, along with methods to evaluate their impact. However, assessing the outcomes and impact from these interventions has remained challenging because of the lack of methods available beyond traditional nutrition assessment tools and techniques. The Social Ecological Model of Health Behavior (**Figure 1**) can be used as a framework to categorize different approaches to evaluating behavioral nutrition interventions.

***Individual-level evaluation.*** Traditional nutritional assessments involve anthropometric, biochemical, clinical, and dietary measurements (2). Anthropometry includes the measurement of bone, muscle, and adipose tissue in the human body (3). Although anthropometric data are crucial to the evaluation of trends over time, they have limitations. For example, skinfold measurements do not provide information on lean mass (4) and BMI does not distinguish between elevated adiposity or lean mass. Biochemical methods include both static tests, which measure either a nutrient in biological fluids or tissues

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**FIGURE 1** The Social Ecological Model provides a framework for considering what and how to evaluate the impact of a community-based nutrition intervention. Adapted from reference 1 with permission.

or the urinary excretion rate of the nutrient or its metabolite, and functional tests, which are used to detect the later stages in nutritional deficiency (2). Limitations include the following: *1*) no biochemical marker alone is an adequate screener for nutritional deficiency (5), *2*) trained personnel are needed to handle biological samples, and *3*) equipment and facilities are potentially expensive (2). Clinical assessments consist of medical history and physical examination to detect signs and symptoms associated with malnutrition (2). Because signs and symptoms are nonspecific and develop during the last stages of nutritional deficiencies, clinical methods depend on biochemical assessments for a comprehensive assessment. Other limitations include respondent bias when reporting medical history, signs produced by multiple nutrient deficiencies, and examiner inconsistencies (2). Individual dietary assessment methods measure food consumption (6). Dietary records, 24-h recalls, and FFQs are the most common (2). In dietary records, the act of recording may affect the foods consumed. In 24-h recalls and FFQs, measurement error can be substantial (6). Other limitations include under- or overreporting due to social bias (7) and participants' literacy (8).

In addition to various nutrition assessment methods, questionnaires are frequently used to collect psychosocial and health-related behaviors. This may include the evaluation of nutrition knowledge, attitudes, beliefs, intentions, self-efficacy, shopping and food-purchasing practices, food-label use, and cooking skills. The appropriateness and effectiveness of the use of questionnaires in evaluating nutrition interventions depend on the following: *1*) the instruments' validity and reliability, *2*) cost, *3*) feasibility, *4*) medium (e.g., paper, online), and *5*) the actual administration (self- or interviewer-administered) of the questionnaire. Respondent burden and comprehension are additional factors to consider (9).

***Interpersonal-level evaluation.*** Peer and family influences can be assessed by using self-report measures via questionnaires and direct observation or video records in laboratory or field settings, such as the home, school, or restaurants (10–14). When younger children or others are unable to complete assessments on their own, a caregiver may be used to collect the data. Data from these types of assessments are subject to comprehension, memory, bias, and recording errors and may reflect hypothetical rather than actual behaviors (15–17).

***Policy-, systems-, and environment-level evaluation.*** Dietary behaviors also reflect the structure of the physical environment typically assessed by using environmental scanning tools on the basis of observational methods, audits, and questionnaires. The availability, accessibility, and cost of various food and beverage options are measured in restaurants, worksites, supermarkets, corner stores, childcare facilities, schools, and homes (18–24). Training is required to ensure consistency in data collection. Tools need to be valid and reliable in specific populations and relevant to local contexts and criteria. These methods are labor intensive and time consuming, although the application of technology to both nutrition intervention and assessment is becoming increasingly common and helpful in reducing costs (25).

Although evaluation tools should be appropriately matched to a nutrition intervention, they must also be valid and reliable (26), feasible to implement, and cost-effective. At the individual level, more objective and individualized evaluation options beyond standard nutrition assessment tools for behavior-based nutrition interventions are needed. Therefore, the objective of this narrative review is to describe the current status of knowledge as it relates to 4 novel methods or tools to assess behavioral nutrition interventions at the individual level. Methods reviewed include the following: *1*) techniques to assess stress and stress responsiveness to enhance the evaluation of nutrition interventions, *2*) eye-tracking technology in nutritional interventions, *3*) smartphone biosensors to assess nutrition and health biochemical-related outcomes, and *4*) skin carotenoid measurements to assess vegetable and fruit (VF)[15] intake. These methods were chosen because of their innovative and evolving applicability to evaluating behavior-based nutrition interventions relevant in the nutrition field.

## Current Status of Knowledge

### Evaluating stress and stress responsiveness to enhance the evaluation of nutrition interventions

***Description and rationale.*** Poor or highly varied responsiveness to interventions aimed at changing nutritional behaviors continues to impede substantial progress in preventing obesity. Although there are several factors that potentially contribute to this variability, the assessment of stress responsiveness, the executive brain, and dietary flexibility provides a model for showing the power in embracing and understanding variability as it potentially applies to refining nutrition intervention strategies and interpreting intervention outcomes. Studies that use fMRI and other measures of stress are reviewed to show how investigating these central nervous system targets can help clarify and even predict behavioral intervention responsiveness.

