Compendium of Abstracts
Raman detection of carotenoids in tissues

Raman spectroscopy in the Eye

Study reference:

ABSTRACT

We describe resonance Raman imaging (RRI) of macular pigment (MP) distributions in the living human eye. MP consists of the antioxidant carotenoid compounds lutein and zeaxanthin, is typically present in high concentrations in the healthy human macula relative to the peripheral retina, and is thought to protect this important central region from age-related macular degeneration. We demonstrate that RRI is capable of quantifying and imaging the spatially strongly varying MP distribution in the human retina. Using laser excitation of the MP molecules at 488nm, and sequential camera detection of light emitted back from the retina at the MP's strongest Raman peak position and at an off-peak position, RRI maps of MP are obtained at a resolution below 50microm within a fraction of a second per exposure. RRI imaging can be carried out with undilated pupils and provides a highly molecule-specific diagnostic imaging approach for MP distributions in human subjects.

Study reference:

ABSTRACT

There is growing evidence that high levels of the macular xanthophyll carotenoids lutein and zeaxanthin may be protective against visual loss from age-related macular degeneration. To study this protective effect further, it is important to measure macular carotenoid levels noninvasively in a wide variety of subjects. We have developed and validated resonance Raman spectroscopy as a sensitive and specific objective method to measure macular carotenoid levels in the living human eye. In this minireview, the principles and implementation of ocular carotenoid resonance Raman spectroscopy are reviewed, and the results of observational cross-sectional studies and of prospective supplementation studies on subjects with and without macular pathology are summarized. We have recently extended this technology to an imaging mode which will further enhance our understanding of the roles of lutein and zeaxanthin in normal macular function and in the prevention of age-related visual loss.
ABSTRACT

PURPOSE: Dietary carotenoids lutein and zeaxanthin may play a protective role against visual loss from age-related macular degeneration (AMD) through antioxidant and light screening mechanisms. We used a novel noninvasive objective method to quantify lutein and zeaxanthin in the human macula using resonance Raman spectroscopy and compared macular pigment levels in AMD and normal subjects. DESIGN: Observational study of an ophthalmology clinic-based population. PARTICIPANTS AND CONTROLS: Ninety-three AMD eyes from 63 patients and 220 normal eyes from 138 subjects. METHODS: Macular carotenoid levels were quantified by illuminating the macula with a low-power argon laser spot and measuring Raman backscattered light using a spectrograph. This technique is sensitive, specific, and repeatable even in subjects with significant macular pathologic features. MAIN OUTCOME MEASURE: Raman signal intensity at 1525 cm\(^{-1}\) generated by the carbon-carbon double-bond vibrations of lutein and zeaxanthin. RESULTS: Carotenoid Raman signal intensity declined with age in normal eyes (P < 0.001). Average levels of lutein and zeaxanthin were 32% lower in AMD eyes versus normal elderly control eyes as long as the subjects were not consuming high-dose lutein supplements (P = 0.001). Patients who had begun to consume supplements containing high doses of lutein (> or =4 mg/day) regularly after their initial diagnosis of AMD had average macular pigment levels that were in the normal range (P = 0.829) and that were significantly higher than in AMD patients not consuming these supplements (P = 0.038). CONCLUSIONS: These findings are consistent with the hypothesis that low levels of lutein and zeaxanthin in the human macula may represent a pathogenic risk factor for the development of AMD. Resonance Raman measurement of macular carotenoid pigments could play an important role in facilitating large-scale prospective clinical studies of lutein and zeaxanthin protection against AMD, and this technology may someday prove useful in the early detection of individuals at risk for visual loss from AMD.
Study reference:

ABSTRACT

The xanthophyll carotenoids lutein and zeaxanthin (see Note 1) are specifically concentrated in the macula of the primate eye, the region of the retina responsible for high-resolution visual acuity necessary for reading, driving, and recognizing faces. They are thought to protect the macula from light-induced oxidative damage by acting as light-screening filters for short wavelength visible light and by acting as in situ antioxidants to prevent oxidative damage to polyunsaturated membrane lipids (1,2). Since high dietary intakes and blood levels of lutein and zeaxanthin have been epidemiologically associated with a lower risk of visual loss from age-related macular degeneration (AMD) (3,4), there has been considerable interest in measuring carotenoid macular pigment levels in living human eyes as a possible early test to detect individuals at high risk for visual loss from AMD. The current most commonly used method, psychophysical heterochromatic flicker photometry, has significant drawbacks since it is a subjective test that requires an attentive observer with good visual acuity, and it has a high intrasubject variability that may exceed ± 50% (5,6), which tends to limit its utility as a screening or diagnostic test. We have developed an alternative objective measurement method based on the principles of resonance Raman spectroscopy. This method is rapid, specific, sensitive, and highly reproducible, characteristics conducive to its use as a screening and diagnostic test on large populations with a wide range of visual acuities.
Study reference:

ABSTRACT

The human macula uniquely concentrates extraordinarily high levels of two xanthophylls carotenoids, lutein and zeaxanthin. The function, metabolism, and physiology of these yellow pigments are incompletely understood, but they are likely to prevent age-related damage to the foveal region by virtue of their ability to act as free-radical quenching antioxidants and to absorb phototoxic blue light with high efficiency. A wealth of circumstantial evidence suggests that high macular levels of these two carotenoids may protect against age-related macular degeneration (AMD), but definitive prospective clinical studies still remain to be conducted. It is imperative to gain a greater knowledge of the basic biochemical and physiological mechanisms underlying the specific uptake and metabolism of lutein and zeaxanthin in the macula and to develop improved methods of quantifying macular carotenoid levels noninvasively in order to facilitate the rational design of successful interventions against the leading cause of irreversible blindness in the elderly in the developed world. The development of resonance Raman spectroscopic methods for the objective measurement of macular carotenoid levels in living humans with and without AMD will be reviewed.
ABSTRACT

PURPOSE: To develop and test a novel noninvasive optical technique suitable for the objective measurement of macular carotenoid levels in human retina. METHODS: A resonance Raman scattering apparatus was constructed to measure carotenoid levels in flat-mounted human retinas and eyecups and in experimental animal eyes. Light from an argon laser was used to resonantly excite the electronic absorption of the carotenoid pigments, and scattered light was collected and analyzed by a Raman spectrometer. After carotenoid Raman measurements were completed on the retinal samples, macular carotenoid levels were determined by high-performance liquid chromatography (HPLC). RESULTS: Carotenoid resonance Raman scattering proved to be a highly sensitive and specific method for the noninvasive measurement of macular pigments in the human retina. Signal strength scaled linearly with actual macular carotenoid content as measured by HPLC. Our apparatus was also used to record resonance Raman signals from xanthophyll carotenoids stored in the retinal pigment epithelium of intact frog eyes. CONCLUSIONS: This new noninvasive optical method will facilitate studies of ocular carotenoid distributions and their role in degenerative diseases of the eye and may allow for the rapid screening of carotenoid levels in large populations at risk for vision loss from age-related macular degeneration, the leading cause of blindness in the elderly in the United States. A prototype clinical instrument is under development.
**ABSTRACT**

Raman spectroscopy holds promise as a novel noninvasive technology for the quantification of the macular pigments (MP) lutein and zeaxanthin. These compounds, which are members of the carotenoid family, are thought to prevent or delay the onset of age-related macular degeneration, the leading cause of irreversible blindness in the elderly. It is highly likely that they achieve this protection through their function as optical filters and/or antioxidants. Using resonant excitation in the visible region, we measure and quantify the Raman signals that originate from the carbon double bond (C=C) stretch vibrations of the pi-conjugated molecule backbone. In this manuscript we describe the construction and performance of a novel compact MP Raman instrument utilizing dielectric angle-tuned band-pass filters for wavelength selection and a single-channel photo-multiplier for the detection of MP Raman responses. MP concentration measurements are fast and accurate, as seen in our experiments with model eyes and living human eyes. The ease and rapidity of Raman MP measurements, the simplicity of the instrumentation, the high accuracy of the measurements, and the lack of significant systematic errors should make this technology attractive for widespread clinical research.

