

# RAMAN MEASUREMENT OF CAROTENOIDS IN LIVING TISSUES

A VALIDATED METHOD FOR DETERMINING MEANINGFUL ASPECTS OF HUMAN HEALTH





## Raman Measurement of Carotenoids in Living Tissues

*A validated method for determining meaningful aspects of human health*

Over 50 full-length peer-review articles validate the use of Raman spectroscopy for the measurement of carotenoids in living tissues. Additional abstracts presented at scientific symposiums further confirm the validity of this method.

The Pharmanex BioPhotonic Scanner uses resonant Raman light to detect concentrations carotenoids in *intact* human skin as an indicator of nutritional intake and *in vivo* antioxidant status. Prior to being adapted for measurements in human skin, Raman resonance was validated for detection of carotenoid concentrations in *intact* human retinas (an indicator of macular health). Well over a dozen peer-reviewed articles have been published on the use of Raman spectroscopy to detect macular carotenoids *in vivo*. Raman spectroscopy has also been validated as an accurate measurement of skin carotenoid concentrations. Pharmanex has published two full-length studies in English, and an additional two full-length studies in Chinese (Chinese publications presented data that is entirely unique from data published in English journals; the Chinese papers are not simply translations of the English publications).

The Pharmanex BioPhotonic Scanner is highlighted in the highly respected textbook: *Krause's Food, Nutrition and Diet Therapy* (12th Edition). It has also been given a complete chapter in the book *Carotenoids and Retinoids: Molecular Aspects and Health Issues*, which was edited by the distinguished Dr. Lester Packer (Father of the Antioxidants).

Pharmanex is not the only research group that has used and validated Raman spectroscopy for the measurement of skin carotenoid concentrations. Other research groups (all disinterested in Pharmanex, Nu Skin, or the sale of dietary supplements) have designed their own Raman spectrometers to measure skin carotenoids.

**THE FOLLOWING FULL-LENGTH STUDIES ARE CO-AUTHORED BY AT LEAST ONE IN-HOUSE PHARMANEX SCIENTIST, AND EACH OF THE FOLLOWING FOUR STUDIES USED THE PHARMANEX BIOPHOTONIC SCANNER.**

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1. Zidichouski JA, Mastaloudis A, Poole SJ, Reading JC, Smidt CR. **Clinical validation of a noninvasive, Raman spectroscopic method to assess carotenoid nutritional status in humans.** J Am Coll Nutr. 2009 Dec;28(6):687-93. Abstract available at: <http://www.ncbi.nlm.nih.gov/pubmed/20516269>

**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  $\geq 48$  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.*

2. Bergeson SD, Peatross JB, Eyring NJ, Fralick JF, Stevenson DN, Ferguson SB. **Resonance Raman measurements of carotenoids using light-emitting diodes.** J Biomed Opt. 2008 Jul-Aug;13(4):044026. Full-length article available at: <http://arxiv.org/pdf/0801.1697v1.pdf>

**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 f1.3 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%.*

3. Li CL, Bi SX, Zhu JS, Zhu ZG. **New functions of carotenoids and clinical assessments.** Shanghai Journal of Preventive Medicine 2006;6:261-264. [Article in Chinese] *No online abstract available- Article in Chinese.*
4. Li CL, Bi SX, Poole S, Smidt C, Zhu JS. **Human Skin Carotenoids in 88,611 subjects measured by Biophotonic Scanner.** Chinese Journal of Clinical Pharmacy 2006;15(2):124-125. [Article in Chinese]

#### **Abstract**

*The 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 ( $n=1116$ ,  $r^2=0.704$ ,  $p<0.001$ ). 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.*

5. Smidt, C.R., Burke, D.S. **Nutritional Significance and Measurement of Carotenoids.** *Current Topics in Nutraceutical Research*. 2004, Vol. 2, No. 2, pp. 79-91. Review **Abstract available at:** [http://ctnr.newcenturyhealthpublishers.com/about/issue\\_2\\_2.php#2](http://ctnr.newcenturyhealthpublishers.com/about/issue_2_2.php#2) Full-length article of the above available at same website.

### **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 blood-skin 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.*

THE FOLLOWING PUBLICATIONS ARE FULL-LENGTH STUDIES CONDUCTED BY THIRD PARTY RESEARCH GROUPS. EACH OF THE STUDIES USED THE PHARMANEX BIOPHOTONIC SCANNER TO MEASURE SKIN CAROTENOIDS. FULL-LENGTH VERSIONS CAN BE PURCHASED AT THE PUBMED LINKS PROVIDED BELOW.

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6. Holt EW, Wei EK, Bennett N, Zhang LM. **Low skin carotenoid concentration measured by resonance Raman spectroscopy is associated with metabolic syndrome in adults.** Nutr Res. 2014 Sep 6. pii: S0271-5317(14)00168-7. doi: 10.1016/j.nutres.2014.08.017. [Epub ahead of print] PubMed PMID: 25249018. <http://www.ncbi.nlm.nih.gov/pubmed/25249018>

#### **Abstract**

*Oxidative stress is increased in patients with metabolic syndrome (MS). Antioxidants, including carotenoids, are decreased in MS. We hypothesized that a low skin carotenoid score (SCS), calculated using resonance Raman spectroscopy, would correlate with the presence of MS. We retrospectively reviewed consecutive patients referred for dietary assessment between 2010 and 2012. For each patient, a nutrition history, medical history, and SCS were recorded.  $\chi^2$  and Student *t* test were used to determine factors associated with MS. Multivariate logistic regression was used to identify factors associated with MS. One hundred fifty-five patients were included. The mean age was  $54.1 \pm 13.1$  years, and the mean body mass index was  $28.3 \pm 6.1$  kg/m<sup>2</sup>. Metabolic syndrome was present in 43.9% of patients. The mean SCS was  $28\,084 \pm 14\,006$  Raman counts (RC), including  $23\,058 \pm 9812$  RC for patients with MS and  $32\,011 \pm 15\,514$  RC for patients without MS ( $P = .0001$ ). In a multivariate analysis, SCS less than 25 000 RC (odds ratio, 3.71; 95% confidence interval, 1.36-10.7;  $P = .01$ ) was independently associated with MS. A higher number of MS components was associated with a progressively lower SCS ( $P = .004$ ). In a consecutive sample of patients referred for dietary assessment, a noninvasively measured SCS was lower among patients with MS.*

7. Aguilar SS, Wengreen HJ, Lefevre M, Madden GJ, Gast J. **Skin carotenoids: a biomarker of fruit and vegetable intake in children.** J Acad Nutr Diet. 2014 Aug;114(8):1174-80. doi: 10.1016/j.jand.2014.04.026. Epub 2014 Jun 18. PubMed PMID: 24951435. Abstract at: <http://www.ncbi.nlm.nih.gov/pubmed/24951435>

#### **Abstract**

**BACKGROUND:** Studies of adult subjects have found a strong correlation between serum carotenoids and skin carotenoids measured by resonance Raman spectroscopy (RRS). No published studies have examined correlations between skin and serum carotenoids among children.

**OBJECTIVES:** We aimed to validate skin RRS methodology against serum carotenoid measurements by high-performance liquid chromatography and to determine whether RRS can be used as a valid biomarker of fruit and vegetable (F/V) intake among children.

**DESIGN:** In our cross-sectional study, participants were 45 healthy children aged 5 to 17 years who provided three blood samples used to assess serum carotenoid concentrations and three RRS skin measurements of the palm within a 4-week period. Dietary intake of F/V was assessed three times within 4 weeks using a 27-item food frequency questionnaire (FFQ) and an automated multiple-pass 24-hour daily recall. Estimates of intake from three FFQs, completed at least 7 days apart, were

averaged. Estimates of intake from 24-hour daily recalls were collected on 2 weekdays and 1 weekend day and averaged.

**RESULTS:** Levels of skin and serum carotenoids were highly correlated ( $R(2)=0.62$ ;  $P<0.001$ ). A linear regression model, controlling for child's weight and scanner unit, predicted that for every unit increase of total F/V from FFQ and total F/V as assessed by 24-hour daily recall, RRS intensity was predicted to increase by 3,798 ( $P=0.001$ ) and 3,504 ( $P=0.001$ ), respectively. Similar results were observed for reported high-carotenoid vegetable intake. Total carotenoid and beta carotene levels from 24-hour daily recalls correlated to total serum carotenoids levels ( $P<0.01$  and  $P<0.05$ , respectively). Total carotenoid, alpha carotene, and beta carotene levels from the 24-hour daily recalls correlated to RRS ( $P<0.01$ ).

**CONCLUSIONS:** Skin carotenoids measured by RRS were strongly correlated with serum carotenoid levels and were positively associated with estimates of intake from FFQ and an automated multiple-pass 24-hour daily recall among children aged 5 to 17 years. Skin carotenoids may be used as valid biomarker of F/V intake among children.

8. Ramírez-Vélez R, González-Ruiz K, García S, López-Alban CA, Escudero N, Agredo-Zúñiga RA. **Non-invasive assessment of  $\beta$ -carotene levels in the skin of colombian adults.** *Endocrinol Nutr.* 2012 Apr 13. <http://www.ncbi.nlm.nih.gov/pubmed/22503818>

#### **Abstract**

**INTRODUCTION:** Carotenoid pigments have antioxidant properties beneficial for human health. Use of resonance Raman spectroscopy (RRS) as a reliable method for measuring carotenoid levels in tissues such as dermis has been suggested. However, data about the variability and reproducibility of this technique should be collected before it can be used.

**OBJECTIVE:** To assess reproducibility of RRS for detection of total  $\beta$ -carotene levels in the skin of Colombian adults.

**DESIGN:** Forty-eight healthy men and 30 healthy women with various pigmentation levels were enrolled into the study. Measurements by RRS were performed in the palmar region and medial and lateral aspects of the arms. Odds ratio and 95% confidence intervals were calculated, adjusting for confounding factors: body mass index, waist circumference, percent body fat, age, race, smoking, and sex. Reproducibility of the technique was estimated using intraclass correlation coefficient (ICC).

**RESULTS:** Mean  $\beta$ -carotene levels were  $29.9 \pm 11.9$  in men and  $30.6 \pm 8.6$  in women ( $P=.787$ ). No differences or significant associations were found of  $\beta$ -carotene levels with confounding factors assessed by sex. ICCs were 0.89 in the palmar region, 0.85 in the medial aspect of arm, and 0.82 in the external aspect of arm.