Stress-response pathways are physiologically linked to and affect brain functions key to an individual's capacity for behavioral change. Understanding the mechanisms that underlie stress may help to improve the effectiveness of behavioral interventions. Acute stress is a transient, brain-derived physiologic response to a stimulus, referred to as the stressor, of a real or perceived, and possibly anticipated, threat to well-being. Chronic stress can lead to adaptive transformations in the brain and body, which trigger a new operational state (allostasis), including responsivity to new acute stressors. That is, chronic stress can alter the degree and direction of stress reactions and therefore the magnitude and type of disease risk and behavioral alteration. Chronic psychological stress and abnormally low or high stress system reactivity (e.g., in the autonomic nervous and neuroendocrine systems) can lead to and reinforce unhealthy habits (e.g., nutritional) and sabotage durable behavior change (e.g., dieting and weight-loss maintenance). These effects of chronic stress are likely due, in part, to the degradative effects of stress on the structure and function of the prefrontal cortex (27). This brain region plays a key role in mediating executive function, a group of cognitive functions that enable one to self-regulate and to make prudent decisions (e.g., short-term reward against long-term consequences). The less flexible or adaptable these functions become, the less receptive an individual will likely be to interventions that aim to change lifestyle nutrition habits.

***Stress system responsiveness, the executive brain, and dietary flexibility.*** Differences in chronic stress and acute stress reactivity have been tested to possibly explain interindividual differences in snacking and the brain's response to food cues. For example, in 2 parallel studies (28, 29), middle-aged women were observed snacking from a voluntary food buffet after a mental stress task (Trier Social Stress Test). Salivary cortisol, another technique to assess stress, was collected at home and during a laboratory visit in response to the Trier Social Stress Test to examine physiologic responsiveness to the tasks. Foods chosen and consumed were highly variable in these women. However, a specific stress phenotype characterized by higher chronic stress exposure, as determined by self-report from the Wheaton Chronic Stress Questionnaire (30), and stress-induced salivary cortisol hyporesponsiveness was associated with greater consumption of chocolate cake from the buffet (**Figure 2**).

In an attempt to further explain the association between this stress phenotype and high-calorie snacking, the brain's response was assessed by using fMRI to view high- and low-calorie food in women who showed a range of self-reported chronic stress exposure and stress-induced salivary cortisol responsiveness. In response to viewing pictures of high-calorie foods, compared with low-calorie foods and nonfood control images, women with more chronic stress who were hypocortisolemic showed enhanced activation in brain regions linked to emotionality (e.g., amygdala) and deactivation in executive brain regions (e.g., Brodmann's area 10; **Figure 3**).

The findings suggest that chronic stress may induce changes in the brain that bias habitual, emotion-based eating compared with goal-directed (executive) decisions about what and possibly how much to eat. Furthermore, these results suggest that phenotypic differences in stress exposure and salivary cortisol responsiveness mark adaptive changes in the brain that influence eating behavior and possibly responsiveness to interventions aimed at improving unhealthy eating habits. Together, chronic stress may lead to detrimental changes in the executive brain (27, 31), which makes it difficult to limit emotionally rewarding behaviors (e.g., eating) and durably change behavior even when people are informed of the health consequences.

Reports have shown a negative correlation between executive function, overeating, and obesity (32–34). This possible link between lower executive function and obesity may result from increased vulnerability to emotion-based overeating at a very early age. In support of this idea, a study in a research-based preschool was conducted to investigate the associations between executive function, emotional arousal, and eating in the absence of hunger (EAH) (35). Executive function was measured through child-completed tasks, parent questionnaires, and standardized teacher reports. Emotional arousal was measured via skin conductance before, during, and after the executive function and eating tasks. There is a direct biological connection between emotional arousal and elevated sympathetic nervous system activity in sweat glands, which leads to measurable changes in electrical conductance of the skin. Changes in skin conductance have long been used as a sensitive marker of emotional arousal (36). The EAH task was conducted after a standardized snack, and fullness was confirmed by the child.

[15] Abbreviations used: Ara h1, *Arachis hypogaea* allergen 1; EAH, eating in the absence of hunger; RRS, resonance Raman light-scattering spectroscopy; RS, pressure-mediated reflection spectroscopy; VF, vegetable and fruit.