**Study reference:**

http://www.ncbi.nlm.nih.gov/pubmed/16053555

**ABSTRACT**

Clinical studies of carotenoid macular pigments (MP) have been limited by the lack of noninvasive, objective instruments. We introduce a novel noninvasive optical instrument, an MP Raman detector, for assessment of the carotenoid status of the human retina in vivo. The instrument uses resonant excitation of carotenoid molecules in the visible wavelength range, and quantitatively measures the highly specific Raman signals that originate from the single- and double-bond stretch vibrations of the pi-conjugated carotenoid molecule's carbon backbone. The instrument is a robust, compact device and suitable for routine measurements of MP concentrations in a clinical setting. We characterized and tested the instrument in clinical studies of human subjects to validate its function and to begin to establish its role as a possible screening test for macular pathologies. We also show that the MP Raman spectroscopy technology has potential as a novel, highly specific method for rapid screening of carotenoid antioxidant levels in large populations at risk for vision loss from age-related macular degeneration, the leading cause of blindness of the elderly in the developed world. (c) 2004 Society of Photo-Optical Instrumentation Engineers.
**ABSTRACT**

We have used resonant Raman scattering as a novel, noninvasive in vivo optical technique to measure the concentration of macular carotenoid pigments in the living human retina. Using a backscattering geometry and resonant molecular excitation in the visible, we measure the Raman peaks that originate from the single- and double-bond stretch vibrations of the p-conjugated molecule's carbon backbone. The Raman signals scale linearly with carotenoid content, whereas the required laser excitation is well under safety limits for macular exposure. The Raman technique is objective and quantitative and may lead to a new method for rapid screening of carotenoid pigment levels in large human populations that are at risk for vision loss from age-related macular degeneration, the leading cause of blindness of the elderly in the United States.

**Study reference:**

**ABSTRACT**

There is currently strong interest in developing noninvasive technologies for the detection of macular carotenoid pigments in the human eye. These pigments, consisting of lutein and zeaxanthin, are taken up from the diet and are thought to play an important role in the prevention of age-related macular degeneration, the leading cause of blindness in the elderly in the Western world. It may be possible to prevent or delay the onset of this debilitating disease with suitable dietary intervention strategies. We review the most commonly used detection techniques based on heterochromatic flicker photometry, fundus reflectometry, and autofluorescence techniques and put them in perspective with recently developed more molecule-specific Raman detection methods.

(c) 2004 Society of Photo-Optical Instrumentation Engineers.
ABSTRACT

We have used resonant Raman scattering spectroscopy as a novel, noninvasive, in vivo optical technique to measure the concentration of the macular carotenoid pigments lutein and zeaxanthin in the living human retina of young and elderly adults. Using a backscattering geometry and resonant molecular excitation in the visible wavelength range, we measure the Raman signals originating from the single- and double-bond stretch vibrations of the pi-conjugated molecule's carbon backbone. The Raman signals scale linearly with carotenoid content, and the required laser excitation is well below safety limits for macular exposure. Furthermore, the signals decline significantly with increasing age in normal eyes. The Raman technique is objective and quantitative and may lead to a new method for rapid screening of carotenoid pigment levels in large populations at risk for vision loss from age-related macular degeneration, the leading cause of blindness in the elderly in the United States.

ABSTRACT

We have imaged the spatial distribution of macular carotenoid pigments (MPs) in the human retina, employing Raman spectroscopy. Using excised human eyecups as initial test samples and resonant excitation of the pigment molecules with narrow-bandwidth blue light from a mercury arc lamp, we record Raman images originating from the carbon-carbon double-bond stretch vibrations of the molecules. Preliminary Raman images reveal significant differences in the MPs of different samples in regard to absolute levels as well as spatial variation. This technique holds promise as a method of rapid screening of MPs in large populations at risk for vision loss from age-related macular degeneration, a leading cause of blindness.
ABSTRACT

PURPOSE: There are several techniques for measuring macular pigment (MP) in vivo, of which Raman spectroscopy (RS) is a recently developed objective METHOD: This study reports the reproducibility, test-retest variability, and validity of RS MP readings, by comparing them with heterochromatic flicker photometry (HFP). METHODS: MP was measured with HFP and RS in 120 healthy subjects, and the latter technique was also used on two separate occasions in a sample of 20 subjects to investigate the intersessional variability of readings. Intrasessional reproducibility of RS MP measurements was also calculated. In addition, serum concentrations of lutein (L) and zeaxanthin (Z) were measured and correlated with both RS and HFP MP readings. RESULTS: Mean (+/−SD) MP in the right eye was 0.279 +/− 0.145 and 0.319 +/− 0.155 with RS and HFP, respectively. The differences between corresponding MP readings taken on RS and HFP lay within the Bland-Altman 95% limits of agreement for the two instruments in 93.6% and 94.4% of cases in the right and left eyes, respectively. Intrasessional reproducibility of RS readings, expressed as the coefficient of variation, was 8.42% +/− 7.12%. Ninety-five percent of MP readings taken with RS on two separate occasions lay within the 95% limits of agreement for the two sessions. A positive, but insignificant, relationship was observed between RS and HFP MP readings and serum concentrations of L and Z (RS, P = 0.356; HFP, P = 0.540). CONCLUSIONS: RS, an objective method of measuring MP levels in vivo, exhibits acceptable reproducibility and test-retest variability. The results demonstrated good correlation between RS and HFP measurements of MP, thus authenticating RS against a validated psychophysical technique of measuring MP. However, investigators should use only one of these instruments for the duration of any given study because of differences in the scientific rationale, and the factors that influence RS and HFP measurements of MP.
Study reference:

ABSTRACT

BACKGROUND: It has been hypothesized that the macular carotenoid pigments lutein and zeaxanthin may protect against macular and retinal degenerations and dystrophies. OBJECTIVE: To test this hypothesis by objectively measuring lutein and zeaxanthin levels in a noninvasive manner in patients who have retinitis pigmentosa (RP), choroideremia (CHM), and Stargardt macular dystrophy and comparing them with an age-matched healthy control population.

METHODS: Using resonance Raman spectroscopy, a novel objective noninvasive laser-optical technique, we measured macular carotenoid levels in 30 patients (54 eyes) who have RP, CHM, and Stargardt macular dystrophy and compared them with 76 age-matched subjects (129 eyes) who did not have macular pathologic conditions in a case-control study.

RESULTS: As a group, patients with RP and CHM had the same macular carotenoid levels as age-matched healthy control subjects (P = .76, 2-way analysis of variance). Patients with Stargardt macular dystrophy tended to have levels of macular carotenoid pigments that, on average, were about 50% lower than healthy controls (P = .02, unpaired 2-tailed t test).

CONCLUSIONS: The patients with RP and CHM had normal levels of macular carotenoids, suggesting that nutritional supplementation with macular carotenoids such as lutein, zeaxanthin, or both will be unlikely to affect the clinical course of RP and CHM. Although the number of patients with Stargardt macular dystrophy examined was limited, their macular carotenoid levels were usually lower than those of subjects of a similar age with no macular pathologic condition.
**Raman Spectroscopy in the Skin**

**Study reference:**

**ABSTRACT**

Variation in the level of the carotenoid antioxidant substances beta-carotene and lycopene in the human skin of ten healthy volunteers was measured with resonance Raman spectroscopy in an in vivo experiment over the course of 12 months. Information on the lifestyle of the volunteers concerning dietary supplementation and stress factors was obtained daily by the completion of questionnaires. The results showed individual variations in the levels of carotenoid antioxidant substances in the skin of the volunteers, which strongly correlated to specific lifestyles, such as the intake of dietary supplementations rich in carotenoids, and the influence of stress factors. A carotenoid-rich nutrition, based on large amounts of fruit and vegetables, increased the measured carotenoid levels of skin, while stress factors such as fatigue, illness, smoking, and alcohol consumption gave rise to a decrease in carotenoid levels of the skin. These decreases occurred relatively quickly over the course of one day, while the subsequent increases lasted for up to 3 days. During the summer and autumn months, an increase in the level of carotenoids in the skin was measured for all volunteers. The average "seasonal increase" of the carotenoid content in the skin was determined to be 1.26-fold.