**CONCLUSION:** RRS spectroscopy is a reliable method for non-invasive measurement of  $\beta$ -carotene levels in skin, and may be used as an important biomarker of antioxidant status in nutritional and health studies in humans.

9. Rerksuppaphol S, Rerksuppaphol L. **Carotenoid intake and asthma prevalence in Thai children.** *Pediatr Rep.* 2012 Jan 2;4(1):e12. Full-length article available free of charge at: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3357611/pdf/pr-2012-1-e12.pdf>

### **Abstract**

*Several antioxidant nutrients have been described to inversely correlate with asthma. In order to quantify the intake of these substances, it is possible to measure skin levels by Raman spectroscopy, a novel non-invasive technique that can also be used in children. This cross-sectional school-based study involved 423 children from a rural area of Thailand. Asthmatic children were diagnosed according to a Health Interview for Asthma Control questionnaire. Skin carotenoid levels were measured with Raman spectroscopy. Demographic data were obtained by directly interviewing children and their parents, whereas anthropometric parameters were measured by trained staff. Intake of carotenoids, vitamin A and C were evaluated by a food frequency questionnaire. Overall incidence of asthma in Thai schoolchildren (aged 3.5-17.8 years) was 17.3%. There was no significant difference in dietary intake of carotenoids and vitamin A and C, and skin carotenoid level between asthmatic and non-asthmatic children. Skin carotenoid level significantly correlated with all carotenoids and vitamin A intake ( $P < 0.05$ ). Carotenoids and vitamin A and C intakes, and skin carotenoid levels were not associated with the risk of asthma in Thai children. Skin carotenoids correlated with all carotenoids and vitamin A intake in mild to moderate degrees. Raman spectroscopy was confirmed to be a useful tool to determine antioxidant skin levels.*

10. Richer SP, Stiles W, Graham-Hoffman K, Levin M, Ruskin D, Wrobel J, Park DW, Thomas C. **Randomized, double-blind, placebo-controlled study of zeaxanthin and visual function in patients with atrophic age-related macular degeneration: The Zeaxanthin and Visual Function Study (ZVF)** FDA IND #78, 973. *Optometry.* Nov 2011; 82(11) 667-680. <http://www.ncbi.nlm.nih.gov/pubmed/22027699>

### **Abstract**

*BACKGROUND: The purpose of this study is to evaluate whether dietary supplementation with the carotenoid zeaxanthin (Zx) raises macula pigment optical density (MPOD) and has unique visual benefits for patients with early atrophic macular degeneration having visual symptoms but lower-risk National Institute of Health/National Eye Institute/Age-Related Eye Disease Study characteristics.*

*METHODS: This was a 1-year, n = 60 (57 men, 3 women), 4-visit, intention-to-treat, prospective, randomized controlled clinical trial of patients (74.9 years, standard deviation [SD] 10) with mild-to-moderate age-related macular degeneration (AMD) randomly assigned to 1 of 2 dietary supplement carotenoid pigment intervention groups: 8 mg Zx (n = 25) and 8 mg Zx plus 9 mg lutein (L) (n = 25) or 9 mg L ("Faux Placebo," control group, n = 10). Analysis was by Bartlett's test for equal variance, 3-way repeated factors analysis of variance, independent t test ( $P < 0.05$ ) for variance and between/within group differences, and post-hoc Scheffé's tests. Estimated foveal heterochromic flicker photometry, 1° macular pigment optical density (MPOD QuantifEye®), low- and high-contrast visual acuity, foveal shape discrimination (Retina Foundation of the Southwest), 10° yellow kinetic visual fields (KVF), glare recovery, contrast sensitivity function (CSF), and 6° blue cone ChromaTest® color thresholds were obtained serially at 4, 8, and 12 months.*



*RESULTS: Ninety percent of subjects completed  $\geq 2$  visits with an initial Age-Related Eye Disease Study report #18 retinopathy score of 1.4 (1.0 SD)/4.0 and pill intake compliance of 96% with no adverse effects. There were no intergroup differences in 3 major AMD risk factors: age, smoking, and body mass index as well as disease duration and Visual Function Questionnaire 25 composite score differences. Randomization resulted in equal MPOD variance and MPOD increasing in each of the 3 groups from 0.33 density units (du) (0.17 SD) baseline to 0.51 du (0.18 SD) at 12 m, ( $P = 0.03$ ), but no between-group differences (Analysis of Variance;  $P = 0.47$ ). In the Zx group, detailed high-contrast visual acuity improved by 1.5 lines, Retina Foundation of the Southwest shape discrimination sharpened from 0.97 to 0.57 ( $P = 0.06$ , 1-tail), and a larger percentage of Zx patients experienced clearing of their KVF central scotomas ( $P = 0.057$ ). The "Faux Placebo" L group was superior in terms of low-contrast visual acuity, CSF, and glare recovery, whereas Zx showed a trend toward significance.*

*CONCLUSION: In older male patients with AMD, Zx-induced foveal MPOD elevation mirrored that of L and provided complementary distinct visual benefits by improving foveal cone-based visual parameters, whereas L enhanced those parameters associated with gross detailed rod-based vision, with considerable overlap between the 2 carotenoids. The equally dosed (atypical dietary ratio) Zx plus L group fared worse in terms of raising MPOD, presumably because of duodenal, hepatic-lipoprotein or retinal carotenoid competition. These results make biological sense based on retinal distribution and Zx foveal predominance.*

11. Lima XT, Kimball AB. **Skin carotenoid levels in adult patients with psoriasis.** J Eur Acad Dermatol Venereol. 2011 Aug;25(8):945-9. <http://www.ncbi.nlm.nih.gov/pubmed/21054574>

### **Abstract**

*BACKGROUND: Psoriasis is an inflammatory disease that not only affects the skin but can also have systemic implications such as obesity and nutritional deficiencies. Carotenoids are vitamin A provitamins with anti-oxidant properties that are present in human tissues including skin.*

*OBJECTIVES: To determine whether psoriasis is associated with lower levels of skin carotenoid levels.*

*METHODS: In this cross-sectional study, skin carotenoid levels were measured on the palms of 44 patients with psoriasis and 72 patients without psoriasis. A linear regression model was used to evaluate the relationship between psoriasis and carotenoid levels (primary aim) and to determine if severity of disease was associated with carotenoid levels (secondary aim). Potential confounders included demographic factors, smoking status, body mass index and multivitamin intake.*

*RESULTS: The mean carotenoid levels in the psoriasis and no psoriasis groups were respectively 22,099 and 29,180 and presence of psoriasis was found to be significantly related to lower levels of carotenoids in both univariable and multivariable analysis ( $P < 0.05$ ). In the psoriasis group, the Psoriasis Area and Severity Index score was not significantly related to carotenoid levels ( $P = 0.07$ ).*

*CONCLUSIONS: Patients with psoriasis appear to have lower skin carotenoid counts than patients without psoriasis.*

12. Harpenau LA, Cheema AT, Zingale JA, Chambers DW, Lundergan WP. **Effects of nutritional supplementation on periodontal parameters, carotenoid antioxidant levels, and serum C-reactive protein.** J Calif Dent Assoc. 2011 May;39(5):309-12, 314-8. <http://www.ncbi.nlm.nih.gov/pubmed/21721475>

#### **Abstract**

*Few studies have focused on the role of nutrition in periodontal disease. The purpose of this trial was to determine the effect of a nutritional supplement on gingival inflammation, bleeding, probing depth, clinical attachment level, carotenoid antioxidant level, and C-reactive protein. The test supplement, consisting of a standard multivitamin formula, as well as several phytonutrients associated with anti-inflammatory/antioxidant effects, provided modest benefits in reducing inflammation; however, further studies with larger populations and longer intervention are warranted.*

13. Rerksuppaphol S, Rerksuppaphol L. **Effect of fruit and vegetable intake on skin carotenoid detected by non-invasive Raman spectroscopy.** J Med Assoc Thai. 2006 Aug;89(8):1206-12. Full-length article available free of charge at: [http://www.mat.or.th/journal/readpdf.php?link=files/Vol89\\_No8\\_1206.pdf](http://www.mat.or.th/journal/readpdf.php?link=files/Vol89_No8_1206.pdf)

#### **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.*

14. *Carotenoids and Retinoids: Molecular Aspects and Health Issues.*

Gellermann W, Zidichouski JA, Smidt CR, Bernstein PS. **Raman Detection of Carotenoids in Human Tissue.** In: Packer L, Obermueller-Jevic U, Kraemer K, and Sies H, eds. Champaign, IL: AOCS Press, 2005: Ch. 6, 86-114.

**Summary**

*The presence of carotenoids in the diet and their role in human health has become a subject of unprecedented interest. The chapters in this book represent an account of the information presented at a recent workshop, combined with several additional invited contributions to cover topics more completely that are currently at the cutting edge of research. Some of the highlights of this book include a thorough review of the special role that vitamin A intake plays in the health status of developing countries, the essential role of vitamin A in cell signaling, the molecular targets involved in carotenoid action in smoke-induced lung pathology, and how carotenoids are beneficial in cardiovascular health.*

15. Mahan LK and Escott-Stump S. (Eds.). *Krause's Food, Nutrition and Diet Therapy*, 12th Ed. Philadelphia, PA: Saunders 2007; Ch. 15, 427-428.