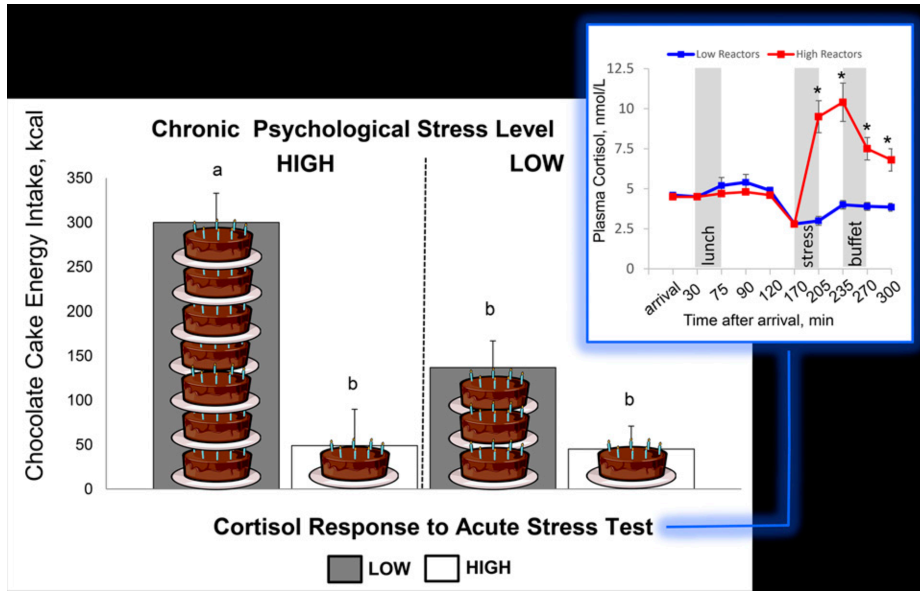


**FIGURE 2** Higher chronic stress exposure, as measured by the Wheaton Chronic Stress Questionnaire, and stress-induced cortisol hyporesponsiveness as associated with greater consumption of highly palatable food (e.g., chocolate cake) from a voluntary snack food buffet. Chocolate cake intake data were adjusted for total energy intake from the buffet. Salivary cortisol was collected at home and during a laboratory visit in response to a standard meal challenge and mental stress test. Low and high chronic stress are indicated above the graphs. Salivary cortisol reactivity is indicated within and below the graph by gray (low reactivity) and white (high reactivity) bars. Means in the main panel without a common letter differ, $P < 0.05$. In the inset, salivary cortisol concentrations during the visit are presented to show low (blue) and high (red) cortisol reactivity. *Different from low reactors, $P < 0.05$. Adapted from reference 28 with permission.

At first glance, no relation was observed between emotionality and EAH. However, increased emotional arousal was associated with increased EAH but only in a subgroup of children who had a lower capacity for emotional regulation as suggested by lower performance on executive function tasks, lower effortful control, and overall lower teacher-reported cognitive development.

*Reliability and validity.* Brain imaging is an accepted technique for examining task-driven changes in regional brain activity, and this imaging technique has been successfully applied to nutrition research (37). Although there has been some dispute about the statistical approaches, brain imaging methods such as fMRI are powerful approaches for examining regional brain activity. In addition, the measurement of salivary cortisol is an accepted method for determining the cortisol response to mental and physical stressors, as well as diurnal fluctuations of cortisol. Finally, changes in skin conductance have long been used as a sensitive marker of emotional arousal (36).

*Advantages and limitations.* Examining markers of stress, which can affect executive functioning, may provide deeper and more useful insight into variable intervention responsiveness and possibly a predictive biomarker of responsiveness to interventions aimed at changing behavior. Interventions aimed at reducing stress (e.g., mindfulness, meditation) and/or strengthening executive function may help improve nutritional habits in some people (38, 39). Although current tools, such as fMRI and neuroendocrine and autonomic nervous system evaluation, are limited to smaller studies and the availability of specialized equipment, their use in subgroups of responders and nonresponders may facilitate new ideas about how to create more effective interventions.

### Eye-tracking technology and evaluating nutrition interventions

*Description.* Eye-tracking is becoming increasingly popular in nutrition research to assess which consumers use nutrition information when and how they make food choices (40–42). Eye-tracking cameras objectively measure an individual's gaze, providing a reliable measure of attention (43, 44). Cameras may be integrated into glasses, allowing for mobility through environments such as supermarkets [e.g. (45, 46)]; alternatively, desk-mounted eye-trackers are used with electronic presentations of food images [e.g. (47–51)]. In addition to specifying precisely where individuals are looking, eye-trackers provide information, including how many times

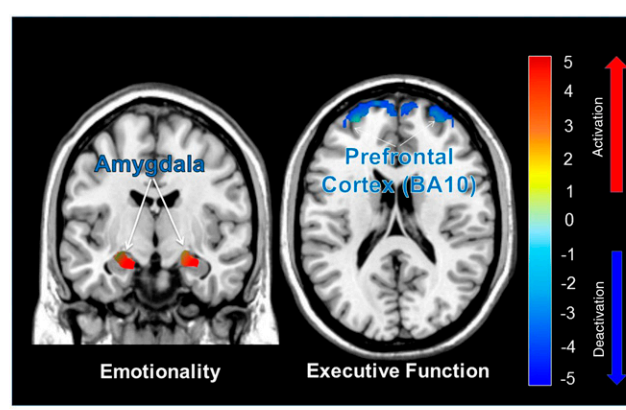


**FIGURE 3** In response to viewing pictures of high-calorie foods, compared with low-calorie foods and nonfood control images, women with more chronic stress and hypocortisolemia showed enhanced activation in brain regions linked to emotionality (e.g., amygdala) and deactivation in executive brain regions (e.g., Brodmann's area 10). BA10, Brodmann's area 10. Adapted from reference 28 with permission.

a given area of interest is viewed, for how long, and in what order. In addition, eye-trackers measure pupil diameter, another objective measure in nutrition research, because pupil diameter is not very amendable to conscious cognitive control and increases in response to arousing stimuli (52), such as desired foods.

Eye-tracking is useful not only for comprehending an individual's attention but also for understanding higher-order cognitive processes revealed through visual attention (53–56). For example, the number of times an area of interest is viewed is related to information processing and to the information's importance to the viewer (56). In addition, attention to various food-related stimuli (e.g., packages, labels, advertisements) predicts highly consequential outcomes, including brand memory, preference, and consumption (49, 55, 57–69). Indeed, highly visually salient products may be more likely to be chosen independently of consumers' preferences (70); thus, visual attention can be a better predictor of food choice than even liking of available foods.

***Rationale.*** Eye-tracking research can help policymakers and researchers design, implement, and evaluate effective nutrition policies and programs in areas including *1*) nutrition labeling, *2*) food marketing, and *3*) food attitudes and disordered eating.

**Nutrition labeling.** Nutrition labeling policy has the potential to improve the dietary behavior of many consumers due to the tremendous reach of labels (71). Eye-tracking can clarify how consumers attend to labels (**Figure 4**) and identify ways to increase consumer attention to nutrition information (40). Several nutrition label components and characteristics related to visual attention have been identified (40), including the following: label location and size (e.g., larger, more prominent labels receive more attention); simplicity, design, and saliency; position of nutrients on label; and visual clutter surrounding the label (72–74).