**Study reference:**

**ABSTRACT**

Reactive free radicals can be produced in the skin by the action of environmental factors, such as sun radiation and toxins. These radicals can damage the DNA, proteins and lipids of the living cells. The consequences can be skin aging, immune suppression and even skin cancer. Humans have developed a protective mechanism against the action of free radicals in the form of antioxidant substances. Several of these antioxidants cannot be produced by humans and have to be acquired via food, such as carotenoids. Optical, non-invasive methods, like resonance Raman spectroscopy, allow a qualitative and quantitative online detection of the kinetics of antioxidants such as carotenoids in the skin. By employing this method it has been shown that the uptake of carotenoids in food can lead to an accumulation in the skin. On the other hand, stress, illness and UV-radiation can reduce the concentration of antioxidant substances in the skin. A high concentration of antioxidant substances is protective and associated with a reduction in skin wrinkling.
ABSTRACT
The predominant long-chain carotenoids found in human skin are lycopene and beta-carotene. They are powerful antioxidants and thought to act as scavengers for free radicals and singlet oxygen formed by normal metabolism as well as excessive exposure of skin to sunlight. The specific importance of the particular representatives of the carotenoid antioxidants regarding skin defense mechanisms is of strong current interest. We demonstrate fast and noninvasive detection of beta-carotene and lycopene concentrations in living human skin using Raman detection of the molecules' carbon-carbon double bond stretch vibrations. Employing excitation with suitable blue and green laser lines, and taking advantage of differing Raman cross sectional profiles for beta-carotene and lycopene, we determine the relative concentration of each carotenoid species. This novel technique permits the quantitative assessment of individual long-chain carotenoid species rather than their composite level in human skin. The obtained results reveal significant differences in the carotenoid composition of the subjects' skin and show that the ratio between beta-carotene and lycopene concentration can vary from 0.5 to 1.6. The technique holds promise as a method for rapid screening of carotenoid compositions in human skin in large populations and should be suitable for clinical studies correlating carotenoid status with risk for cutaneous diseases.

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ABSTRACT
We have used resonance Raman scattering as a novel noninvasive optical technology to measure carotenoid antioxidants in living human tissues of healthy volunteers. By use of blue-green laser excitation, clearly distinguishable carotenoid Raman spectra superimposed on a fluorescence background are obtained. The Raman spectra are obtained within less than a minute, and the required laser light exposure levels are well within safety standards. Our technique can be used for rapid screening of carotenoid levels in large populations and may have applications for assessing antioxidant status and the risk for diseases related to oxidative stress.
Study reference:
Gellermann, W., Ermakov, I.V., Scholz, T.A. and Bernstein, P. S. Noninvasive laser Raman

ABSTRACT

Carotenoids are an important part of the endogenous antioxidant system in the skin and other areas of the human body. Carotenoid molecules, provided by fruits and vegetables, are potent free-radical quenchers that accumulate in the body. If not balanced by carotenoids and other antioxidants, free radicals may cause premature skin aging, oxidative cell damage, and even skin cancers. In animal models, carotenoids inhibit carcinoma formation in skin and other tissues. As carotenoid depletion may predispose a person to cancer or other disease, rapid and noninvasive measurement of carotenoid levels in skin may be of preventative or diagnostic help. At the very least, such measurements can be used to obtain a biomarker for healthy levels of fruit and vegetable consumption. We use a noninvasive optical technique, based on resonance Raman spectroscopy, to rapidly screen carotenoid levels in skin and to assess antioxidant status. This technique has the potential to help in assessing risk for cutaneous disease and other diseases related to oxidative stress.

Study reference:
Hata TR, Scholz TA, Ermakov IV, McClane RW, Khachik F, Gellermann W, Pershing LK. Non-
invasive Raman spectroscopic detection of carotenoids in human skin. J Invest Dermatology
2000;115:441-448.
http://www.ncbi.nlm.nih.gov/pubmed/10951281

ABSTRACT

Carotenoids are thought to play a significant part in the skin's anti-oxidant defense system, and may help prevent malignancy. Inability to measure skin carotenoid content readily has, however, made it difficult to establish the relationship between carotenoid concentration and the occurrence of cutaneous malignancy. We have measured in vivo carotenoid concentration using a noninvasive optical method, Raman spectroscopy. To validate our instrumentation, abdominoplasty skin was evaluated by both Raman spectroscopy and high-performance liquid chromatography determination for carotenoid content. Evaluation of the Raman signal in specific carotenoid solutions was also performed. Precision of Raman measurements within skin sites, within subjects, and between subjects was measured. Sensitivity of the method was evaluated as a function of anatomical region and the distribution of carotenoids within the stratum corneum. Lastly, we evaluated the Raman signal in actinic keratosis and basal cell carcinoma lesions and perilesional skin and compared this with region-matched sites in healthy subjects. Our results indicate that the Raman scattering method reflects the presence of carotenoids in human skin and is highly reproducible. Evaluation of five anatomical regions demonstrated significant differences in carotenoid concentration by body region with the highest carotenoid concentration noted in the palm. Comparison of carotenoid concentrations in basal cell carcinomas, actinic keratosis, and their perilesional skin demonstrate a significantly lower carotenoid concentration than in region-matched skin of healthy subjects. These results represent the first evidence that carotenoid concentration in the skin correlate with the presence or absence of skin cancer and precancerous lesions.
**Study reference:**

http://www.fasebj.org/cgi/content/meeting_abstract/21/5/A709?maxtoshow=&hits=10&RESULTFORMAT=&author1=Bi&andorexacttitle=and&andorexacttitleabs=and&andorexactfulltext=and&searchid=1&FIRSTINDEX=0&sortspec=relevance&volume=21&fdate=7/1/2006&tdate=6/30/2008&resourcetype=HWCIT

**ABSTRACT**

This study was to assess the effects of life styles and dietary supplement LifePak on human skin carotenoids measured by Biophotonic Scanner using a non-invasive Resonance Raman Spectroscopy (RS) technique. We examined skin carotenoids of 88,721 volunteers, and monitored changes in skin carotenoids as a function of life styles and in response to daily consumption of fruits, vegetables, and dietary supplement LifePak. RS skin carotenoids scores are closely, positively correlated with serum carotenoids determined by use of HPLC ($r^2=0.704$, $p<0.001$). Non-tobacco users and subjects with less sunlight exposure had significantly higher scores than those for current or former tobacco users or people with high level sunlight exposure ($p<0.001$). The higher the BMI, the lower the scores ($p<0.001$), indicating diluted fat-soluble carotenoids in the body as a function of increased body fat mass. We found that the more daily consumption of fruits-vegetables and dietary supplements, the higher the scores ($p<0.01$). Daily LifePak intake increased the RS scores by 24% at Week 4 and by 44% at Week 8 ($p<0.001$). In conclusion, RS skin carotenoids scores reflect the steady state levels of antioxidant carotenoids in human skin. Fruits-vegetables intake and LifePak supplementation increase antioxidant capacity in human body, but tobacco use and sunlight exposure reduce it.
Study reference:

ABSTRACT

Background- Biophotonic Scanner was designed for clinical use to specifically determine skin antioxidant carotenoids, on the basis of a non-invasive technique of Resonance Raman Spectroscopy. Skin carotenoids represent steady state levels of antioxidant define capability in human bodies.

