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Review

Resonance Raman spectroscopic evaluation of skin carotenoids as a biomarker of carotenoid status for human studies

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ABSTRACT

Resonance Raman spectroscopy (RRS) is a non-invasive method that has been developed to assess carotenoid status in human tissues including human skin *in vivo*. Skin carotenoid status has been suggested as a promising biomarker for human studies. This manuscript describes research done relevant to the development of this biomarker, including its reproducibility, validity, feasibility for use in field settings, and factors that affect the biomarker such as diet, smoking, and adiposity. Recent studies have evaluated the response of the biomarker to controlled carotenoid interventions, both supplement-based and dietary [e.g., provision of a high-carotenoid fruit and vegetable (F/V)-enriched diet], demonstrating consistent response to intervention. The totality of evidence supports the use of skin carotenoid status as an objective biomarker of F/V intake, although in the cross-sectional setting, diet explains only some of the variation in this biomarker. However, this limitation is also a strength in that skin carotenoids may effectively serve as an integrated biomarker of health, with higher status reflecting greater F/V intake, lack of smoking, and lack of adiposity. Thus, this biomarker holds promise as both a health biomarker and an objective indicator of F/V intake, supporting its further development and utilization for medical and public health purposes.

Introduction to skin carotenoids: health effects

Carotenoids accumulate in human skin, with the levels of carotenoids reflecting dietary intake and bioavailability from food sources [1]. The most common carotenoids in the Western diet are alpha-carotene, beta-carotene, beta-cryptoxanthin, lycopene, lutein, and zeaxanthin [2]. After absorption in the intestine, carotenoids are transported through the bloodstream by lipoproteins to various target tissues [3,4], including skin. Cholesterol transporters, such as scavenger receptor class B1 type 1 protein (SR-B1)[1] and cluster of differentiation 36 membrane protein (CD 36), appear to facilitate absorption of carotenoids in the intestine [5], and these transporters may also facilitate carotenoid absorption in the epidermal layers of the skin [6]. Some have suggested that sweat and sebum may also transport carotenoids to the skin surface, allowing the carotenoids to subsequently penetrate back into the skin [7]. Carotenoids are lipophilic molecules found in many tissues, including skin – especially in skin sites where the stratum corneum, the upper-most skin layer, is thick [8]. Body sites highest in total skin carotenoid levels include the sole of the foot, forehead, and palm of the hand [9] (e.g., vegetarians are commonly noted to have "yellow" palms due to visible carotenoid accumulation). All of the major carotenoids have been detected in human skin using chemical (e.g., HPLC) analysis [8,10].

There has been and continues to be considerable interest in possible health effects of carotenoids in skin as reviewed elsewhere [11–13]. Arguably the best-studied potential health effect of carotenoids beyond a role in provitamin A activity is a role in photoprotection, that is, the protection against erythema and sunlight damage [11–15]. Beta-carotene has established efficacy in the treatment of erythropoietic protoporphyria, a photosensitivity disease [16,17]. In humans without this disease, there is also evidence from controlled studies that carotenoids such as beta-carotene have efficacy in the protection from sunburn [18], although the

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[1] *Abbreviations used*: RRS, Resonance Raman spectroscopy; SR-B1, scavenger receptor class B1; CD 36, cluster of differentiation 36; SPF, sun protection factor; ICCs, intraclass correlation coefficients; BMI, body mass index; SNPs, single nucleotide polymorphisms.

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sun protection factor (SPF) is modest (i.e., approximately equal to 2). Carotenoids are known to quench singlet oxygen and other free radical species, which are generated in the skin by exposure to UVA and can cause skin damage [16]. Several recent studies have examined the potential protective effects of carotenoids against premature photoaging of the skin, marked by signs such as wrinkling, pigmentation, dryness, and inelasticity. There is suggestive evidence for a protective effect of beta-carotene on photoaging [19]. It has also been suggested that other carotenoids, such as lycopene [20] and astaxanthin [21] may also protect against photoaging. Tobacco smoking, which also generates free radical species, is another cause of premature skin aging [22], further suggesting that free radical quenching ability of carotenoids may contribute to photoprotection/reducing premature skin aging.

Introduction to skin carotenoids as a biomarker of fruit and vegetable intake

Not only is skin carotenoid assessment of interest for studying the direct effects of carotenoids on skin, but there is great interest in assessing skin carotenoids as a biomarker of fruit and vegetable intake. Higher relative to lower fruit and vegetable intake has been associated with a reduction in the risk of a number of chronic diseases, including various cancers [23], cardiovascular disease [24], age-related degenerative diseases [25], and obesity [26]. Many countries, including the U.S., are supporting interventions to increase fruit and vegetable intake, and the recent *Dietary Guidelines for Americans 2010* [27] recommendation states that individuals should "increase vegetable and fruit intake." In order to identify populations at particular risk for inadequate intake of fruits and vegetables, and to evaluate the success of interventions aimed at increasing fruit and vegetable intake, objective indicators of fruit and vegetable intake are critically needed. This is especially true in studies involving children, where it is extraordinarily difficult to obtain valid dietary intake data [28]. Parents and caregivers typically do not observe all meals consumed, and young children lack the cognitive ability to self-report diet.