Eye-tracking has also been useful for evaluating existing policy labeling proposals (46, 75–77). For example, front-of-package nutrition labels receive more attention than back- and side-of-package nutrition information (46, 78), suggesting that requiring nutrition information to be displayed on package fronts would substantially increase consumer attention to this information. Furthermore, "traffic light" labels that use red, amber, and green coloring to visually convey nutritional information receive more attention and are more helpful for consumers than are monochromatic labels (49, 79–81). Similar nutrition policy issues (e.g., calorie labeling on menus) could benefit from eye-tracking research.

Consumer motivation also affects attention to nutrition information (47, 49, 82, 83). Interventions to increase consumer motivation to eat healthfully or to provide nutrition education could also benefit by using visual attention data to optimize communication strategies, such as educational signage, public service announcements, etc. It is, however, important to note that despite the ability of attention to predict product choice, greater attention to nutrition information does not always predict healthier choices (84).

**Food marketing.** Eye-tracking can also be used to increase consumer attention to healthful options. Frequently, stimuli compete for attention in food choice settings (e.g., supermarket shelves, online retailers); thus, gaining and retaining attention are challenging (55). Eye-tracking research can identify ways in which healthful options can be more attractively advertised, packaged, located, and presented. Increasing the ability of healthful options to attract consumer attention is critical because the most-viewed products are selected most (49, 63–66, 70). This visual saliency bias is particularly pronounced under conditions that typify many supermarket purchases—high cognitive load and rapid decision making (70).

Attention to advertising predicts food choice (85) and is affected by visual factors, including the spatial location of marketing stimuli, color, lines and edges, movement, size, and context of ad components. Similarly, modifying package characteristics, such as contour and shape, contrast, simplicity, and ratio, or including familiar or liked stimuli (e.g., cartoon characters) can increase attention (86, 87), which is important because most products receive no visual attention, thus failing to be considered as potential purchases (45). In addition, a product's location within a store or an online array affects whether and how much it is viewed and its likelihood of being chosen (63, 88). These location effects can be large: for example, in a $3 \times 3$ array, the product in the center receives more than double the visual attention and is 60% more likely to be chosen than peripheral items (63). Finally, food products themselves can be modified (e.g., via coloring) to attract more attention, increasing their likelihood of being chosen (65, 89).

**Food attitudes and disordered eating.** Eye-tracking data can identify attentional biases that reveal implicit food attitudes and can guide intervention efforts aimed at modifying diet by overcoming these biases. Whereas total attention and attention deployed later in visual tasks are likely to show controlled, explicit processing, attention during early stages of visual processing and changes in pupil dilation can reflect automatic, implicit responses (90, 91). Eye-tracking data can thus provide insights unavailable through traditional self-report measures.

Individuals with eating disorders show attentional avoidance of food information that increases with disorder severity (92). Individuals with (compared with those without) eating disorders also spend substantially more time looking at body and weight stimuli (93). These attentional patterns can cause and exacerbate body dissatisfaction and/or dietary restriction (94); however, these attentional biases can be overcome through training (94). Attentional training may also help to reduce impulsive eating or overeating because biased attention may be a key cognitive mechanism by which the food environment promotes overeating (95, 96). Although attentional bias for food is difficult to modify (97) and appears among healthy-weight as well as overweight individuals (98), individuals with problematic eating behaviors may be able to train themselves to deploy patterns of visual attention that reduce their susceptibility to particular foods (63, 99, 100).