Design and outcomes- We examined skin carotenoids of 88,721 volunteers, and monitored changes in skin carotenoids as a function of life styles and in response to daily consumption of fruits, vegetables, and dietary supplements LifePak. Skin carotenoids presented as Biophotonic scanner scores are closely, positively correlated with serum carotenoids determined by use of HPLC (r²=0.704, P<0.001). Non-smokers and subjects with less sun-light exposure had significantly higher scores than those for cigarette smokers/former smokers and people with high exposure to sun light (P<0.001). The higher the BMI is, the lower the scores are (P<0.001), indicating diluted fat-soluble carotenoids in the body as a function of increases in body fat mass. We also found that The more daily consumption of fruits-vegetables and dietary supplements, the higher the scores are (P<0.01). Daily LifePak intake resulted in increases in the scores by 24% after 4 weeks of LifePak and by 44% after 8 weeks (P<0.001).

Conclusions- Biophotonic scanner scores reflect steady state levels of antioxidant carotenoids in human’s skin. Fruits-vegetables intakes and LifePak supplementations increase body’s antioxidant capacity, but smoking and sun-light exposure reduce it.
ABSTRACT

Micronutrients, such as beta-carotene and vitamins A and E, are potential chemopreventive agents; however, their concentrations in human target tissues are largely unknown. Because these micronutrients may exert their action at the site of target tissues, the tissue concentrations of the micronutrients need to be determined. In this cross-sectional study, we have measured the concentrations of seven carotenoids, two retinoids, and two tocopherols in paired plasma, buccal mucosal cells (BMC), and skin samples from 96 healthy subjects (ages 26-82 yrs). The plasma-tissue, as well as the diet-plasma and diet-tissue relationships of the micronutrients, and the impact of various potential confounders on the micronutrient concentrations were evaluated. The micronutrient concentrations of plasma and BMC used in the evaluation were the average of three measurements over a one-month period. Our data indicated that 1) the correlations between the plasma and BMC (Spearman r = 0.40-0.91, p < 0.05) and the plasma and skin (r = 0.24-0.75, p < 0.05) concentrations of most micronutrients were significant in all subjects, suggesting that the status of these micronutrients in the BMC and skin may be estimated from their plasma concentrations; 2) the correlations between the diet and plasma/tissue concentrations of the micronutrients were generally not as strong as the plasma-tissue relationships; the diet-plasma and diet-tissue relationships of the carotenoids were particularly poor in the smokers; 3) the plasma and tissue concentrations of most micronutrients were lower in smokers than in nonsmokers and higher in vitamin supplement users than in nonsupplement users; the differences remained significant after adjustment for age, gender, and diet intake estimates; 4) among the seven carotenoids examined, lycopene was unique, because its concentration was not lower in smokers or higher in supplement users but was inversely associated with age.
ABSTRACT

Purpose: Increasing evidence from epidemiological studies suggest that measures of chronic inflammation are associated with an increased risk of cardiovascular disorders, including ischemic heart disease, stroke and other thromboembolism. Oxidative stress may play a role in the development of the inflamed state, and previous studies have shown a possible protective effect of high levels of cardiorespiratory fitness. The purpose of this study was to examine the relationship between oxidative stress, markers of inflammation and cardiorespiratory fitness (VO2max) in the obese. Methods: A total of four hundred eighty three obese individuals (mean age 43.34 ± 8.32) were assessed for VO2max, oxidative stress as measured photometrically in the blood with a Free Radical Analysis System (FRAS), C-Reactive protein (CRP), which was used as a measure of inflammation and Body Defense Score (BDS), which was used as an indicator of antioxidant status and measured non-invasively using Raman Spectroscopy (Biophotonic Scanner, Pharmanex. Descriptive data is shown below in table 1. Results: Body weight was negatively correlated with FRAS (r=−0.199, p=0.009) and BDS (r=−0.171, p=0.002), but not CRP. Additionally CRP was positively correlated with FRAS (r=0.551, p<0.001) and negatively with VO2max (r=−0.260, p<0.001). No other associations among these variables were observed. Conclusions: These data support the concept that obesity stimulates an inflammatory response. Part of the inflammatory response may be due to oxidative stress. The strong negative correlation between body weight and BDS suggests that a diet rich in fruits and vegetables or antioxidant supplementation may be particularly important in obese individuals. Increased fitness may also protect against inflammation in these individuals.

Work Supported by: Pharmanex.
ABSTRACT

Introduction: The protective effects of dietary antioxidants on aging, cancer, cardiovascular disease and other disease conditions have been well documented. We have previously reported data from our laboratory that revealed an inverse relationship between skin carotenoid status (SCS), weight, BMI, and adiposity. During a calorie restricted diet it is possible that a decline in dietary quality may be observed despite a well planned diet. The purpose of this study was to determine the effect of weight loss on dietary carotenoids and what effect this has on SCS in overweight or obese subjects.

Methods: One hundred and eighty-one overweight or obese adults (143 females and 38 males, age 42.2 ± 8.96, BMI 30.9 ± 2.47) were randomized into 3 intervention groups; exercise only (E), exercise with caloric restriction of 500Kcal/day (D) and exercise and caloric restriction of 500Kcal/day including a high fiber, whole grain cereal (HF). One hundred and twenty-six subjects were tested at week 24. We report data only for those who completed the trial. A 3-day food record collected at baseline and again at week 24 examined the dietary carotenoids; Beta-carotene, Lutein and Zeaxanthin, and Lycopene. SCS was measured non-invasively using Raman Spectroscopy (Biophotonic Scanner, Pharmanex), which fires a low powered laser positioned on the palm of the hand. Data was analyzed by repeated measures analysis of variance using SPSS version 11.0. Statistical significance was set at p<0.05.

Results: Data for weight, SCS and dietary carotenoid intake is shown in Table 1.

Conclusion: Decreases in SCS occurred with weight loss similarly in all intervention groups. Despite the reduced calorie diet no decreases in any of the dietary carotenoids were observed. This data suggests that weight loss significantly decreases the skin carotenoid level, but the decrease in the skin carotenoid level was due to factors other than changes in dietary carotenoids. Further studies are needed to examine the mechanisms responsible for the decreases in SCS due to weight loss in overweight and obese individuals.

Supported by grants from Kraft Foods, Inc (primary) and Pharmanex (secondary)
Raman spectroscopy (RS) has been used to determine human carotenoid status based on non-invasive skin measurements. In this study, we compared RS with serum HPLC to monitor effects of a comprehensive dietary vitamin/mineral/antioxidant supplement (LifePak, Pharmanex). Fifty-three healthy adults (age 55.4±8.4 yrs) were randomly assigned in a 42-day double-blind study to assess the effects of the dietary supplement (S: n=25) vs placebo (P: n=28) on serum carotenoids and antioxidant status. Serum carotenoids were determined using HPLC. Skin carotenoids were assessed using RS (Pharmanex BioPhotonic Scanner) using 473 nm excitation in stratum corneum layer of a standardized location on the palm. Significant increases (p<0.05) were observed with S for total serum carotenoids (2.48 ± 1.1 to 3.35±1.6 ìmol/L), serum β-carotene (0.56 ± 0.3 to 1.26 ± 0.6 ìmol/L), lutein (0.29 ± 0.1 to 0.34 ± 0.1 ìmol/L), lycopene (0.94 ± 0.3 to 1.31 ± 0.4 ìmol/L), ascorbate (50 ± 23 to 85 ± 29 ìmol/L), α-tocopherol (34 ± 9.4 to 54 ± 13 ìmol/L) and skin carotenoids (18353 ± 4827 to 25358 ± 5229 units). Only serum ascorbate changed in P (53 ± 24 to 67 ± 39 ìmol/L). Significant correlations (p<0.01) between total serum and skin carotenoids were observed in S (baseline: r= 0.73; day 42: r= 0.57) and in P (baseline: r= 0.91; day 42: r= 0.87). The significant correlations between serum and skin carotenoid levels suggest that RS may be useful as an effective non-invasive tool to monitor carotenoid and possibly antioxidant status during dietary antioxidant interventions.