**Summary**

*Krause's Food, Nutrition, & Diet Therapy is a classic textbook in the field of nutrition and diet therapy, providing a wealth of information on nutrition basics, nutrition throughout the life cycle, nutrition care, nutrition for health and fitness, and medical nutrition therapy. Always up-to-date with the most current information available, this outstanding resource recognizes the increasing importance of nutrition in achieving and maintaining optimal health and fitness and as a component of complete and effective healthcare. It is universally recognized as an essential text for nutrition and diet therapy students and practicing registered dietitians. It features extensive appendixes, tables, illustrations, figures, and clinical insight boxes that provide practical hands-on information and clinical tools for use throughout a student's education and career.*

*Krause's Food & Nutrition Therapy has been considered one of the most authoritative nutrition texts for over 50 years worldwide. It provides a basic overview of nutrition as well as in-depth information on up-to-date nutrition therapies for medical conditions. Krause's is a text used by students in many allied health programs as well as other disciplines interested in the theoretical and clinical knowledge of the nutrition care process. It is commonly used as a reference for dietitians, nurses, doctors, dentists, life coaches, health educators and child development specialists.*

ALTHOUGH PHARMANEX (NU SKIN) OWNS EXCLUSIVE RIGHTS FOR USE OF RAMAN SPECTROSCOPY IN FOR PROFIT SETTINGS, OTHER RESEARCH GROUPS ARE PERMITTED TO DEVELOP THEIR OWN RAMAN SKIN CAROTENOID DEVICES FOR RESEARCH PURPOSES. THE FOLLOWING STUDIES USE RAMAN SPECTROSCOPY TO MEASURE SKIN CAROTENOID CONCENTRATIONS. THE RAMAN DEVICES USED IN THESE STUDIES WERE NOT THE PHARMANEX BIOPHOTONIC SCANNER. THE FACT THAT RESEARCH GROUPS OTHER THAN PHARMANEX (NU SKIN) HAVE VALIDATED RAMAN MEASUREMENT OF SKIN CAROTENOIDS FURTHER CONFIRMS THE LEGITIMACY OF THIS METHOD. FULL-LENGTH VERSIONS OF THE FOLLOWING STUDIES CAN BE PURCHASED AT THE PUBMED LINKS PROVIDED BELOW (ABSTRACTS AVAILABLE FREE OF CHARGE):

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16. Jahns L, Johnson LK, Mayne ST, Cartmel B, Picklo MJ Sr, Ermakov IV, Gellermann W, Whigham LD. **Skin and plasma carotenoid response to a provided intervention diet high in vegetables and fruit: uptake and depletion kinetics.** Am J Clin Nutr. 2014 Sep;100(3):930-7. <http://www.ncbi.nlm.nih.gov/pubmed/25008856?dopt=Abstract>

### **Abstract**

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

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

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

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

*CONCLUSION: Skin carotenoid status assessed by resonance Raman spectroscopy is a noninvasive, objective biomarker of changes in vegetable and fruit intake*

17. Darvin ME, Richter H, Ahlberg S, Haag SF, Meinke MC, Le Quintrec D, Doucet O, Lademann J. **Influence of sun exposure on the cutaneous collagen/elastin fibers and carotenoids: negative effects can be reduced by application of sunscreen.** J Biophotonics. 2014 Mar 18. <http://www.ncbi.nlm.nih.gov/pubmed/24639418>

#### **Abstract**

*Resonance Raman spectroscopy and multi-photon tomography were used in vivo to analyse the influence of sun exposure on the cutaneous carotenoids and collagen/elastin fibers. Comparing Berlin (low sun exposure) and Monegasque (high sun exposure) volunteers, it could be demonstrated that extended sun exposure significantly reduces the cutaneous carotenoids and collagen/elastin concentration ( $p < 0.05$ ). The tendency towards correlation ( $R^2 = 0.41$ ) between the dermal collagen/elastin (SAAID) and carotenoids confirms the important role of antioxidants in the protection against sun-induced negative effects. The application of sunscreen was shown to be effective, protecting cutaneous carotenoids and collagen/elastin from being damaged subsequent to sun exposure. (© 2014 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim).*

18. Ermakov IV, Ermakova MR, Rosenberg TD, Gellermann W. **Optical detection of carotenoid antioxidants in human bone and surrounding tissue.** J Biomed Opt. 2013 Nov 1;18(11):117006. <http://www.ncbi.nlm.nih.gov/pubmed/24213478>

#### **Abstract**

*Carotenoids are known to play an important role in health and disease state of living human tissue based on their antioxidant and optical filtering functions. In this study, we show that carotenoids exist in human bone and surrounding fatty tissue both in significant and individually variable concentrations. Measurements of biopsied tissue samples with molecule-specific Raman spectroscopy and high-performance liquid chromatography reveal that all carotenoids that are known to exist in human skin are also present in human bone. This includes all carotenes, lycopene,  $\beta$ -cryptoxanthin, lutein, and zeaxanthin. We propose quantitative reflection imaging as a noncontact optical method suitable for the measurement of composite carotenoid levels in bone and surrounding tissue exposed during open surgeries such as total knee arthroplasty, and as a proof of concept, demonstrate carotenoid measurements in biopsied bone samples. This will allow one to establish potential correlations between internal tissue carotenoid levels and levels in skin and to potentially use already existing optical skin carotenoid tests as surrogate marker for bone carotenoid status.*

19. Ermakov IV, Ermakova MR, Bernstein PS, Chan GM, Gellermann W. **Resonance Raman based skin carotenoid measurements in newborns and infants.** J Biophotonics. 2013 Oct;6(10):793-802. <http://www.ncbi.nlm.nih.gov/pubmed/23193015>

#### **Abstract**

*We describe Resonance Raman based skin carotenoid measurements in newborns and infants. Skin- and serum carotenoid levels correlate with high statistical significance in healthy newborns and infants, and with reduced accuracy also in prematurely born infants, who in general feature very low carotenoid levels and thin transparent skin giving rise to large background absorption effects.*

*Skin carotenoid levels can be easily compared among subjects and/or tracked in longitudinal studies with the highly molecule-specific Raman method. It therefore holds promise as a rapid, non-invasive, carotenoid antioxidant assessment method for newborns and infants in the field of pediatrics.*

20. Mayne ST, Cartmel B, Scarmo S, Jahns L, Ermakov IV, Gellermann W. **Resonance Raman spectroscopic evaluation of skin carotenoids as a biomarker of carotenoid status for human studies.** Arch Biochem Biophys. 2013 Jun 30. <http://www.ncbi.nlm.nih.gov/pubmed/23823930>

#### **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.*

21. Chan GM, Chan MM, Gellermann W, Ermakov I, Ermakova M, Bhosale P, Bernstein P, Rau C. **Resonance Raman spectroscopy and the preterm infant carotenoid status.** J Pediatr Gastroenterol Nutr. 2013 May;56(5):556-9. <http://www.ncbi.nlm.nih.gov/pubmed/23254446>

#### **Abstract**

*OBJECTIVE: The aim of the study was to validate the noninvasive resonance Raman spectroscopy (RRS) method in infants in comparison with the high-performance liquid chromatography (HPLC) method, and to evaluate the carotenoid status in preterm infants fed with mother's milk or formula.*

*METHODS: In the first phase of the study, resonance Raman measurements were made on male term infants' skin and correlated with tissue harvested at the time of circumcision. Each baby's foreskin was weighed, enzymatically digested, and the total carotenoids were extracted and quantitated by the HPLC. Next, to evaluate the carotenoid status of preterm infants (BW <1500 g), the skin and serum carotenoids in infants fed with either human milk or preterm formula were studied from the start of feedings and every 2 weeks until hospital discharge. Skin carotenoids were measured by RRS and the serum total carotenoids by HPLC.*

*RESULTS: Foreskin carotenoid levels measured by RRS correlated with HPLC measurements of total serum carotenoids ( $R = 0.52$ ,  $P < 0.01$ ,  $n = 16$ ). Forty preterm infants were studied for their carotenoid status. Thirty-two infants were fed mother's milk, whereas 8 were fed a preterm infant formula that was not enriched with carotenoids. The gestation and birth weight of the 2 feeding groups were similar. The infants fed human milk had a higher serum total carotenoid concentration*

and skin Raman counts than formula-fed infants. The skin Raman counts and total serum carotenoid correlated ( $R = 0.44$ ,  $P = 0.01$ ). The human milk-fed infants' serum total carotenoid concentrations and Raman values did not change during the study period; however, the formula-fed group's total serum and skin carotenoid decreased significantly during the study.

**CONCLUSIONS:** RRS of infant's skin reliably assesses total carotenoid status noninvasively. Human milk-fed preterm infants have higher serum and skin carotenoids than formula-fed infants suggesting that formula-fed infants may benefit from carotenoid supplementation.

22. Scarmo S, Cartmel B, Lin H, Leffell DJ, Ermakov IV, Gellermann W, Bernstein PS, Mayne ST. **Single v. multiple measures of skin carotenoids by resonance Raman spectroscopy as a biomarker of usual carotenoid status.** *Br J Nutr.* 2013 Sep;110(5):911-7. Full length article available free of charge at: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3696054/>

### Abstract

Resonance Raman spectroscopy (RRS) is a non-invasive method of assessing carotenoid status in the skin, which has been suggested as an objective indicator of fruit/vegetable intake. The present study assessed agreement and identified predictors of single v. multiple RRS measures of skin carotenoid status. A total of seventy-four participants had their skin carotenoid status measured in the palm of the hand by RRS at six time points over 6 months. Questionnaires were administered to collect information on demographic, lifestyle and dietary data. Mean age of the participants was 36.6 years, 62.2% were female, 83.8% Caucasian and 85.1% were non-smoking at baseline. There was a good agreement between a single measure of skin carotenoids by RRS and multiple measures (weighted  $\kappa = 0.80$ ; 95% CI 0.72, 0.88). The same variables were significantly associated with carotenoid status based on single or multiple measures, including a positive association with intake of total carotenoids ( $P < 0.01$ ) and an inverse association with season of measurement ( $P \leq 0.05$ ). The exception was recent sun exposure, which emerged as a significant predictor of lower carotenoid status only when using multiple RRS measures ( $P \leq 0.01$ ). A single RRS measure was reasonably accurate at classifying usual skin carotenoid status. Researchers using RRS may want to take into account other factors that are associated with the biomarker, including season of measurement and recent sun exposure.

23. Darwin ME, Sandhagen C, Koecher W, Sterry W, Lademann J, Meinke MC. **Comparison of two methods for noninvasive determination of carotenoids in human and animal skin: Raman spectroscopy versus reflection spectroscopy.** *J Biophotonics.* 2012 Jan 23. <http://www.ncbi.nlm.nih.gov/pubmed/22271669>

### Abstract

Based on compelling *in vivo* and *in vitro* studies on human skin, carotenoids are thought to be of great interest as powerful antioxidants acting to prevent free-radical-induced damages, including premature skin ageing and the development of skin diseases such as cancer. Among the available techniques that are suitable for noninvasive determination of carotenoids in human skin, are resonance Raman spectroscopy (RRS) and reflection spectroscopy (RS). For RS, a LED-based miniaturized spectroscopic system (MSS) was developed for noninvasive measurement of carotenoids in human skin. The optimization and subsequent calibration of the MSS was performed with the use of RRS. A strong correlation between the carotenoid concentration determined by the RS and for the RRS system was achieved for human skin *in vivo* ( $R = 0.88$ ) and for bovine udder skin *in vitro* ( $R = 0.81$ ).