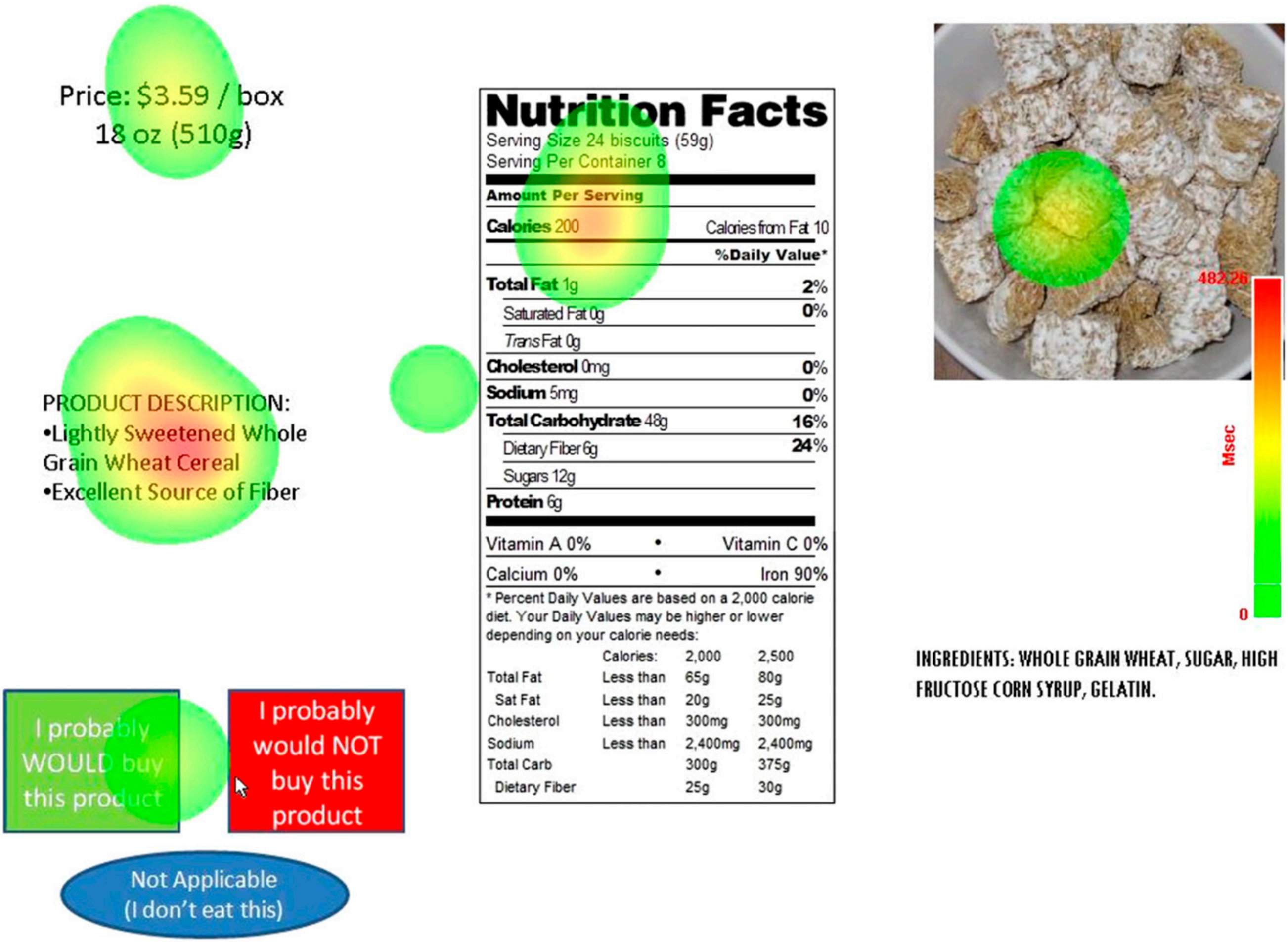


**FIGURE 4** Heat map showing visual attention aggregated via an eye-tracking camera. Warmer colors (e.g., red) on the heat map represent higher concentrations of visual attention.

***Reliability and validity.*** Because eye movements are the behavioral manifestation of the underlying process of visual attention (55), eye-tracking is a reliable and valid measure of visual attention to the extent that actual eye movements are consistently and accurately measured by the eye-tracking equipment. In the eye-tracking literature, reliability and validity are discussed in terms of precision (eye-tracker's ability to reliably reproduce a measurement) and accuracy (difference between tracker-estimated gaze and actual gaze position), respectively (101). Although the accuracy and precision of eye-trackers varies, and at present all systems are subject to some degree of error, the accuracy and precision of research-grade systems are high and improving according to third-party estimates reported in a recent comparative analysis (101).

***Advantages and limitations.*** Eye-tracking provides an objective attention measure that is not subject to self-report biases and elucidates decision-making processes that can occur without conscious awareness and which consumers may later fail to recall (102). It provides novel opportunities in nutrition research to optimize food labeling, food marketing, and dietary interventions.

Although there are many novel contributions that eye-tracking can make to nutrition research, it has limitations. Eye-tracking can be expensive, and trained researchers are needed to operate and calibrate equipment. In addition, eye tracking may itself affect behavior. Some eye-trackers require users to remain stationary and may use a head-and/or chinrest. Eye-tracking technology may prompt individuals to behave unnaturally because others can see where they are looking (i.e., users may intentionally view healthier foods or nutrition information). However, eye-tracking's impact on behavior may be smaller than the impact of self-report bias (e.g., visual attention to nutrition information typically does not reach the high self-reported levels) (50). Finally, it is not always obvious what the amount of viewing conveys (40, 86).

### Smartphone biosensors to assess nutrition and health biochemical-related outcomes

***Description and rationale.*** There are several important challenges in nutrition that could benefit from the development of detection instruments and tests that can be performed in the field. This is particularly relevant to the collection of dietary data as a primary marker for intervention success. Broad classes of tests range from extremely simple analyses that could be performed by consumers at home or in restaurants to those requiring the skill of trained technicians. Specific to nutrition interventions, mobile diagnostic tests could be used to potentially monitor the nutritional status of people. A variety of chemical and biomolecular analytes are commonly used in clinical trials to monitor the effects of specific diets on study participants that include the measurement of concentrations of minerals (e.g., iron and magnesium), vitamins, and metabolites (e.g., urea and creatinine). Advanced testing can consider the role of diet on the concentration of soluble proteins and gene expression as measured by monitoring the presence of specific sequences of circulating mRNA and DNA.

Laboratory-based tests for each of the analytes mentioned are currently available in which an assay protocol is performed on a person's fluid sample (e.g., serum or saliva), which results in a liquid-based chemical reaction that causes a liquid to change color. The most commonly used assay method is the ELISA. Although ELISA assays offer excellent specificity, and thousands of ELISA test kits have been commercially developed, they require a complex test protocol and an expensive ($5000–$50,000) laboratory instrument. Smartphone biosensors could serve as a less expensive alternative.