Bioavailable carotenoids and other antioxidants were provided by LifePak.
Study reference:
Duan L, Lu J, Li G, Zhu JS. Improvement of Skin Carotenoids Antioxidant Scores with G3 Drink and LifePak is affected by Endurance Training Intensity in Young Athletes. *FASEB J.* 2009 23:1007.3

http://www.fasebj.org/cgi/content/meeting_abstract/23/1_MeetingAbstracts/1007.3?maxtoshow=10&RESULTFORMAT=&andorexacttitle=and&andorexacttitleabs=and&fulltext=pharmanex&andorexactfulltext=and&searchid=1&FIRSTINDEX=0&sortspec=relevance&resourcetype=HWCIT

ABSTRACT

Intensive endurance exercise training increases O2 consumption in athletes and generates excessive ROS, which may cause fatigue and exercise-induced injury. Carotenoids are known as an important class of antioxidants (Sh J Prevent Med 6:261, 2006). By use of a noninvasive BioPhotonic Scanner (Pharmanex), we assessed skin carotenoids as a clinical marker of antioxidant status in young endurance athletes in response to supplementation of Pharmanex G3 drink and LifePak (enriched in carotenoids and antioxidant nutrients). Young athlete volunteers (19.6 yrs on average) were recruited from China skating and cross country ski teams, 32 males and 27 females, and received 120 mL of G3 and 2 sachets of LifePak per day for 8 wks. All subjects were on the same diet programs during the study. Skin carotenoids scores were increased by 28% (32,695±1,250 to 40,051±1,239; p<0.001) after 4 wks of G3 and LifePak, under intensive training of 20 hrs per wk. On Wk 8, skin scores remained 19% higher (38,618±1,853) above that on Wk 0 (p<0.001), when training intensity was increased to 25 hrs per wk (p=0.048). Increases in training intensity affected the skin scores in males (+38% & +16% on Wks 4 & 8) greater than in females (+18% & +22%). Our data indicate that improvement of the antioxidant capability with G3 and LifePak is affected by intensity of endurance training in young athletes, in particular in males.
Study reference:

ABSTRACT

Carotenoids are an important group of dietary antioxidants with many health benefits. Serum or plasma carotenoid measurements are commonly used to assess human carotenoid status and to monitor reported intake of fruits, vegetables and dietary supplements. Recently, a Raman spectroscopy (RS) method was developed to safely assess skin carotenoids non-invasively (Biophotonic Scanner, Pharmanex). To help validate this method, 104 healthy adults (64 men, 40 women) were recruited for this study. After an overnight fast, each subject provided a blood sample, and skin carotenoids were assessed at the palm of the hand using RS (473 nm excitation). Blood serum was analyzed for carotenoids by HPLC. Results show a highly significant correlation between serum total carotenoids and skin carotenoids as assessed with RS (r = 0.78, p < 0.001). Mean serum total carotenoid concentration was 1.44 mcg/ml (range: 0.37 – 3.36) and the mean Raman response for skin measurements was 28,808 counts (range: 14,524 – 56,298). Among individual carotenoids, correlations were strongest for beta-carotene, followed by alpha-carotene, lutein/zeaxanthin, lycopene and beta-cryptoxanthin. Based on these results, RS is able to estimate serum total carotenoids with a variability of +/- 10 % and 95 % confidence. This high correlation between serum and skin carotenoid measurements helps validate RS as a novel, non-invasive, rapid, and field-usuable tool to assess human carotenoid status.

Supported by Pharmanex, LLC
ABSTRACT

Carotenoids are found in many foods, fruits, and are partly responsible for the well-documented health benefits of diets rich in fruits and vegetables. For example, lutein and zeaxanthin prevent cataracts and macular degeneration; b-carotene and lycopene protect the skin from ultraviolet radiation damage; lutein and lycopene may benefit cardiovascular health, and lycopene may help prevent prostate cancer. Because of these and other marked health benefits, an accurate assessment of human carotenoid status is important. Carotenoid status can serve as a tool to monitor compliance to healthy diets rich in fruits and vegetables or dietary supplements. Currently, carotenoid levels are assessed with blood serum or plasma HPLC measurements. However, such methods are invasive, expensive and impractical for general use in large populations. Skin carotenoid levels correlate well with blood levels and may more accurately indicate carotenoid status, because unlike bloodskin carotenoids are not influenced by postprandial fluctuations. Recently, a convenient, rapid and non-invasive measurement of skin carotenoid status using Raman spectroscopy has been developed. This method can become a strong motivator for people to consume the recommended five to nine fruits and vegetables daily and well-balanced dietary supplements.

Study reference:

ABSTRACT

A novel, non-invasive biophotonic Raman spectroscopy method (Hata et al., J Invest Dermatology 115:441, 2000) was used for the first time to help establish relationships between biophotonic skin carotenoid response (BSCR) as a biomarker of antioxidant status, and demographic, dietary, lifestyle and oxidative stress parameters in 1,375 volunteers. Methods: Subjects completed a lifestyle and diet questionnaire, performed a home urinary MDA oxidative stress test (VesPro, Lenexa, KS), and underwent biophotonic BSCR measurement in the palm of the hand. Body fat was determined using a near-infrared device (Futrex Inc., Galthersburg, MD). Results: BSCR measurements were positively associated with fruit and vegetable consumption and antioxidant supplement intake. BSCR was inversely associated with urinary MDA, smoking habits, sunlight exposure, BMI and body fat, independently of reported fruit and vegetable or carotenoid intake. BSCR was not confounded by general demographic variables, such as age, sex, race or ethnicity. Subjects taking an antioxidant-rich multivitamin/mineral supplement (n=59; LifePak®, Pharmanex, LLC, Provo, UT) had a 61% higher BSCR than non-supplement users. Conclusions: This study supports that BSCR is a highly convenient method to determine skin carotenoids, which appear to be a valuable biomarker of antioxidant status in humans.

Supported by Pharmanex, LLC, Provo, UT.
Study reference:

ABSTRACT

Carotenoids are an important group of dietary nutrients demonstrated by research and epidemiological studies to provide human health benefits. HPLC quantification of carotenoids from blood serum (invasive) is the current accepted means of assessment. Recently, a Raman-spectroscopic (RS) method was introduced as a non-invasive alternative to assess carotenoid status in humans (Smidt et al, 2004). To further validate the RS method, 372 healthy adults participated in a clinical trial (IRB approval #1053052). Within an 8-day period, each subject provided 3 fasted blood samples and 3 same-day RS determinations of skin carotenoids. The primary clinical endpoint was to measure the intra-individual variability (IIV) for each testing method. The IIV for RS method was significantly (p=0.031) less (9.48%) than that observed using the serum/HPLC method (10.44%). Three separate correlation plots were produced and all showed highly significant correlations (range .78 – .82, p<.0001) between total serum carotenoid level and RS-derived skin carotenoid scores. For one such plot, the mean serum total carotenoid concentration was 1.08 ± .51μg/ml (range: 0.21 – 3.74) and the mean Raman response for skin measurements was 19,640 ± 7754 counts (range: 5,933 – 56,606). Among individual carotenoids, correlations were strongest for beta-carotene, followed by alpha-carotene, beta-cryptoxanthin, lutein/zeaxanthin, and lycopene and all were significant. Based on these results, RS appears to estimate the level of skin carotenoids with a variability that was significantly less than carotenoid determination using serum. These data provide further validation the RS technology as a viable non-invasive and alternative method to rapidly assess carotenoid status in humans.
ABSTRACT