24. Fluhr JW, Sassning S, Lademann O, Darvin ME, Schanzer S, Kramer A, Richter H, Sterry W, Lademann J. **In vivo skin treatment with tissue-tolerable plasma influences skin physiology and antioxidant profile in human stratum corneum.** *Exp Dermatol.* 2012 Feb;21(2):130-4. <http://www.ncbi.nlm.nih.gov/pubmed/22142271>

#### **Abstract**

*The antimicrobial treatment of wounds is still a major problem. Tissue-tolerable electrical plasma (TTP) is a new approach for topical microbial disinfection of the skin surface. The aim of the present study was to investigate the influence of TTP on a carotenoid profile in relation to skin physiology parameters (epidermal barrier function, stratum corneum (SC) hydration, surface temperature and irritation parameters). We were interested in the interaction of TTP and the antioxidative network, as well as the consequences for skin physiology parameters. These parameters are also indicative of TTP safety in vivo. For plasma application, 'Kinpen 09' was used (surface exposure 30-43°C) for 3 s. Beta-carotene and water profiles were assessed by in vivo Raman microspectroscopy (skin composition analyzer 3510). Skin physiology parameters were measured with Tewameter TM 300, Corneometer CM 825, skin thermometer and Chromameter CR 300. All parameters were assessed non-invasively on seven healthy volunteers before and after plasma application in vivo. We could show that TTP application leads to a decrease in beta-carotene especially in the superficial SC. Skin-surface temperature increased by 1.74°C, while the transepidermal water loss (TEWL) increase indicated an impaired barrier function. SC hydration decreased as seen in water profile especially in the superficial layers and capacitance values. A slight increase in skin redness was measurable. The induction of reactive oxygen species is probably the major contributor of TTP efficacy in skin disinfection. Skin physiology parameters were influenced without damaging the skin or skin functions, indicating the safety of TTP under in vivo conditions.*

25. Scarmo S, Henebery K, Peracchio H, Cartmel B, Lin H, Ermakov IV, Gellermann W, Bernstein PS, Duffy VB, Mayne ST. **Skin carotenoid status measured by resonance Raman spectroscopy as a biomarker of fruit and vegetable intake in preschool children.** *Eur J Clin Nutr.* 2012 May;66(5):555-60. Full length article available free of charge at: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3380427/pdf/nihms381137.pdf>

#### **Abstract**

*BACKGROUND/OBJECTIVE: Dietary assessment in children is difficult, suggesting a need to develop more objective biomarkers of intake. Resonance Raman spectroscopy (RRS) is a non-invasive, validated method of measuring carotenoid status in skin as a biomarker of fruit/vegetable intake. The purpose of this study was to examine the feasibility of using RRS in preschool children, to describe inter-individual variability in skin carotenoid status and to identify factors associated with the biomarker in this population.*

*SUBJECTS/METHODS: We conducted a cross-sectional study of 381 economically disadvantaged preschoolers in urban centers in Connecticut (USA). In all, 85.5% were black non-Hispanic or Hispanic/Latino, and 14.1% were obese and 16.9% were overweight by age- and sex-specific body mass index (BMI) percentiles. Children had their skin carotenoid status assessed by RRS in the palm of the hand. Fruit/vegetable consumption was assessed by a brief parent/guardian-completed food frequency screener and a liking survey.*



*RESULTS: We observed inter-individual variation in RRS values that was nearly normally distributed. In multiple regression analysis, higher carotenoid status, measured by RRS, was positively associated with fruit/vegetable consumption ( $P=0.02$ ) and fruit/vegetable preference ( $P<0.01$ ). Lower carotenoid status was observed among younger children, those participating in the US Supplemental Nutrition Assistance Program, and those with greater adiposity ( $P<0.05$  for all).*

*CONCLUSIONS: We observed wide variability in skin carotenoid status in a population of young children, as assessed by RRS. Parent-reported fruit/vegetable intake and several demographic factors were significantly associated with RRS-measured skin carotenoid status. We recommend further development of this biomarker in children, including evaluating response to controlled interventions.*

26. Vierck HB, Darwin ME, Lademann J, Reißhauer A, Baack A, Sterry W, Patzelt A. **The influence of endurance exercise on the antioxidative status of human skin.** Eur J Appl Physiol. 2012 Jan 22. <http://www.ncbi.nlm.nih.gov/pubmed/22270481>

#### **Abstract**

*Oxidative stress is supposed to be responsible for a diversity of diseases. For protection purposes, the human organism exhibits a line-up of antioxidant substances functioning as radical catchers. As a result of neutralization of free radicals, antioxidants are destroyed. Therefore, the degradation of the antioxidants can be utilized as an indirect parameter for the measurement of free radical formation. As physical exercise may also induce oxidative stress, the aim of the present study was to determine the antioxidant substances, and more precisely, the carotenoid concentration in the skin of male volunteers during different sportive exposures (cycling and running with two different exercise intensities) with resonance Raman spectroscopic measurements. The results revealed that moderate and high intensity cycling and running decrease the carotenoid concentration of the skin, whereas both sport disciplines and both exercise intensities revealed similar results. It can be concluded that above a certain threshold, physical exercise leads to oxidative stress also in the skin associated with the decrease in the antioxidant concentration. This gives rise to the impairment of the first defence line of the skin and means an increase in the risk of sun exposure-induced damage, e.g., when exercise training is performed outside. Nevertheless, it has to be emphasized that sport in general applied at moderate loads has predominantly positive effects on the health of humans especially concerning cardiovascular and metabolic diseases.*

27. Darwin ME, Fluhr JW, Meinke MC, Zastrow L, Sterry W, Lademann J. **Topical beta-carotene protects against infra-red-light-induced free radicals.** Exp Dermatol. 2011 Feb;20(2):125-9. <http://www.ncbi.nlm.nih.gov/pubmed/21255091>

#### **Abstract**

*The influence of stress factors on human skin induces the production of free radicals. Free radicals react immediately with antioxidants contained in the skin, giving rise to their depletion and with the surrounding molecules, resulting in their damage, disorganization and even destruction. High amounts of free radicals are produced in the upper skin layers, i.e. mainly in the epidermis, subsequent to sun irradiation. Irradiation of the skin in the infra-red (IR) range of the spectra, applied at physiological doses, can produce free radicals. The magnitude of destruction of antioxidants, such as carotenoids, can serve as a marker of the extent of the stress factor,*

characterized by the quantity of produced free radicals. In this study, measurements on the degradation of cutaneous carotenoids following IR skin irradiation of 12 healthy volunteers (skin type II), with two IR sources (standard infrared radiator = SIR and water filter infrared = wIRA) were taken using resonance Raman spectroscopy. Topical application of the antioxidant beta-carotene (2 mg/cm<sup>2</sup>) provided protection for the human skin when exposed to IR radiation. The magnitude of the degradation of dermal carotenoids after IR irradiation was significantly higher for SIR than for wIRA irradiation, for both non-treated and cream-treated skin areas. The amount of destroyed carotenoids after IR irradiation was higher in the case of pretreatment with beta-carotene than for the untreated skin, indicating that the superficial part of antioxidants is most important for protecting against external stressors. The direct comparison of beta-carotene content was significantly higher for the cream-treated compared to untreated areas for all pairs: baseline, wIRA, after wIRA, baseline SIR and after SIR. Additionally, topically applied carotenoids as a single antioxidant component are less stable than the carotenoids in the skin incorporated by nutrition and accumulated in a mixture with different antioxidant substances. Resonance Raman spectroscopy can be used for the non-invasive measurements of carotenoids, which can be rated as marker substances of redox processes.

28. Darvin ME, Fluhr JW, Schanzer S, Richter H, Patzelt A, Meinke MC, Zastrow L, Golz K, Doucet O, Sterry W, Lademann J. **Dermal carotenoid level and kinetics after topical and systemic administration of antioxidants: enrichment strategies in a controlled in vivo study.** J Dermatol Sci. 2011 Oct;64(1):53-8. <http://www.ncbi.nlm.nih.gov/pubmed/21763110>

### **Abstract**

**BACKGROUND:** High doses of sun-emitted UV-radiation induce reactive oxygen species (ROS) as major pro-oxidants thus inducing premature skin aging. The best prevention of the destructive action of free radicals in human skin is textile coverings, topical sunscreens and the development of a high antioxidative protective network.

**OBJECTIVE:** The effects of topical, systemic and combined application of antioxidants (AO) were investigated on human skin in vivo.

**METHODS:** Topical application of creams and systemic incorporation of tablets both containing AO was investigated in vivo by resonance Raman spectroscopy.

**RESULTS:** Topical, systemic and combined AO-treatments induced a statistically significant increase of AO levels in human skin while placebo did not show any changes. The highest accumulation was induced by the combination of topical and systemic AO. Carotenoid-tablets combined with placebo-cream induced less carotenoid accumulation than carotenoid-tablets alone. Carotenoid levelling after the end of treatment lasted for around 2 weeks following the topical application of AOs, and up to 5 weeks after systemic administration, depending on the BMI of volunteers.

**CONCLUSION:** Topically applied AO are stored in the SC for a short time only due to the rapid AO-depletion by desquamation, textile contact, washing and environmental stress. In contrast to topical application, the systemically applied carotenoids are stored in the body fat tissue and slowly released onto the skin surface with sweat and sebum. The combined topical and systemic application of AO represents an optimal form of protection of the AO-network.