Fruits and vegetables are concentrated sources of carotenoids. Carotenoids can be measured in blood and in other tissues, and levels in blood and tissues are correlated with dietary intake of both total and specific carotenoids [29]. Plasma concentrations of carotenoids also increase significantly in response to fruit/vegetable behavioral interventions [30,31]. Given their widespread distribution in fruits and vegetables, carotenoids have been used as an objective biomarker of fruit and vegetable intake. The National Academy of Sciences [2] stated "blood concentrations of carotenoids are the best biological markers for consumption of fruits and vegetables."

Thus, blood concentrations of carotenoids can and have served as concentration biomarkers of fruit and vegetable intake, and are used in nutritional surveillance (e.g., NHANES biochemical components), in observational research studies, and as a marker of adherence in relevant behavioral interventions. Concentration biomarkers can be used to calibrate and correct for measurement error in self-reported nutrient intake data, improving the quality of dietary exposure data for epidemiologic research [32]. Despite these advantages of blood carotenoids as a biomarker, there are real and significant disadvantages to the use of plasma or serum carotenoid concentrations, including cost (of sample collection, processing, storage, and chemical analysis, usually by HPLC); the need for study participants to agree to submit to venipuncture, which may introduce participation bias; sample lability during processing and analysis; and the relatively short half-life of carotenoids in blood [33]. Adipose tissue is thought to be a more stable depot of carotenoids [34], and a few epidemiologic studies have utilized adipose tissue to assess carotenoid status. However, this approach requires biopsies and extensive sample preparation prior to HPLC analysis (to remove lipid) and thus is even more prohibitive to use in the setting of large population studies. Despite the costs and other limitations, blood carotenoids have been the biomarker of choice for fruit and vegetable intake for human studies.

Noninvasive assessment of skin carotenoids

As described above, skin carotenoids are of current interest as a biomarker associated with better health, as well as a potential biomarker of fruit and vegetable intake. Both of these areas of research would be greatly facilitated by the availability of noninvasive approaches to rapidly assess carotenoid status in living human skin. Because of some of the unique properties of carotenoids, both reflectance based methods [35] as well as methods utilizing resonance Raman light scattering spectroscopy (RRS) have been evaluated.

RRS is a form of laser spectroscopy that detects the characteristic vibrational/rotational energy levels of a molecule. Carotenoids are particularly well suited to RRS, as all have a conjugated carbon backbone molecular structure, strongly absorbing in the blue wavelength region and thus providing the basis for efficient resonant laser excitation of the molecules with visible laser lines. The backbone consists of alternating carbon double- and single-bonds, with the conjugation length differing between particular carotenoid species. The stretch vibration frequencies of the carbon double and single bonds can be detected with RRS, where they appear as sharp spectral lines that are shifted by the vibration frequencies relative to the frequency of the excitation laser [9]. In homogeneous, optically thin solvent systems, the intensity of the resonance Raman scattered light is linearly related to the carotenoid concentration, thus serving as an optical measure for carotenoid content.

These light scattering properties have led us to explore the use of RRS for the non-invasive quantitative optical measurement of carotenoids and their spatial distributions in living human tissue, initially in the human macula (retina) [36–38], and shortly after also in human skin [8,9,39–41] and oral mucosal tissue [9]. Typical spectra for skin are shown in Fig. 1 and compared with an RRS spectrum for a beta-carotene solution. A growing number of studies, done by our group as well as other research groups over the past several years, support the promise of this approach. Below we summarize what is known about skin carotenoid status as assessed by RRS, including reproducibility, validity, feasibility of use in field settings, and factors known to affect skin carotenoid status. We conclude with a discussion of future research needs with regard to assessment of skin carotenoid status.

Biomarker development: intra- versus inter-subject variability

An early step in the development of any biomarker is characterizing the intra- (within) subject variability as well as the inter- (between) subject variability. Ideal biomarkers would vary widely across different individuals within a population, but be relatively constant over time within an individual. To understand the variability in skin carotenoids as assessed by RRS, we conducted two initial surveys in which we recruited 57 and 1375 healthy adults, respectively, and measured the carotenoid status by RRS, choosing the palm of the hand as a convenient tissue site. Wide distributions were evident, with large variation throughout the populations [9,42]. More recently, 74 healthy adults were recruited and their carotenoid status assessed by RRS longitudinally six times over the ensuing six months [43]. As shown in Fig. 2a, we observed again a wide distribution of skin carotenoid status that was nearly