BACKGROUND: Carotenoids are an important group of phytonutrients that are abundant in fruits and vegetables. Epidemiological and clinical intervention studies have implied the presence of protective qualities of these nutrients against the development of a variety of chronic diseases. Previously, human carotenoid status has been assessed in serum and tissue using high-performance liquid chromatography (HPLC) methodology. Recently, a Raman spectroscopy (RS)-based photonic method has been developed to accurately and noninvasively measure the carotenoid concentration in human skin. OBJECTIVES: (1) To validate skin RS methodology against standard serum carotenoid measurements by HPLC and (2) to establish and compare the reliability of the 2 methods. DESIGN: This study included 372 healthy adults who provided 3 blood samples and 3 RS skin carotenoid measurements within an 8-day period; each day-matched blood sample and RS determination was spaced by >or=48 hours. RESULTS: Consistent positive correlations were observed for each of 3 separate same-day correlation plots of total serum versus RS skin carotenoids. Overall estimate of the line of best fit from analysis of covariance, using all 3 samples (n = 1116), yielded a Pearson correlation of R = 0.81 (r(2) = 0.66; p < 0.001). Based on analysis of variance, RS skin carotenoid methodology exhibited 0.9% less variance over the 3 tests than serum carotenoids by the HPLC method (p < 0.03). CONCLUSIONS: RS accurately measures total carotenoids in human skin with less intra-individual variability than measurement of serum carotenoids by HPLC analysis. RS technology is a valid and reliable noninvasive method to rapidly assess carotenoid nutritional status in humans.
**ABSTRACT**

We evaluated the associations of: 1) human skin carotenoids measured by RS with conventionally measured serum carotenoids, 2) RS with serum levels of vitamins C and E, and markers of antioxidant capacity (ORAC) and oxidative stress (TBARs, urinary isoprostanes). Following approval by the University of Utah IRB, consent was obtained from 320 apparently healthy male and female corporate employees participating in their annual health risk assessment. Skin carotenoids were measured with RS 473nm excitation at a standardized location in the palm of the hand. Blood and urine samples were collected to assess serum antioxidants, ORAC and oxidative stress markers. Co-variates included BMI, dietary and lifestyle behaviors. Pearson correlations and regression analyses (n = 295) indicated a significant correlation with skin levels and a composite serum carotenoid score (r = .80; p < 0.001). RS skin measures were also associated with serum levels of vitamins C (r = .33; p < 0.001) and E (alpha tocopherol; r = .30; p < 0.001) and inversely associated with urinary isoprostanes (r = .23; p < 0.001), but not TBARS. There was no significant difference by gender, however, older and heavier subjects had lower serum carotenoid and RS carotenoid levels than their leaner counterparts. The data suggest RS offers a safe, non-invasive alternative to drawing blood for assessing carotenoid status, and also modestly correlates with other antioxidant nutrients (vitamins C and E).
Study reference:

ABSTRACT

PURPOSE: To develop and test a novel noninvasive optical technique suitable for the objective measurement of macular carotenoid levels in human retina. METHODS: A resonance Raman scattering apparatus was constructed to measure carotenoid levels in flat-mounted human retinas and eyecups and in experimental animal eyes. Light from an argon laser was used to resonantly excite the electronic absorption of the carotenoid pigments, and scattered light was collected and analyzed by a Raman spectrometer. After carotenoid Raman measurements were completed on the retinal samples, macular carotenoid levels were determined by high-performance liquid chromatography (HPLC). RESULTS: Carotenoid resonance Raman scattering proved to be a highly sensitive and specific method for the noninvasive measurement of macular pigments in the human retina. Signal strength scaled linearly with actual macular carotenoid content as measured by HPLC. Our apparatus was also used to record resonance Raman signals from xanthophyll carotenoids stored in the retinal pigment epithelium of intact frog eyes. CONCLUSIONS: This new noninvasive optical method will facilitate studies of ocular carotenoid distributions and their role in degenerative diseases of the eye and may allow for the rapid screening of carotenoid levels in large populations at risk for vision loss from age-related macular degeneration, the leading cause of blindness in the elderly in the United States. A prototype clinical instrument is under development.
Study reference:

ABSTRACT

Purpose: To describe the cross-sectional association of body composition with carotenoid antioxidants in overweight individuals in 176 overweight (BMI ≥ 27 kg.m⁻²) participants.

Methods: Carotenoid antioxidant concentration in the skin was measured non-invasively using Raman Spectroscopy (Biophotonic Scanner, Pharmanex). Briefly, by shining a low power blue laser into the skin an individual's carotenoid antioxidant levels were determined. Body composition (i.e., percent fat, fat mass, lean body mass) was determined with air displacement plethysmography. Data were analyzed using SPSS version 9.0. Statistical significance was set at p<0.05.

Results: Carotenoid level was inversely correlated with Body Weight (r=−0.228, p=0.002), BMI (r=−0.176, p=0.019), and Fat Mass (r=−0.228, p=0.002). However correlations with Lean Body Mass (r=−0.121, p=0.109) and percent fat (r=−0.092, p=0.223) were not significant.

Conclusion: This study confirms that in overweight and obese individuals the level of adipose tissue accumulation negatively influences skin carotenoid levels, and thus antioxidant status. A component of the known adverse health consequences from excess adiposity may thus arise from free radical damage to tissues. Effective strategies to support antioxidant status may be appropriate for overweight and obese individuals. Future studies must examine the effect of weight loss on Carotenoid antioxidant concentration.
**ABSTRACT**

We evaluated the associations of fruit and vegetable intake with both conventionally measured serum carotenoids and skin carotenoids measured by RS. Following approval by the University of Utah IRB, consent was obtained from 320 apparently healthy male and female corporate employees participating in their annual health risk assessment. Skin carotenoids were measured with RS 473nm excitation in standardized location in the palm of the hand. Blood samples were taken to assess serum nutrients including carotenoids. Fruit and vegetable intake was assessed with a modified Block fruit and vegetable food frequency questionnaire (FVFFQ). Co-variates included BMI, lifestyle behaviors and dietary supplement intake. Pearson correlations and regression analyses revealed similar modest significant correlations with both the FVFFQ composite serving score and a composite serum carotenoid score (r=.21; p=0.0003; n=285) and with the RS skin carotenoid score (r=.28; p<0.0001; n=296). These relationships were independent of supplement intake which had a stronger significant relationship with serum carotenoid levels (r=.52; p<0.0001; n=285) and RS carotenoid levels (r=.48; p<0.0001; n=296). These results indicate RS scanner has potential utility as a rapid screening method that reflects fruit and vegetable intake similar to a FVFFQ as well as serum blood measures, without the risk of time associated with collecting and analyzing blood samples.

**Study reference:**

**ABSTRACT**

We report on the development of a compact commercial instrument for measuring carotenoids in skin tissue. The instrument uses two light-emitting diodes (LEDs) for dual-wavelength excitation and four photomultiplier tubes for multichannel detection. Bandpass filters are used to select the excitation detection wavelengths. The f/1.3 optical system has high optical throughput and single photon sensitivity, both of which are crucial in LED-based Raman measurements. We employ a signal processing technique that compensates for detector drift and error. The sensitivity and reproducibility of the LED Raman instrument compares favorably to laser-based Raman spectrometers. This compact, portable instrument is used for noninvasive measurement of carotenoid molecules in human skin with a repeatability better than 10%.