29. Darvin ME, Haag SF, Meinke MC, Sterry W, Lademann J. **Determination of the influence of IR radiation on the antioxidative network of the human skin.** J Biophotonics. 2011 Jan;4(1-2):21-9. <http://www.ncbi.nlm.nih.gov/pubmed/20151398>

#### **Abstract**

*Production of free radicals in the human skin subsequent to IR irradiation has been demonstrated by means of two different methods. The first technique, based on resonance Raman spectroscopy, enables the non-invasive measurements of the kinetics of cutaneous carotenoid antioxidants beta-carotene and lycopene, subsequent to IR irradiation. Obtained degradation of the cutaneous carotenoids was a hint but not evidence that IR irradiation can produce free radicals in the skin. Therefore, the direct observation sustaining the production of free radicals subsequent to IR irradiation in the skin was performed in-vitro by electron paramagnetic resonance spectroscopy. Enzymatic processes as well as heat shock-induced radicals in the human skin are presumably involved in the energy transfer from IR irradiation into the molecules of the skin. Protection strategy for human skin against IR-induced free radicals based on the increase in the concentration of antioxidants by means of antioxidant-rich supplementation is discussed.*

30. Dayan SH, Arkins JP, Sharma V, Paterson E, Barnes D. **A phase 2, double-blind, randomized, placebo-controlled trial of a novel nutritional supplement product to promote healthy skin.** J Drugs Dermatol. 2011 Oct;10(10):1106-14. <http://www.ncbi.nlm.nih.gov/pubmed/21968660>

#### **Abstract**

*BACKGROUND: Despite an abundance of nutritional supplements, very few well-controlled trials have assessed their beneficial effect on the skin, such as hydration, antioxidant levels, texture or appearance. The objective of the following placebo-controlled, double-blind study was to determine the effects of the Skin Health Experimental Product (SHEP) on skin health.*

*METHODS: The study enrolled healthy men and women aged 30 years or older. Subjects were randomized to receive a twice-daily regimen of SHEP or placebo. The effects SHEP had on overall skin appearance and health were assessed by measuring improvements in: (1) skin hydration using a closed-aperture transepidermal water-loss moisture meter and a vapometer; (2) skin texture using silicon profilometry; (3) skin carotenoid concentration using Raman spectrometry; and (4) reported self-image assessments using the Global Aesthetic Improvement Scale (GAIS).*

*RESULTS: SHEP-treated subjects demonstrated a significant reduction in fine lines compared to the placebo-treated group. Raman spectroscopy showed that SHEP increased carotenoids at some measurement sites. Based on the GAIS, SHEP-treated subjects were three times more likely to perceive an improvement in their appearance compared to placebo-treated subjects ( $P > 0.049$ ).*

*CONCLUSION: The orally-administered SHEP nutritional supplement improves skin texture, carotenoid levels in specific areas of the hand, and improves patients' perception of skin health.*

31. Fluhr JW, Caspers P, van der Pol JA, Richter H, Sterry W, Lademann J, Darvin ME. **Kinetics of carotenoid distribution in human skin in vivo after exogenous stress: disinfectant and wIRA-induced carotenoid depletion recovers from outside to inside.** J Biomed Opt. 2011 Mar;16(3):035002. <http://www.ncbi.nlm.nih.gov/pubmed/21456863>

#### **Abstract**

*The human organism has developed a protection system against the destructive effect of free radicals. The aim of the present study was to investigate the extent of exogenous stress factors such as disinfectant and IR-A radiation on the skin, and their influence on the kinetics of carotenoids distribution during the recovery process. Ten healthy volunteers were assessed with resonance spectroscopy using an Argon-laser at 488 nm to excite the carotenoids in vivo. Additionally, Raman-confocal-micro-spectroscopy measurements were performed using a model 3510 Skin Composition Analyzer with spatially resolved measurements down to 30  $\mu\text{m}$ . The measurements were performed at a baseline of 20, 40, 60, and 120 min after an external stressor consisting either of water-filtered infrared A (wIRA) with 150 mW/cm<sup>2</sup> or 1 mJ/cm<sup>2</sup> of an alcoholic disinfectant. Both Raman methods were capable to detect the infrared-induced depletion of carotenoids. Only Raman-microspectroscopy could reveal the carotenoids decrease after topical disinfectant application. The carotenoid-depletion started at the surface. After 60 min, recovery starts at the surface while deeper parts were still depleted. The disinfectant- and wIRA-induced carotenoid depletion in the epidermis recovers from outside to inside and probably delivered by sweat and sebaceous glands. We could show that the Raman microscopic spectroscopy is suited to analyze the carotenoid kinetic of stress effects and recovery.*

32. Haag SF, Taskoparan B, Darvin ME, Groth N, Lademann J, Sterry W, Meinke MC. **Determination of the antioxidative capacity of the skin in vivo using resonance Raman and electron paramagnetic resonance spectroscopy.** Exp Dermatol. 2011 Jun;20(6):483-7. <http://www.ncbi.nlm.nih.gov/pubmed/21366704>

#### **Abstract**

*BACKGROUND: Non-invasive measurements are of major interest for investigating the effects of stress, nutrition, diseases or pharmaceuticals on the antioxidative capacity of the human skin. However, only a few non-invasive methods are available.*

*MATERIAL AND METHODS: The resonance Raman spectroscopy is well established to monitor carotenoids in the skin, but correlations with other antioxidants have not yet been described. Electron paramagnetic resonance spectroscopy used for measurements of free radicals has already been used elsewhere to investigate the reduction of applied long-living nitroxide radicals, caused by skin antioxidants and UV irradiation, but only a single or up to four volunteers were included in these studies. Therefore, in this study, the two methods were applied in parallel on 17 volunteers, and the rate constant of the nitroxide decrease was correlated with the cutaneous carotenoid concentration.*

*RESULTS AND DISCUSSION: A correlation with  $R = 0.65$  was found, supporting the thesis that different antioxidants protect each other and build an antioxidative network in the skin. The results also give first indications that the carotenoids serve as marker substances for the antioxidative capacity, if the nutrition is well balanced.*

33. Lademann J, Meinke MC, Sterry W, Darvin ME. **Carotenoids in human skin.** Exp Dermatol. 2011 May;20(5):377-82. <http://www.ncbi.nlm.nih.gov/pubmed/21366698>

#### **Abstract**

*The interaction of free radicals with antioxidants is a topic of increasing interest in the development of prevention strategies against skin ageing. Carotenoids can serve as marker substances for the complete antioxidative network of human skin. Recently, it has become possible to measure the carotenoids non-invasively and online using resonance Raman spectroscopy. This method has been used in various studies to investigate the interaction of carotenoid antioxidants and free radicals in human skin. In this review, the results of the selected studies are summarized and compared. It could be demonstrated that the carotenoid concentration of the skin reflects the lifestyle of individuals. A high level of carotenoids can be achieved with a healthy diet rich, for instance, in fruit and vegetables. Stress factors such as illness, UV and IR radiation of the sun, and smoking and alcohol consumption reduce the concentration of the carotenoids in the skin. It could be demonstrated that premature skin ageing was less in people with a high level of antioxidants in their tissue. Consequently, the furrows and wrinkles were not so deep and dense as in the skin of individuals with a low antioxidant level. The measurements are highly suited for the development of anti-ageing strategies and can be efficiently used in the medical diagnostics and therapy control.*

34. Lademann J, Schanzer S, Meinke M, Sterry W, Darvin ME. **Interaction between carotenoids and free radicals in human skin.** Skin Pharmacol Physiol. 2011;24(5):238-44. <http://www.ncbi.nlm.nih.gov/pubmed/21447993>

#### **Abstract**

*Environmental factors like air pollutants, radiation of the sun and stress factors such as illness, smoking, or alcohol abuse produce free radicals in the human tissue as well as in the skin. Free radicals serve as the main cause for premature skin aging. Additionally, they also contribute towards immunosuppression and the formation of skin diseases including cancer. The human organism has developed a protection system against the destructive action of free radicals by means of the antioxidant network. In the present study, the interaction of free radicals and carotenoid antioxidants in the human skin under in vivo conditions was investigated and summarized. The measurement of carotenoids in human skin was performed in vivo using resonance Raman spectroscopy.*

35. Darvin ME, Haag S, Meinke M, Zastrow L, Sterry W, Lademann J. **Radical production by infrared A irradiation in human tissue.** *Skin Pharmacol Physiol.* 2010;23(1):40-6.. <http://www.ncbi.nlm.nih.gov/pubmed/20090407>

#### **Abstract**

*The influence of the ultraviolet (UV) irradiation of the sun on the formation of free radicals in human skin is well investigated. Up to now, only small amounts of data are available stating that infrared (IR) irradiation can produce free radicals in the skin. In the present study, the formation of free radicals in human skin, subsequent to IRA irradiation (600-1,500 nm), has been demonstrated by means of two different methods. Firstly, the radical formation was detected indirectly by the degradation of the cutaneous carotenoid antioxidants beta-carotene and lycopene, which was investigated in vivo by resonance Raman spectroscopic measurements. Secondly, the direct observation of produced radicals subsequent to IRA irradiation of the skin was performed in vitro by electron paramagnetic resonance spectroscopy. Taking into account the results of the present study and previous UV light studies, it can be expected that also solar irradiation in the visible spectral range will produce free radicals in the human skin. Therefore, the current sun protection strategies should be reconsidered. Furthermore, it was shown in the present study that the side effect in the form of radical formation could be significantly reduced by increasing the protection system of the human organism in form of the antioxidant network.*

36. Ermakov IV, Gellermann W. **Validation model for Raman based skin carotenoid detection.** *Arch Biochem Biophys.* 2010 Dec 1;504(1):40-9. <http://www.ncbi.nlm.nih.gov/pubmed/20678465>

#### **Abstract**

*Raman spectroscopy holds promise as a rapid objective non-invasive optical method for the detection of carotenoid compounds in human tissue in vivo. Carotenoids are of interest due to their functions as antioxidants and/or optical absorbers of phototoxic light at deep blue and near UV wavelengths. In the macular region of the human retina, carotenoids may prevent or delay the onset of age-related tissue degeneration. In human skin, they may help prevent premature skin aging, and are possibly involved in the prevention of certain skin cancers. Furthermore, since carotenoids exist in high concentrations in a wide variety of fruits and vegetables, and are routinely taken up by the human body through the diet, skin carotenoid levels may serve as an objective biomarker for fruit and vegetable intake. Before the Raman method can be accepted as a widespread optical alternative for carotenoid measurements, direct validation studies are needed to compare it with the gold standard of high performance liquid chromatography. This is because the tissue Raman response is in general accompanied by a host of other optical processes which have to be taken into account. In skin, the most prominent is strongly diffusive, non-Raman scattering, leading to relatively shallow light penetration of the blue/green excitation light required for resonant Raman detection of carotenoids. Also, sizable light attenuation exists due to the combined absorption from collagen, porphyrin, hemoglobin, and melanin chromophores, and additional fluorescence is generated by collagen and porphyrins. In this study, we investigate for the first time the direct correlation of in vivo skin tissue carotenoid Raman measurements with subsequent chromatography derived carotenoid concentrations. As tissue site we use heel skin, in which the stratum corneum layer thickness exceeds the light penetration depth, which is free of optically confounding chromophores, which can be easily optically accessed for in vivo RRS measurement, and which can be easily removed for subsequent biochemical measurements. Excellent correlation (coefficient  $R=0.95$ ) is obtained for this tissue site which could serve as a model site for scaled up future validation studies of large populations. The obtained results provide proof that resonance Raman spectroscopy is a valid non-invasive objective methodology for the quantitative assessment of carotenoid antioxidants in human skin in vivo.*