***Reliability and validity.*** Smartphone biodetection capability is provided by placing an optical diffraction grating in front of the back-facing camera, which disperses the wavelength components of light passing through the grating so that a "rainbow" appears as the captured image, as shown in **Figure 5B**. For the diffraction grating to function correctly, the light incident on it must be collimated (i.e., neither focused nor spreading out), which is achieved by having the light enter the system as a single point source, and then passing the light from that source through a collimating lens (103). The point source may simply be a small hole in an opaque object, or the light that emits from the tip of an optical fiber. To use a smartphone camera as an absorption spectrometer for measuring the colored liquid of an ELISA, a white light source is first passed through the colored test sample, and next is gathered into an optical fiber that, when the light emerges from the opposite end, passes through the lens and diffraction grating before entering the camera. Similarly, to measure the fluorescent spectrum from the light emitted by a fluorescent assay, a laser (e.g., a green laser pointer) illuminates the test sample, and a portion of the light is gathered into the optical fiber.

The system shown in Figure 5A, B can be used to measure the output of assays used in food and nutrition analysis with the same resolution and laboratory-based systems, although it is handheld and contains only ~$300 of components in a three-dimensional–printed plastic enclosure. For point-of-use applications, the data-gathering capability of the system could be used to communicate and share data with a smart service system that can facilitate epidemiologic studies, track the spread of pathogens, monitor societal trends, and provide feedback from physicians.

Figure 5C summarizes the dose-response characterization of the peanut allergen *Arachis hypogaea* allergen 1 (Ara h1) measured in water extract from cookies (104) by an ELISA in which the smartphone spectrometer provided identical limits of detection as a laboratory instrument. Similar results have been obtained for the detection of protein biomarkers for inflammation, cardiac health, and early term pregnancy. The same system, slightly adapted, can perform the detection of a fluorescent assay, in which sensing of an mRNA sequence that is specific to a strain of bacteria, where a fluorescence resonance energy transfer molecular beacon probe is used to indicate the presence of the target molecule in a liquid test sample (105). In this case, the smartphone-based system showed even lower detection limits than a laboratory fluorimeter.

***Advantages and limitations.*** Although these experiments show the powerful capabilities of smartphone cameras for performing accurate molecular analysis that has primarily been the domain of expensive laboratory instruments, for point-of-use testing to become widely adopted for applications in food and nutrition the assays themselves must be automated. Researchers are currently working on the development of plastic cartridges in which the necessary reagents are "printed" into small fluid compartments so they are activated by exposure to the test sample. Combined with innovations in the fields of microfluidics and molecular biology methods, many diagnostic tests can be simplified to 1 or 2 simple steps that can be performed with minimal training. Together, these systems have the potential to revolutionize the ability to monitor the nutritional status of people without convenient access to laboratory- or hospital-based diagnostic facilities.

### Skin carotenoid measurements to assess VF intake

***Rationale.*** As previously discussed, one difficulty in measuring the effectiveness of nutrition interventions to improve diet is in the primary outcome assessment: change in intake. Carotenoids are appealing as biomarkers of VF intake because they are found predominantly in VFs and are not synthesized in the body, and they are also readily deposited into body tissues (106, 107), including skin (108, 109). The major dietary carotenoids are α- and β-carotene, lycopene, β-cryptoxanthin, and the xanthophylls lutein and zeaxanthin (110). Blood carotenoid concentrations are considered the best biomarker of VF intake (110) but are invasive and expensive to measure. Skin carotenoid status is a method of assessing VF intake that is noninvasive and is becoming more available and acceptable for research use, particularly for assessing VF interventions. Currently, there are 2 optical methods of assessing skin carotenoid status for nutritional studies: resonance Raman light-scattering spectroscopy (RRS) and pressure-mediated reflection spectroscopy (RS). Each method is discussed below.