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ABSTRACT

BACKGROUND: Epidemiologic studies found the inverse correlation between fruit and vegetable intake and the risk of cardiovascular disease, various cancers, insulin resistance, and other chronic conditions. Skin carotenoid levels are highly correlated with serum levels; however, the direct measurement of skin carotenoids is difficult to perform. Raman spectroscopy has been described as a highly sensitive, specific and accurate method of skin carotenoid detection. OBJECTIVE: The authors assessed the relation between fruit and vegetable intake and skin carotenoid levels measured by Raman spectroscopy. MATERIAL AND METHOD: Twenty-nine healthy volunteers were enrolled in the present study. Demographic data and fruit and vegetable intake were recorded. Skin carotenoid levels were measured by Raman spectroscopy and were reported as Skin Carotenoid Score (SCS). The data were compared and were reported as 3 groups based on the amounts of fruit and vegetable intake. RESULTS: There were no significant differences of age, body weight, height and body mass index among the groups. Mean skin carotenoid score of low fruit and vegetable intake (25,733 +/- 2,956) was significantly lower than SCS of moderate intake (31,333 +/- 4,792, p = 0.03) and high fruit and vegetable intake (35,125 +/- 6,081, p < 0.01). Mean SCS of underweight participants (29,250 +/- 4,621) was not significantly different from normal (33,384 +/- 6,614) and overweight participants (27,575 +/- 3,811), p = 0.06. CONCLUSION: Using Raman spectroscopy, the authors found that skin carotenoid levels were directly correlated with the degree of fruit and vegetable intakes. We suggest that Raman spectroscopy should be possible to replace the invasive chemical technique for the dermatologic carotenoid measurement.
Study reference:


Objective: To observe the variety of carotenoids level in the body through the detection of skin carotenoids. Methods: 120 adult subjects were paired according to age, gender and the level of skin carotenoids at entry, and divided randomly into treatment and control groups. The treatment subjects took 12.6 mg β-carotene per day for 8 weeks. At the beginning, 4th and 8th week, the skin carotenoids were detected with resonance Raman spectroscopy. Meanwhile, 25h-dietary questionnaires for all participants were carried out. Results: After supplementation, the value of the skin carotenoids went up 23.3% at the 4th week and 44% at the 8th week over the beginning of the treatment group, and there was a very significant difference comparing with the control group (P < 0.001). The intakes of food and dietary β-carotene were not significantly different between two study groups during study. Conclusion: The increase of skin carotenoids in treatment group may relate to the supplement of β-carotenoid. Raman scattering method, as a non-invasive method, is useful to reflect the level of carotenoids in human body through the measurement of carotenoids in skin.

Study reference:

ABSTRACT

Biophotonic Scanner was designed by use of a technique of Resonance Raman Spectroscopy, a non-invasive, easy-to-use tool to specifically determine skin antioxidant carotenoids. We examined skin carotenoids of 88,611 volunteers, and monitored changes in human skin carotenoids as a function of life styles and in response to daily consumption of fruits, vegetables, and a dietary supplement LifePak. We found that skin carotenoids presented as Biophotonic Scanner scores are significantly closely, positively correlated with serum carotenoids determined by use of HPLC (n=1116, r²=0.704, p<0.001). Non-smokers and subjects with less sun-light exposure had significantly higher scores than those for cigarette smokers and former smokers and people with high exposure to sun light (p<0.001). The higher the BMI, the lower the scores (p<0.001), indicating diluted fat soluble carotenoids in the skin associated with increased body fat mass. The more daily consumption of fruits and vegetables and dietary supplements, the higher the scores (p<0.01). Daily LifePak intake resulted in increases in the scores by 24.3% after 4 weeks of supplementation and by 44.0% after 8 weeks (p<0.001). In conclusion, Biophotonic scanner scores reflect steady state levels of antioxidant carotenoids in human’s skin. Fruits and vegetables intake and LifePak supplements increase the antioxidant capacity, but smoking and sun-light exposure reduce it.
Study reference:

ABSTRACT

Increasing evidence points to the beneficial effects of carotenoid antioxidants in the human body. Several studies, for example, support the protective role of lutein and zeaxanthin in the prevention of age-related eye diseases. If present in high concentrations in the macular region of the retina, lutein and zeaxanthin provide pigmentation in this most light sensitive retinal spot, and as a result of light filtering and/or antioxidant action, delay the onset of macular degeneration with increasing age. Other carotenoids, such as lycopene and beta-carotene, play an important role as well in the protection of skin from UV and short-wavelength visible radiation. Lutein and lycopene may also have protective function for cardiovascular health, and lycopene may play a role in the prevention of prostate cancer. Motivated by the growing importance of carotenoids in health and disease, and recognizing the lack of any accepted noninvasive technology for the detection of carotenoids in living human tissue, we explore resonance Raman spectroscopy as a novel approach for noninvasive, laser optical carotenoid detection. We review the main results achieved recently with the Raman detection approach. Initially we applied the method to the detection of macular carotenoid pigments, and more recently to the detection of carotenoids in human skin and mucosal tissues. Using skin carotenoid Raman instruments, we measure the carotenoid response from the stratum corneum layer of the palm of the hand for a population of 1,375 subjects and develop a portable skin Raman scanner for field studies. These experiments reveal that carotenoids are a good indicator of antioxidant status. They show that people with high oxidative stress, like smokers, and subjects with high sunlight exposure, in general, have reduced skin carotenoid levels, independent of their dietary carotenoid consumption. We find the Raman technique to be precise, specific, sensitive, and well suitable for clinical as well as field studies. The noninvasive laser technique may become a useful method for the correlation between tissue carotenoid levels and risk for malignancies or other degenerative diseases associated with oxidative stress.
**Presentation reference:**

**ABSTRACT**

Measurement of skin carotenoids (SC) by Raman Spectroscopy (RS) has been proposed as a way to evaluate carotenoid status non-invasively and quickly. The objective of this work was to evaluate the association of human skin carotenoids with conventional serum carotenoids measurements. Furthermore, we measured markers of oxidative stress and body composition (Body Mass Index-BMI) in order to understand their relationships with carotenoid status.

Two studies were undertaken to determine the correlation of SC measurement by RS (excitation at 473 and detection at 511 nm on the palm of the right hand) with serum carotenoid levels (HPLC). First we compared intra-individual variability of 372 healthy subjects who donated 3 blood samples along with simultaneous SC measurement for each draw within an 8 day period [1]. Secondly, we measured (n = 286) blood carotenoids (α- and β-carotene, lutein, zeaxanthin, lycopene and α- and β-cryptoxanthin), and correlated them with SC measurements [2]. Measurement of other serum nutrients (tocopherols and ascorbic acid) and markers of oxidative stress [serum oxygen radical absorbance capacity (ORAC) and urinary F2-isoprostanes (F2-IsopS), malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS)] were measured. All blood samples were taken following at least an 8 hour fast. Comparison of SC levels with BMI was also examined.

For the first study, consistent positive correlations were observed for each of three separate same-day measurements and correlations (Pearson’s correlation of r = 0.82, 0.81, 0.80; p<0.001). Overall estimate of the line of best fit from ANCOVA using all three samples (n = 1,116) yielded a significant correlation (r = 0.81; p < 0.001). Based on ANOVA, SC by RS methodology exhibited 0.9% less variance over the three tests than serum carotenoids by the HPLC method (p<0.03; p<0.001). Similar correlation of SC and serum carotenoid levels was found in the second study (r=0.81). Regression analysis showed the strongest association of serum carotenoids was with β-carotene and skin carotenoids (r=0.76). SC measures were associated with serum levels of vitamins C (r = 0.37; p < 0.001) and E (alpha tocopherol); r = 0.34; p < 0.001) and inversely associated with urinary F2-IsopS (r = -0.19; p = 0.001), serum ORAC (r = -0.11, p= 0.06), urinary TBARS (r=-0.09, p=0.13) and MDA (r=0.10, p = 0.11). BMI was inversely correlated with SC.