37. Meinke MC, Darvin ME, Vollert H, Lademann J. **Bioavailability of natural carotenoids in human skin compared to blood.** Eur J Pharm Biopharm. 2010 Oct;76(2):269-74.  
<http://www.ncbi.nlm.nih.gov/pubmed/20558286>

#### **Abstract**

*Skin functions and structure are significantly influenced by nutrients. Antioxidants protect the supportive layer of the skin against any damaging irradiation effects and the action of free radicals. A lack of suitable methods means that the pharmacokinetic properties of systemically applied carotenoids transferred into the skin remain poorly understood. In this study, a natural kale extract or placebo oil were given orally to 22 healthy volunteers for 4 weeks. Carotenoid bioaccessibility was evaluated using non-invasive resonance Raman spectroscopy on the palm and forehead skin. For the analysis of the blood serum, the standard HPLC method was used. The blood and skin levels of the carotenoids increased significantly during the study but compared to the blood serum values, increases in skin were delayed and depended on the dermal area as well as on the carotenoid. Lycopene, measured as being low in the extract, increases more in the skin compared to the blood indicating that the natural mixture of the extract stabilizes the antioxidative network in the skin. After supplementation had ended, the carotenoids decreased much faster in the blood than in the skin. The delayed decrease in the skin may indicate a peripheral buffer function of the skin for carotenoids.*

38. Scarmo S, Cartmel B, Lin H, Leffell DJ, Welch E, Bhosale P, Bernstein PS, Mayne ST. **Significant correlations of dermal total carotenoids and dermal lycopene with their respective plasma levels in healthy adults.** Arch Biochem Biophys. 2010 Dec 1;504(1):34-9. Full length article available free of charge at: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2957565/pdf/nihms229903.pdf>

#### **Abstract**

*Carotenoids in skin have been known to play a role in photoprotection against UV radiation. We performed dermal biopsies of healthy humans (N=27) and collected blood samples for pair-wise correlation analyses of total and individual carotenoid content by high performance liquid chromatography (HPLC). The hydrocarbon carotenoids (lycopene and beta-carotene) made up the majority of carotenoids in both skin and plasma, and skin was somewhat enriched in these carotenoids relative to plasma. Beta-cryptoxanthin, a monohydroxycarotenoid, was found in similar proportions in skin as in plasma. In contrast, the dihydroxycarotenoids, lutein and zeaxanthin, were relatively lacking in human skin in absolute and relative levels as compared to plasma. Total carotenoids were significantly correlated in skin and plasma ( $r=0.53$ ,  $p<0.01$ ). Our findings suggest that human skin is relatively enriched in lycopene and beta-carotene, compared to lutein and zeaxanthin, possibly reflecting a specific function of hydrocarbon carotenoids in human skin photoprotection.*

39. Mayne ST, Cartmel B, Scarmo S, Lin H, Leffell DJ, Welch E, Ermakov I, Bhosale P, Bernstein PS, Gellermann W. **Noninvasive assessment of dermal carotenoids as a biomarker of fruit and vegetable intake.** Am J Clin Nutr. 2010 Oct;92(4):794-800. Full length article available free of charge at: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3133234/pdf/ajcn9240794.pdf>

#### **Abstract**

*BACKGROUND: Resonance Raman spectroscopy (RRS) has been suggested as a feasible method for noninvasive carotenoid measurement of human skin. However, before RRS measures of dermal carotenoids can be used as a biomarker, data on intra- and intersubject variability and validity are needed.*

*OBJECTIVE: The purpose of this study was to evaluate the reproducibility and validity of RRS measures of dermal total carotenoids and lycopene in humans.*

*DESIGN: In study 1, 74 men and women with diverse skin pigmentation were recruited. RRS measures of the palm, inner arm, and outer arm were obtained at baseline, 1 wk, 2 wk, 1 mo, 3 mo, and 6 mo (to maximize seasonal variation). The RRS device used visible light at 488 nm to estimate total carotenoids and at 514 nm to estimate lycopene. Reproducibility was assessed by intraclass correlation coefficients (ICCs). In study 2, we recruited 28 subjects and assessed dietary carotenoid intake, obtained blood for HPLC analyses, performed RRS measures of dermal carotenoid status, and performed dermal biopsies (3-mm punch biopsy) with dermal carotenoids assessed by HPLC.*

*RESULTS: ICCs for total carotenoids across time were 0.97 (palm), 0.95 (inner arm), and 0.93 (outer arm). Total dermal carotenoids assessed by RRS were significantly correlated with total dermal carotenoids assessed by HPLC of dermal biopsies ( $r = 0.66$ ,  $P = 0.0001$ ). Similarly, lycopene assessed by RRS was significantly correlated with lycopene assessed by HPLC of dermal biopsies ( $r = 0.74$ ,  $P < 0.0001$ ).*

*CONCLUSION: RRS is a feasible and valid method for noninvasively assessing dermal carotenoids as a biomarker for studies of nutrition and health.*

40. Blume-Peytavi U, Rolland A, Darvin ME, Constable A, Pineau I, Voit C, Zappel K, Schäfer-Hesterberg G, Meinke M, Clavez RL, Sterry W, Lademann J. **Cutaneous lycopene and beta-carotene levels measured by resonance Raman spectroscopy: high reliability and sensitivity to oral lactolycopene deprivation and supplementation.** Eur J Pharm Biopharm. 2009 Sep;73(1):187-94. <http://www.ncbi.nlm.nih.gov/pubmed/19442725>

#### **Abstract**

*Carotenoids, naturally occurring lipophilic micronutrients, possess an antioxidant activity associated with protection from damage induced by free radicals. The present study investigated an innovative non-invasive method to measure cutaneous levels of lycopene and beta-carotene and to monitor the distribution of orally administered lactolycopene in human skin and plasma. A double-blind placebo-controlled randomized study was performed in 25 volunteers, who were under a lycopene-deprived diet (4 weeks prior to study until end of the study) and orally received either lactolycopene or placebo for 12 weeks. Skin and plasma levels of lycopene and beta-carotene were monitored monthly using Raman spectroscopy and HPLC, respectively. Cutaneous levels of lycopene and beta-carotene monitored by resonance Raman spectroscopy showed high reliability. Irrespective of the investigated area, cutaneous levels were sensitive to lycopene deprivation and to oral*



*supplementation; the forehead showed the closest correlation to lycopene variation in plasma. Plasma and skin levels of lycopene were both sensitive to oral intake of lycopene and, interestingly, also skin levels of beta-carotene. Thus, oral supplementation with lycopene led to an enrichment of beta-carotene in human skin, possibly due to the fact that carotenoids act in the skin as protection chains, with a natural protection against free radicals*

41. Darvin ME, Fluhr JW, Caspers P, van der Pool A, Richter H, Patzelt A, Sterry W, Lademann J. **In vivo distribution of carotenoids in different anatomical locations of human skin: comparative assessment with two different Raman spectroscopy methods.** *Exp Dermatol.* 2009 Dec;18(12):1060-3. <http://www.ncbi.nlm.nih.gov/pubmed/19650865>

#### **Abstract**

*BACKGROUND: The cutaneous antioxidants form an efficient protection system against the destructive potential of free radicals, produced by environmental factors, such as UV-sun irradiation, hazardous substances and lifestyle habits. Most of the antioxidants cannot be produced by the human organism. Thus, they have to be incorporated by food and beverages.*

*MATERIAL AND METHODS: In the present manuscript, the distribution of carotenoids as a marker for antioxidative potential in human skin was investigated with two different in vivo Raman spectroscopy methods with an excitation wavelength of 785 nm (Skin Analyzer) and at 488 nm (resonance Raman spectroscopy). The carotenoid profile was assessed at three different anatomical locations (palm, forehead and volar forearm) in 12 healthy volunteers.*

*RESULTS: In untreated skin, the major fraction of the carotenoids is located in the upper part of the stratum corneum (SC). The amount of carotenoid is lower in the upper part of the SC on the forearm compared to forehead and palm shown with both methods. Both methods detect similar distinction patterns of carotenoid levels for the three anatomical locations.*

*CONCLUSION: The present study supports the hypothesis that antioxidative substances; here carotenoids, are secreted via eccrine sweat glands and/or sebaceous glands to the skin surface. Raman spectroscopic methods are an efficient tool to analyze the distribution of carotenoids in the human skin over time and with the Skin Analyzer over different layers of the epidermis. Resonance Raman spectroscopy is suited to analyze deeper parts of the skin.*

42. Hesterberg K, Lademann J, Patzelt A, Sterry W, Darvin ME. **Raman spectroscopic analysis of the increase of the carotenoid antioxidant concentration in human skin after a 1-week diet with ecological eggs.** *J Biomed Opt.* 2009 Mar-Apr;14(2):024039. <http://www.ncbi.nlm.nih.gov/pubmed/19405767>

#### **Abstract**

*Skin aging is mainly caused by the destructive action of free radicals, produced by the UV light of the sun. The human skin has developed a protection system against these highly reactive molecules in the form of the antioxidative potential. Carotenoids are one of the main components of the antioxidants of the human skin. From former studies, it is known that skin aging is reduced in individuals with high levels of carotenoids. Because most of the antioxidants cannot be produced by the human organism, they must be up taken by nutrition. Using noninvasive Raman spectroscopic measurements it is demonstrated that not only fruits and vegetables but also eggs contain high*

concentrations of antioxidants including carotenoids, which are even doubled in the case of ecological eggs. After a 1-week diet with ecological eggs performed by six volunteers, it is found that the concentration of the carotenoids in the skin of the volunteers increased by approx. 20%. Our study does not intend to recommend exorbitant egg consumption, as eggs also contain harmful cholesterol. But in the case of egg consumption, ecological eggs from hens kept on pasture should be preferred to also receive a benefit for the skin.