***Description.***

**RRS.** The potential uses of measuring Raman scattering was first recognized by Sir CV Raman in 1928 as he watched

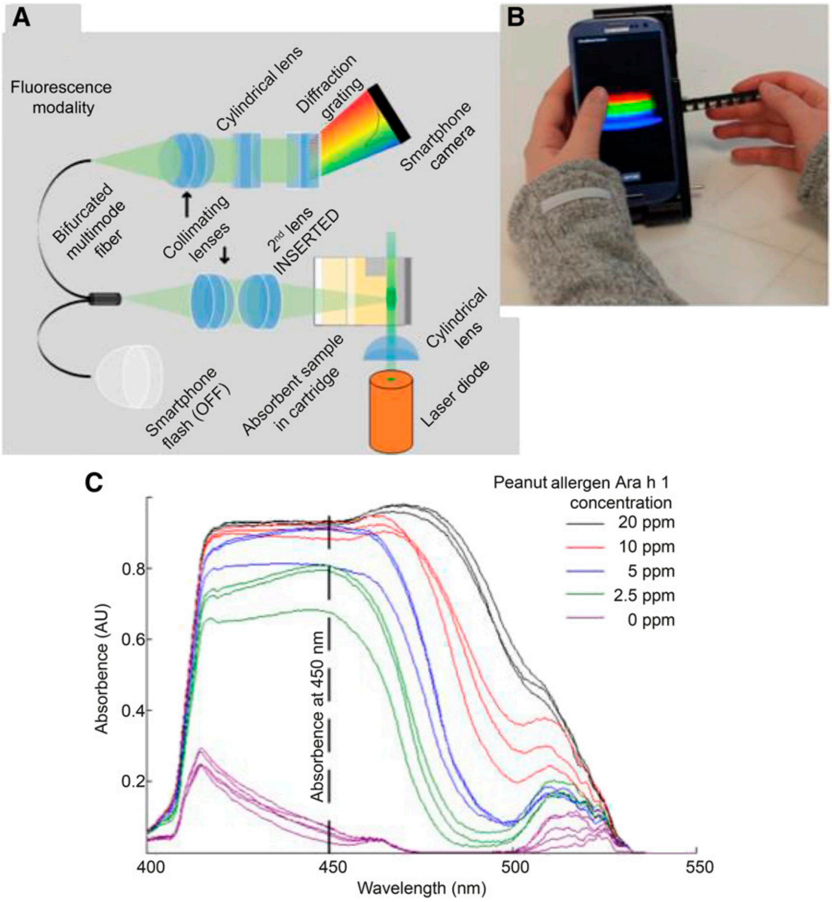


**FIGURE 5** Schematic diagram (A) and photo (B) of a cradle that interfaces with the back-facing camera of a smartphone that enables the camera to function as an optical spectrometer. (C) Absorption spectra for a series of ELISA assays taken with the system for the detection of peanut allergen Ara h1, which shows increasing absorption over a wide band of wavelengths with increasing Ara h1 concentration. Ara h 1, *Arachis hypogaea* allergen 1.

sunlight scattering off ocean waves. RRS is now a technique that is used to observe vibrations of molecules in vivo. It was subsequently adapted for use to assess carotenoid status in the skin and in the macula of the eye (111–116). The carotenoid detection device uses a 488-nm blue-light laser to excite tissue carotenoids, causing their long carbon double-bond backbones to vibrate. Light backscattered from the skin is routed to a spectrograph interfaced with a cooled charge-coupled detector array. The recorded spectrum is then analyzed for Raman response of the skin total carotenoids. Although the surface skin at any anthropometric location may be measured, validation studies have measured at the palm of the hand, an easily accessible location where the stratum corneum is thick and melanin content is lighter and less variable among different race-ethnic groups. Units are reported as Raman counts, or Raman intensities.

**RS.** RS is another method of measuring skin carotenoid status (117, 118). Instead of a laser, the RS methodology uses a broadband white light to excite carotenoid molecules in the 460–500 nm spectral window. The light then is routed to a spectrograph coupled with a cooled charge-coupled detector array. Current commercially available technology takes measurements at the fingers. The subject applies pressure against a lens to temporarily squeeze blood out of the measured tissue, which reduces the potentially confounding effects of chromophores such as oxygenated hemoglobin. The device also adjusts for the potentially confounding effects of melanin. These devices (Figure 6) are approximately the size of a toaster, are light and portable, and cost ~$15,000. They do not require trained personnel or advanced data-processing techniques.

***Reliability and validity.*** A recent review evaluated the use of RRS as a biomarker of carotenoid status in humans (119). Ermakov et al. (112, 113) developed the RRS method for skin carotenoid detection and found that Raman intensity scores are widely distributed among individuals and correlated with excised strata cornea (114) and blood carotenoids ($r$ = 0.78, $P$ < 0.001) (112). Mayne and colleagues conducted a series of experiments to evaluate skin carotenoid status as measured by RRS (120–122). They showed that skin biopsy and plasma carotenoid concentrations were significantly correlated (121). To assess reliability, they tested several areas of the body and reported an intraclass correlation coefficient of 0.97 for the palm of the hand over a period of 6 mo, indicating that the RRS intensities in the palm are stable over time during a self-selected diet (122). To assess validity, they compared RRS total carotenoid measurements with both skin biopsy results and blood carotenoids and found significant correlation coefficients of $r$ = 0.66 ($P$ = 0.0001) and 0.062 ($P$ = 0.006), respectively, and they concluded that skin carotenoid status was a valid and reliable proxy for blood carotenoids (122). They also compared skin carotenoid intensities with dietary carotenoids measured by a

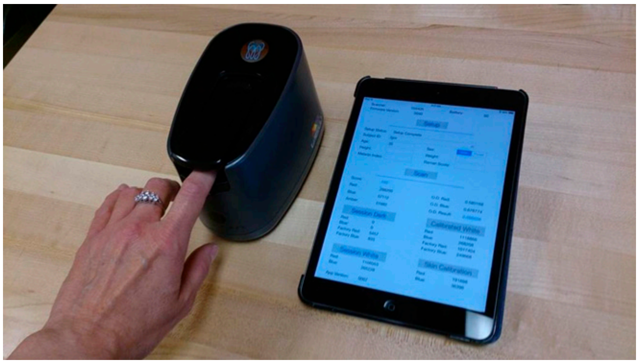

**FIGURE 6** Pressure-mediated reflection spectrometer (the "Veggie Meter").

self-reported FFQ and reported a significant correlation coefficient of $r = 0.52$ ($P < 0.001$) (122). In further research, they reported reasonable agreement between quartiles of intake over 6 mo (κ = 0.80) and that intake of carotenoids, race-ethnicity, season of measurement, and recent sun exposure exerted some influence at baseline and/or over time (120).

The next step was to evaluate whether skin carotenoid status is a valid biomarker of change in VF intake. Jahns et al. (123) conducted a community-based 28-wk, single-arm experimental feeding intervention in 29 men and women designed to compare changes in skin carotenoid status with changes in plasma carotenoid concentrations during a controlled feeding intervention with varying levels of carotenoid intake from foods. The intervention was conducted in 4 phases: during phases 1 and 3, participants were asked to follow a low-carotenoid diet for 6 wk (depletion phases); in phase 2, they consumed a provided high-VF diet for 8 wk (repletion phase); and finally in phase 4, they returned to a self-selected diet but were followed for a final 8 wk (natural repletion phase). Skin carotenoid status was measured by using RRS ≥2 times/wk during phases 1, 3, and 4 and each weekday during phase 2. Blood carotenoids were measured at baseline and the mid- and endpoints of each phase. Both skin and blood carotenoids decreased during each depletion phase and increased during the repletion phase (**Figure 7A, B**). Overall, the within-individual correlation was $r = 0.70$ ($P < 0.001$) between skin and blood carotenoid concentrations and the between-individual correlation was $r = 0.72$ ($P < 0.001$). This study showed that, at least in a high-carotenoid VF intervention, skin carotenoids are a reliable biomarker of change in VF intake.