SC is highly correlated with measurements made in human serum samples and less variable than serum carotenoids. Serum β-carotene is the carotenoid most highly correlated with SC. Additionally SC was modestly correlated with other key antioxidants (vitamins C and E), and inversely correlated with urinary (F2-IsopS) indicating the SC’s potential to give insight on a person’s overall antioxidant status. The inverse association of SC BMI warrant further investigation into influence i.e. oxidative stress, inflammation, etc on carotenoid status.

**Acknowledgements**

The authors are grateful to Neil Craft, PhD and his staff at Craft Technologies (Wilson, NC) for their expertise in HPLC analysis of serum carotenoids. The studies would have not been possible without the committed effort of the participants.

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INTRODUCTION

Raman spectroscopy is a highly specific form of vibrational spectroscopy that can be used to identify and quantify chemical compounds. Carotenoid molecules are especially suitable for Raman measurements because they can be excited with light overlapping their visible absorption bands; under these conditions they exhibit a very strong resonance Raman scattering (RRS) response, with an enhancement factor of ~5 orders of magnitude relative to nonresonant Raman spectroscopy (1). This allows one to detect in a noninvasive manner the characteristic vibrational energy levels of the carotenoids through their corresponding spectral fingerprint signature, even in complex biological systems.

Motivated to find a noninvasive, objective, in vivo method for the detection of carotenoid antioxidants in human tissue, we started developing RRS for this purpose ~6 years ago. Using excised eyecups as test samples, we applied the technology initially to the detection of the macular pigment (MP). Comprised of the carotenoids, lutein and zeaxanthin, and bound to the macular tissue in very high concentrations, the species are thought to play a major role in the prevention of age-related macular degeneration (2). Since that time, we extended the technology to in vivo MP measurements in the laboratory and clinical settings (3-6). Independent trials using the ocular Raman technology are now in progress at several sites worldwide. Recently we began to develop RRS spectroscopy for the detection of carotenoid antioxidants in human skin and mucosal tissue (7-10), tissues in which other carotenoid species such as lycopene and β-carotene are thought to play an important protective role, such as in the protection of skin from UV and short-wavelength visible radiation. The carotenoids, lutein and lycopene may also have protective functions for cardiovascular health, and lycopene may play a role in the prevention of prostate cancer. It is conceivable that skin levels of these species are correlated with corresponding levels in the internal tissues.
Raman Spectroscopy Used to Measure Antioxidant Capacity

Noninvasive measurements of clinical parameters are always preferable to those requiring blood, urine, or tissue. Raman spectroscopy is just such a measurement technique and may become widely used in the future. Using a laser light that is pointed toward the fat pad of the palm, as the laser light penetrates the skin, the amount of carotenoids (all-trans-beta-carotene, lycopene, alpha-carotene, gamma-carotene, phytoene, phytofluene, sepapreno-beta-carotene, 7,7’dihydro-betacarotene, astaxanthin, canthanxanthin, zeaxanthin, lutein, beta-apo8’carotenal, violaxanthins, and rhodoxanthin) is measured at the cellular level. Because all carotenoids have a carbon backbone with alternating carbon double and single bonds, the vibration of these bonds can be detected with Raman spectroscopy. Raman spectroscopy has been used to assess carotenoids in precancerous skin lesions as well as in the retina to assess early stages of macular degeneration (Ermakov et al., 2005). Caotenoids are powerful antioxidants, and because they are part of the “antioxidant network” a measure of their presence can give a good assessment of the antioxidant capacity of the cell. The Raman spectroscopy score also correlates inversely with urinary isoprostanes, a measure of oxidative stress (Carlson et al., 2006). Serum carotenoids significantly correlate with skin carotenoids, as measured using Raman spectroscopy and the Biophotonic Laser scanner (Smidt et al., 2004; Zidichouski et al., 2004). Serum carotenoids are a good measure of the absorptive capacity of the individual (see Chapter 3). Thus an individual with a diet high in fruits and vegetables and therefore large amounts of dietary carotenoids usually has a high carotenoid antioxidant score. The antioxidant score, or the numeric result from this scan, can be used to determine how well a person is processing carotenoid antioxidants and whether the antioxidants are reaching the cell where they exert their protective functions. The number, which seems to be in the range of 25,000 and higher in those with optimal health, increases with greater consumption of carotenoid-containing fruits and vegetables, consumption of carotenoid-containing nutritional supplements, smoking cessation, and loss of excess body fat (Carlson et al., 2006). The measurement is quick, easy, and inexpensive, making it a likely assessment tool for nutritional professionals in the future.
The following studies did not use the biophotonic scanner, however they establish carotenoid-status as an important biological marker

**Study reference:**
*Full length article available at: [http://www.ajcn.org/cgi/reprint/82/4/879](http://www.ajcn.org/cgi/reprint/82/4/879)*

**ABSTRACT**

BACKGROUND: Only a few observational studies have related plasma carotene and alpha-tocopherol to mortality in elderly subjects. OBJECTIVE: The objective was to study the association of plasma carotene (alpha-and beta-carotene) and alpha-tocopherol with all-cause and cause-specific mortality in elderly subjects who participated in a European prospective study. DESIGN: Plasma concentrations of carotene and alpha-tocopherol were measured in 1168 elderly men and women. After a follow-up period of 10 y, 388 persons had died. The association between plasma antioxidants and mortality was analyzed by using Cox proportional hazard models. To put our results in context, we performed a meta-analysis of 5 studies on plasma antioxidants and all-cause mortality in elderly populations. RESULTS: Plasma carotene concentrations were associated with a lower mortality risk [adjusted rate ratio (RR) for an increment of 0.39 micromol/L: 0.79; 95% CI: 0.70, 0.89]. This lower mortality risk was observed for both cancer (RR: 0.59; 95% CI: 0.44, 0.79) and cardiovascular disease (RR: 0.83; 95% CI: 0.70, 1.00). The lower risk of cardiovascular death was confined to those with a body mass index (in kg/m2) <25 (RR: 0.67; 95% CI: 0.49, 0.94). Plasma concentrations of alpha-tocopherol were not associated with all-cause or cause-specific mortality. The results for both plasma antioxidants and all-cause mortality were confirmed by the meta-analysis. CONCLUSIONS: This prospective study suggests that high plasma concentrations of carotene are associated both with lower mortality from all causes and with cancer in the elderly. For cardiovascular mortality, the inverse association was confined to elderly with body mass indexes <25.
**Study reference:**

*Full length article available at:* [http://jn.nutrition.org/cgi/reprint/134/3/562](http://jn.nutrition.org/cgi/reprint/134/3/562)

**ABSTRACT**

The consumption of fruits and vegetables reduces the risk of major chronic degenerative diseases. The active compounds and the mechanisms involved in this protective effect have not been well defined. The objective of this study was to determine the contribution of various food groups to total antioxidant intake, and to assess the correlations of the total antioxidant intake from various food groups with plasma antioxidants. We collected 7-d weighed dietary records in a group of 61 adults with corresponding plasma samples, and used data from a nationwide survey of 2672 Norwegian adults based on an extensive FFQ. The total intake of antioxidants was approximately 17 mmol/d with beta-carotene, alpha-tocopherol, and vitamin C contributing <10%. The intake of coffee contributed approximately 11.1 mmol, followed by fruits (1.8 mmol), tea (1.4 mmol), wine (0.8 mmol), cereals (i.e., all grain containing foods; 0.8 mmol), and vegetables (0.4 mmol). The intake of total antioxidants was significantly correlated with plasma lutein, zeaxanthin, and lycopene. Among individual food groups, coffee, wine, and vegetables were significantly correlated with dietary zeaxanthin, beta-carotene, and alpha-carotene. These data agree with the hypothesis that dietary antioxidants other than the well-known antioxidants contribute to our antioxidant defense. Surprisingly, the single greatest contributor to the total antioxidant intake was coffee.