43. Darvin M, Patzelt A, Gehse S, Schanzer S, Benderoth C, Sterry W, Lademann J. **Cutaneous concentration of lycopene correlates significantly with the roughness of the skin.** Eur J Pharm Biopharm. 2008 Aug;69(3):943-7. <http://www.ncbi.nlm.nih.gov/pubmed/18411044>

#### **Abstract**

*Antioxidant substances in the skin are expected to slow down photo ageing. We therefore developed the hypothesis that high levels of antioxidant substances may be correlated to lower levels of skin roughness. By utilizing modern optical non-invasive in vivo methods, the structures of the furrows and wrinkles as well as the concentration of lycopene were analyzed quantitatively on the forehead skin of 20 volunteers aged between 40 and 50 years. In a first step, the age of the volunteers was correlated to their skin roughness. Here, no significant correlation was found. In a second step, a significant correlation was obtained between the skin roughness and the lycopene concentration ( $R=0.843$ ). These findings indicate that higher levels of antioxidants in the skin effectively lead to lower levels of skin roughness, and therefore support our hypothesis.*

44. Darvin ME, Patzelt A, Knorr F, Blume-Peytavi U, Sterry W, Lademann J. **One-year study on the variation of carotenoid antioxidant substances in living human skin: influence of dietary supplementation and stress factors.** J Biomed Opt. 2008 Jul-Aug;13(4):044028. <http://www.ncbi.nlm.nih.gov/pubmed/19021355>

#### **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.*

45. Shao YH, He YH, Ma H, Nan N, Qian LS, Wang SX. **Carotenoid levels measured by resonance Raman in vivo**. *Guang Pu Xue Yu Guang Pu Fen Xi*. 2007 Nov;27(11):2258-61. Chinese. <http://www.ncbi.nlm.nih.gov/pubmed/18260408>

#### **Abstract**

*Carotenoid molecules are powerful antioxidants which can act as scavengers for free radicals, singlet oxygen, and other harmful reactive oxygen species in human body. Studies have shown an inverse correlation between the level of carotenoid and the risk of cancers, cardiovascular diseases, and degenerative diseases. High-performance liquid chromatography is used for measuring carotenoid levels as a standard method, but it is not noninvasive and real-time detecting. The authors have developed a novel noninvasive optical technology to measure carotenoid level in vivo by detecting the resonance Raman spectra, which can be used for high sensitivity and real-time detecting. When a low noise 473 nm laser with power less than the exposure limit set by ANSI Z136.1-2000 standards, a clearly distinguishable low resonance Raman spectra superimposed on a strong fluorescence background is produced. The carotenoid level is assessed by measuring the resonance Raman intensity. Using penetrating tissue technology, the authors improved the signal-to-noise ratio in the setup. The experimental results from different volunteers confirmed that the carotenoid level is proportional to the intake of it. The technology provided important values for clinic applications and science research.*

46. Darwin M, Schanzer S, Teichmann A, Blume-Peytavi U, Sterry W, Lademann J. **Functional food and bioavailability in the target organ skin**. *Hautarzt*. 2006 Apr;57(4):286, 288-90. German. <http://www.ncbi.nlm.nih.gov/pubmed/16485123>

#### **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.*

47. Ermakov IV, Sharifzadeh M, Ermakova M, Gellermann W. **Resonance Raman detection of carotenoid antioxidants in living human tissue.** J Biomed Opt. 2005 Nov-Dec;10(6):064028. Review. Full length article available free of charge at: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3086339/pdf/nihms277219.pdf>

#### **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 1375 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.*

48. Ermakov, I.V. Ermakova, M.R., Gellermann, W., Lademann, J. **Noninvasive selective detection of lycopene and beta-carotene in human skin using Raman spectroscopy.** J Biomed Opt. 2004 Mar;9(2):332-8. <http://www.ncbi.nlm.nih.gov/pubmed/15065899>

#### **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.*

49. Gellermann, W., Ermakov, I.V., Scholz, T.A. and Bernstein, P. S. **Noninvasive laser Raman detection of carotenoid antioxidants in skin.** *Cosmetic Dermatology* 2002;15(9):65-68. [no online abstract available]
50. Ermakov IV, Ermakova MR, McClane RW, Gellermann W. et al. **Resonance Raman detection of carotenoid antioxidants in living human tissues.** *Optics Letters* 2001;26:1179-1181.  
<http://www.ncbi.nlm.nih.gov/pubmed/18049555>

#### **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.*

51. 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 Dermatol.* 2000 Sep;115(3):441-8. Full length article available free of charge at:  
<http://www.nature.com/jid/journal/v115/n3/pdf/5600819a.pdf>

#### **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.*

## RAMAN SPECTROSCOPY IN THE EYE

Prior to being adapted for measurements in human skin, Raman resonance was validated for detection of carotenoid concentrations in intact human retinas (an indicator of macular health). Well over a dozen full-length peer-reviewed articles have been published on the use of Raman spectroscopy to detect macular carotenoids. Many of the following full-length articles are available free of charge; all other links provide PubMed abstracts from which full-length articles may be purchased:

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52. Bernstein PS, Ahmed F, Liu A, Allman S, Sheng X, Sharifzadeh M, Ermakov I, Gellermann W. **Macular Pigment Imaging in AREDS2 Participants: An Ancillary Study of AREDS2 Subjects Enrolled at the Moran Eye Center.** Invest Ophthalmol Vis Sci. 2012 Sep 14;53(10):6178-86. Abstract available at: <http://www.ncbi.nlm.nih.gov/pubmed/22879423>

### Abstract

*PURPOSE: Age-Related Eye Disease Study 2 (AREDS2) is a randomized, placebo-controlled study designed to determine whether supplementation with 10 mg of lutein and 2 mg of zeaxanthin per day can slow the rate of progression of age-related macular degeneration (AMD). Although some biomarkers of response to carotenoid supplementation such as serum concentrations are part of the AREDS2 protocol, measurement of carotenoid concentrations in the eye and other tissues is not. In this approved ancillary study, macular pigment optical density (MPOD), macular pigment distributions, and skin carotenoid levels at enrollment and at each annual visit were measured to assess baseline carotenoid status and to monitor response to assigned interventions.*

*METHODS: All subjects enrolled at the Moran Eye Center had MPOD and macular pigment spatial distributions measured by dual-wavelength autofluorescence imaging and total skin carotenoids measured by resonance Raman spectroscopy. Results. Baseline MPOD in enrolled subjects was unusually high relative to an age-matched control group that did not consume carotenoid supplements regularly, consistent with the high rate of habitual lutein and zeaxanthin consumption in Utah AREDS2 subjects prior to enrollment. MPOD did not correlate with serum or skin carotenoid measurements.*

*CONCLUSIONS: Useful information is provided through this ancillary study on the ocular carotenoid status of AREDS2 participants in the target tissue of lutein and zeaxanthin supplementation: The macula. When treatment assignments are unmasked at the conclusion of the study, unique tissue-based insights will be provided on the progression of AMD in response to long-term, high-dose carotenoid supplementation versus diet alone.*

53. Ward MS, Zhao da Y, Bernstein PS. **Macular and serum carotenoid concentrations in patients with malabsorption syndromes.** J Ocul Biol Dis Infor. 2008 Mar;1(1):12-8. Full length article available free of charge at: [http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2600549/pdf/12177\\_2008\\_Article\\_9008.pdf](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2600549/pdf/12177_2008_Article_9008.pdf)

### Abstract

*The carotenoids lutein and zeaxanthin are believed to protect the human macula by absorbing blue light and quenching free radicals. Intestinal malabsorption syndromes such as celiac and Crohn's disease are known to cause deficiencies of lipid-soluble nutrients. We hypothesized that subjects with nutrient malabsorption syndromes will demonstrate lower carotenoid levels in the macula and*

blood, and that these lower levels may correlate with early-onset maculopathy. Resonance Raman spectrographic (RRS) measurements of macular carotenoid levels were collected from subjects with and without a history of malabsorption syndromes. Carotenoids were extracted from serum and analyzed by high performance liquid chromatography (HPLC). Subjects with malabsorption ( $n = 22$ ) had 37% lower levels of macular carotenoids on average versus controls ( $n = 25$ ,  $P < 0.001$ ). Malabsorption was not associated with decreased serum carotenoid levels. Convincing signs of early maculopathy were not observed. We conclude that intestinal malabsorption results in lower macular carotenoid levels.

54. Sharifzadeh M, Zhao DY, Bernstein PS, Gellermann W. **Resonance Raman imaging of macular pigment distributions in the human retina**. J Opt Soc Am A Opt Image Sci Vis. 2008 Apr;25(4):947-57. Full length article available free of charge at: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3079576/pdf/nihms277266.pdf>

#### **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 488 nm, 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 50  $\mu\text{m}$  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.

55. Ermakov IV, Ermakova MR, Gellermann W. **Simple Raman instrument for in vivo detection of macular pigments**. Appl Spectrosc. 2005 Jul;59(7):861-7. Full length article available free of charge at: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3079574/pdf/nihms277046.pdf>

#### **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 photomultiplier 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.