Early work with RS for skin carotenoid detection found that measurements responded well to the consumption of a β-carotene supplement (118). Ermakov and Gellermann (124) refined the technology and compared RS measurements in the thumb with excised stratum corneum carotenoids and reported that the measurements were similar. They also found that RS correlated with RRS and was responsive to a juice intervention (117). RS measurement of skin carotenoids is a relatively recent development; therefore, it has not been as thoroughly verified as RRS. However, it is highly correlated with RRS.

***Advantages and limitations.*** It is important to remember that skin carotenoid measurement, like blood carotenoid concentration, is a biomarker of carotenoid status and is affected by many factors in addition to VF intake. Single nucleotide polymorphisms in genes associated with the absorption, transport, and metabolism of carotenoids are known to affect tissue concentrations (125). Race/ethnicity may affect either the skin carotenoid measurements or reflect differences in metabolism of carotenoids. Season and recent sun exposure may influence skin carotenoid status (120), although some studies have not found this relation (126). Other potential confounders include smoking, illness, stress, and alcohol consumption (127, 128). In addition, the sensitivity of the method has not been determined. Strengths of skin carotenoids as a measure of change in VF intake include being safe and noninvasive, having immediate results, and in the case of RS, being portable and easy to use, without requiring intensive

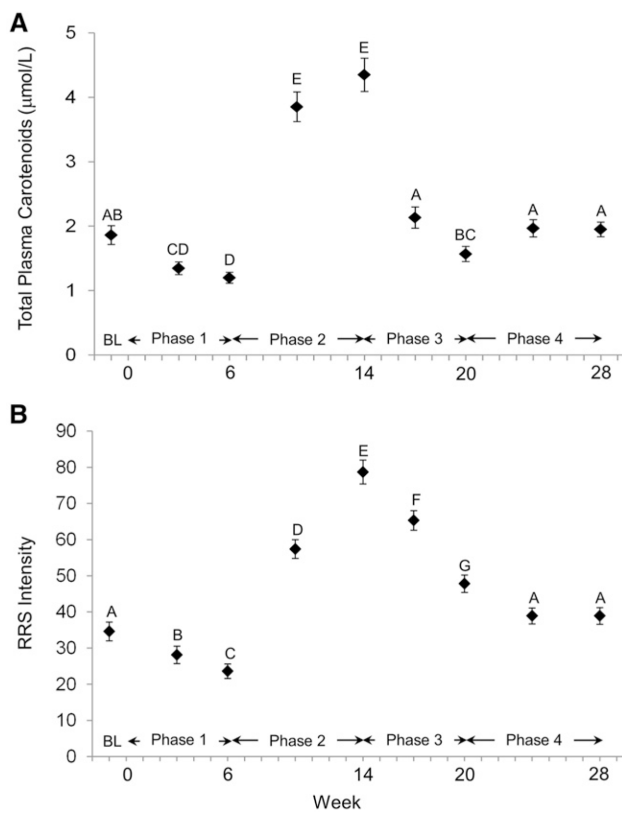


**FIGURE 7** (A) Plasma carotenoid concentrations in men and women ($n = 29$) assessed by HPLC at baseline and at the mid- and endpoints of each phase of the study (phase 1: depletion of carotenoid-rich foods; phase 2: experimental feeding; phase 3: second depletion; phase 4: return to usual diet). (B) RRS intensities at the same 9 time points. Values are means ± SEMs, $n = 29$. Repeated-measures ANOVA, followed by Tukey contrasts for post hoc comparisons of means, was used to test for changes over phases of the study in plasma total carotenoid concentrations and RRS intensities. Means not sharing a common letter differ, $P < 0.05$. BL, baseline; RRS, resonance Raman light-scattering spectroscopy. Adapted from reference 123 with permission.

training. Skin carotenoid status has several qualities of a good biomarker. It has good interindividual variation among both children and adults; is correlated with skin biopsy, blood carotenoid concentrations, and self-reported dietary intake; and is repeatable, which makes it an ideal tool for both observational and intervention studies of VF intake.

## Conclusions

This review provides a brief overview of 4 novel tools and techniques that can be used to evaluate or improve the assessment of behavioral nutrition-related outcomes. Although each method has its own advantages and disadvantages, they offer new options for evaluating behavioral nutrition interventions either alone or in conjunction with traditional assessment methods. For example, although it may not be feasible to scan many participants in an fMRI, by characterizing the brain's responsiveness to intervention, researchers can develop programs with greater efficacy. Similarly, with eye-tracking technology, if researchers can get a better sense as to how participants view materials, they may better tailor materials to create an optimal impact. The latter 2 techniques discussed, the use of smartphone biosensors and detection of skin carotenoids, provide the research community with portable, effective, nonbiased ways to assess dietary intake and quality and other variables in the field.

Overall, these novel tools provide an opportunity to utilize more objective measures of individual nutrition behavior changes that can be applied in a community or a subset of a community population. Furthermore, because some of these methods or tools were originally developed in fields outside of nutrition, this review also provides insight into potential interdisciplinary collaborations for assessing behavioral nutrition outcomes in the community.

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