56. Neelam, K.; O'Gorman, N.; Nolan, J.; O'Donovan, O.; Wong, H.B.; Au Eong, K.G. and Beatty, S. **Measurement of Macular Pigment: Raman Spectroscopy versus Heterochromatic Flicker Photometry**. Invest Ophthalmol Vis Sci 2005;46(3):1023-1032.  
Full length article available free of charge at: <http://www.iovs.org/cgi/reprint/46/3/1023>

#### **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.

57. Ermakov I, Ermakova M, Gellermann W, Bernstein PS. **Macular pigment Raman detector for clinical applications**. J Biomed Opt 2004; 9(1):139–48. Full length article available free of charge at: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3086335/pdf/nihms277261.pdf>

#### **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.*

58. Bernstein PS, Zhao DY, Sharifzadeh M, Ermakov IV, Gellermann W. **Resonance Raman measurement of macular carotenoids in the living human eye.** Arch Biochem Biophys 2004;15;430(2):163-9. Abstract available at: <http://www.ncbi.nlm.nih.gov/pubmed/15369814>

#### **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.*

59. Gellermann, W., Bernstein PS. **Noninvasive detection of macular pigments in the human eye.** J Biomed Opt. 2004 Jan-Feb;9(1):75-85. Review. Abstract available at: <http://www.ncbi.nlm.nih.gov/pubmed/14715058>

#### **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.*

60. Zhao DY, Wintch SW, Ermakov IV, Gellermann W, Bernstein PS. **Resonance Raman measurement of macular carotenoids in retinal, choroidal, and macular dystrophies.** Arch Ophthalmol 2003;121(7):967-72. Abstract available at: <http://www.ncbi.nlm.nih.gov/pubmed/12860799>

#### **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.*

61. Gellermann, W., Ermakov, I.V., McClane, R.W. **Raman imaging of human macular pigments.** Optics Letters 2002; 27(1):833–835. Abstract available at: <http://www.ncbi.nlm.nih.gov/pubmed/18007943>

#### **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.*

62. Gellermann W, Ermakov IV, Ermakova MR, McClane RW, Zhao DY, Bernstein PS. **In vivo resonant Raman measurement of macular carotenoid pigments in the young and the aging human retina.** J Opt Soc Am A Opt Image Sci Vis. 2002;19(6):1172-86. Abstract available at: <http://www.ncbi.nlm.nih.gov/pubmed/12049355>

#### **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.*

63. Bernstein PS, Zhao DY, Wintch SW, Ermakov IV, McClane RW, Gellermann W. **Resonance Raman measurement of macular carotenoids in normal subjects and in age-related macular degeneration patients.** Ophthalmology 2002;109(10):1780-7. Full length article available free of charge at: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3079575/pdf/nihms277255.pdf>

#### **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<sup>-1</sup> 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 ( $> \text{ or } = 4 \text{ mg/day}$ ) 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.*

64. Bernstein, P.S., Gellermann W. **Measurement of carotenoids in the living primate eye using resonance Raman spectroscopy.** *Methods Mol Biol.* 2002;196:321-9. Abstract available at: <http://www.ncbi.nlm.nih.gov/pubmed/12152212>

#### **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  $\pm 50\%$  (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.*

65. Bernstein, P.S. **New insights into the role of the macular carotenoids in age-related macular degeneration.** *Resonance Raman studies. Pure and Applied Chemistry* 2002;74(8):1419-1425. Full length article available free of charge at: <http://www.iupac.org/publications/pac/2002/pdf/7408x1419.pdf>

#### **Abstract**

*The human macula uniquely concentrates extraordinarily high levels of two xanthophyll 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.*

66. Ermakov IG, McClane RW, Gellermann W. **Resonant Raman detection of macular pigments in the living human retina.** Optics Letters 2001;26(4):202–204. Abstract available at: <http://www.ncbi.nlm.nih.gov/pubmed/18033547>

#### **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.*

67. Bernstein PS, Yoshida MD, Katz NB, McClane RW, Gellermann W. **Raman detection of macular carotenoid pigments in intact human retina.** Invest Ophthalmol Vis Sci 1998;39(11):2003-11. Full length article available free of charge at: <http://www.iovs.org/cgi/reprint/39/11/2003>

#### **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.*

**THE FOLLOWING 14 ABSTRACTS ARE AVAILABLE AS ABSTRACTS ONLY (WITH THE EXCEPTION OF WENGREEN 2010 WHICH IS AVAILABLE AS A POSTER); ALL WERE PRESENTED AT SCIENTIFIC SYMPOSIUMS. THE TOOL USED TO MEASURE SKIN CAROTENOIDS WAS THE PHARMANEX BIOPHOTONIC SCANNER. ALL BUT THREE OF THE FOLLOWING WERE CO-AUTHORED BY AT LEAST ONE PHARMANEX SCIENTIST:**

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68. Wengreen H, Aguilar S, Lefevre M. **Skin Carotenoids as a Biomarker of Fruit and Vegetable Intake in Children.** Presented at American Dietetic Association's Food & Nutrition Conference & Expo. Boston, Ma. November 6-9, 2010. *[no online abstract available]*

69. 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. *Abstract available at:*  
[http://www.fasebj.org/cgi/content/meeting\\_abstract/23/1\\_MeetingAbstracts/1007.3](http://www.fasebj.org/cgi/content/meeting_abstract/23/1_MeetingAbstracts/1007.3)

#### **Abstract**

*Intensive endurance exercise training increases O<sub>2</sub> 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.*

70. Wood SM, Mastaloudis A, Carlson J, Zidichouski JA, Holubkov R, Reading J, Stavens S, Askew EW, Morrow JD, Milne GL, Poole SL, Bartlett M. **Consistency of correlations of serum and skin carotenoids as measured by high performance liquid chromatography and Raman spectroscopy.** Presented at 15th International Symposium on Carotenoids, Okinawa Japan, June 2008. *[no online abstract available]*

71. Bi SX, Li CL, Guo HW, Poole SL, Zhu JS. **The effects of life styles and LifePak on human skin carotenoids scores measured by resonance Raman spectroscopy BioPhotonic Scanner.** FASEB Journal 2007;21(4):A709. Abstract available at: [http://www.fasebj.org/cgi/content/meeting\\_abstract/21/5/A709](http://www.fasebj.org/cgi/content/meeting_abstract/21/5/A709)

#### **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.*

72. Carlson J, Stavens S, Holubkav R, Zidichouski J, Mastaloudis A, Smidt CR. and Askew, E. **Associations of Antioxidant Status, Oxidative Stress, with Skin Carotenoids Assessed by Raman Spectroscopy (RS).** Experimental Biology meeting abstracts. FASEB Journal 20: A824.3; 2006. Abstract available at: [http://www.fasebj.org/cgi/content/meeting\\_abstract/20/5/A1318-c](http://www.fasebj.org/cgi/content/meeting_abstract/20/5/A1318-c)

#### **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).*

73. Li CL, Guo HW, Bi SX, Zhu, ZG, Zhu JS. **Skin Carotenoids Measured by Resonance Raman Spectroscopy BioPhotonic Scanner and the Effects of Life Styles and LifePak on Human Carotenoids Nutritional Status and Skin Scores.** Asian Pacific Journal of Clinical Nutrition 2006;15(Suppl.):S79. Abstract available at: [http://apjcn.nhri.org.tw/server/APJCN/Volume15/vol15apcns/IUNS-APCNS2006\\_HEC.pdf](http://apjcn.nhri.org.tw/server/APJCN/Volume15/vol15apcns/IUNS-APCNS2006_HEC.pdf) (see page 92 out of 207 of the PDF at previous link)

#### **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  $42.2 \pm 8.96$ , BMI  $30.9 \pm 2.47$ ) 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.*

74. Stavens S, Carlson J, Holubkav R, Zidichouski J, Mastaloudis A, Smidt CR. and Askew, E. **Associations of Fruit and Vegetable Intake with Serum Carotenoids and Skin Carotenoids Measured with Raman Spectroscopy (RS).** FASEB Journal 20: A669.4;2006. Abstract available at: [http://www.fasebj.org/cgi/content/meeting\\_abstract/20/5/A1058-d](http://www.fasebj.org/cgi/content/meeting_abstract/20/5/A1058-d)

#### **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 a 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-variables 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 and time associated with collecting and analyzing blood samples.

75. Zukley LM, Nguyen V, Lowndes J, Smidt C, Angelopoulos TJ, Rippe JM. **Effects of antioxidant supplementation on skin and serum carotenoids.** FASEB Journal 2006;20:A145. Abstract available at: [http://www.fasebj.org/cgi/content/meeting\\_abstract/20/4/A145-a](http://www.fasebj.org/cgi/content/meeting_abstract/20/4/A145-a)

#### **Abstract**

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  $55.4 \pm 8.4$  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 ( $2.48 \pm 1.1$  to  $3.35 \pm 1.6$   $\mu\text{mol/L}$ ), serum  $\beta$ -carotene ( $0.56 \pm 0.3$  to  $1.26 \pm 0.6$   $\mu\text{mol/L}$ ), lutein ( $0.29 \pm 0.1$  to  $0.34 \pm 0.1$   $\mu\text{mol/L}$ ), lycopene ( $0.94 \pm 0.3$  to  $1.31 \pm 0.4$   $\mu\text{mol/L}$ ), ascorbate ( $50 \pm 23$  to  $85 \pm 29$   $\mu\text{mol/L}$ ),  $\{\alpha\}$ -tocopherol ( $34 \pm 9.4$  to  $54 \pm 13$   $\mu\text{mol/L}$ ) and skin carotenoids ( $18353 \pm 4827$  to  $25358 \pm 5229$  units). Only serum ascorbate changed in P ( $53 \pm 24$  to  $67 \pm 39$   $\mu\text{mol/L}$ ). 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.

76. Zukley LM, Lowndes J, Greenstone CL, Melton R, Nguyen V, TJ Angelopoulos, Rippe JM. **Assessment of The Relationship Between Oxidative Stress, Antioxidant Status, Inflammation and Cardiorespiratory Fitness in the Obese.** Medicine & Science in Sports & Exercise: May 2005. 37(5); pS385. [no online abstract available]
77. Fiutem J, Zukley L, Geise T, Legowski P, Nguyen V, Dube T, Yount B, Smidt C, Angelopoulos T, Rippe J. **Adiposity Negatively Influences Carotenoids and Antioxidant Status in Overweight Individuals.** Medicine and Science in Sports and Exercise. 36 (5) Supplement S302, 2004. [no online abstract available]

78. Smidt, C.R., Gellermann, W., Zidichouski, J.A. **Non-invasive Raman spectroscopy measurement of human carotenoid status.** Pharmanex Research Institute. FASEB 18(4):A480, 2004. *[no online abstract available]*
79. Zidichouski, J.A., Poole, S.L., Gellermann, W. Smidt, C.R. (2004) **Clinical validation of a novel Raman spectroscopic technology to non-invasively assess carotenoid status in humans.** Journal of Am. Coll. Nutr. 23 (5): p.468. *[no online abstract available]*
80. Zukley L, Legowski P, Nguyen V, Geise T, Lowndes J, Melanson K, Angelopoulos T, Rippe J. **The Effect of Weight Loss on Dietary Carotenoid and Skin Carotenoid Levels in Subjects Participating in a Weight Loss Study.** Obesity Research Suppl 2004;12:A57. *[no online abstract available]*
81. Smidt C.R. and Shieh D. **Non-invasive biophotonic assessment of skin carotenoids as a biomarker of human antioxidant status.** FASEB J 2003, 17 (5): A1115. *[no online abstract available]*

Updated: 30 September, 2014