Clinical Screening Study: Use of the Pharmanex® BioPhotonic Scanner to Assess Skin Carotenoids as a Marker of Antioxidant Status

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A new, non-invasive BioPhotonic Raman spectroscopy method was used to establish relationships between skin (palm) carotenoid levels as a biomarker of antioxidant health status and demographic, dietary and lifestyle parameters. A total of1,375 subjects entered into this population study. Results confirmed and helped validate all expectations for this health assessment instrument. Specifically, measurements appear not be confounded by general demographic variables, such as age, sex and race/ethnicity, and they show the expected relationships with body composition, oxidative stress (urinary MDA, smoking) and dietary habits (fruit and vegetable consumption and LifePak® usage). Subjects habitually using LifePak® had a 61% higher BioPhotonic skin response than non-users, and similar or higher responses than subjects who reported eating more than five servings of fruits and vegetables daily.

Introduction

Advancements in BioPhotonic laser technology have offered new opportunities for the health care industry. Most recently, BioPhotonic laser technology has been used for non-invasive nutrition assessment of dietary habits and antioxidant status by measuring skin carotenoids.

Raman spectroscopy is a powerful laser spectroscopy that detects the characteristic vibrational/rotational energy levels of a molecule. Inelastically scattered light ("Raman" scattering) originates when energy is exchanged between incident light photons and the scattering molecules, resulting in a characteristic red shift when comparing the incoming with the scattered photon. Raman spectroscopy generates a spectral fingerprint, which depends on a molecule's unique vibrational energy scheme. Since Raman scattering is linear, the intensity of a Raman spectroscopy measurement is directly proportional to the amount of molecules.

Carotenoids are a family of antioxidant nutrients responsible for most of the red, orange, and yellow colors found in nature. Carotenoids play an important role in human health (Gerster, *Int J Vitam Nutr Res* 63:93, 1993). Recently, the protective effects of carotenoids against free radical damage have stimulated intensive research on several specific carotenoids. Beta-carotene, alpha-carotene, lycopene, lutein, and zeaxanthin are of particular importance in human nutrition. Alpha- and beta-carotene are vitamin A provitamins and act as antioxidants (Mortensen et al., *Arch Biochem Biophys* 385:13, 2001; Paiva and Russell, *J Am Coll Nutr* 118:426, 1999). Lutein and zeaxanthin are important for eye health (Mares-Perlman et al., *J Nutr* 132:518S, 2002), while lycopene, the most potent antioxidant carotenoid, may have far-reaching cell-protective benefits (Rao and Agarwal, *J Am Coll Nutr* 19:563, 2000; Heber et al., *Adv Exp Med Biol* 492:29, 2001).

Carotenoids are present in the epidermal and stratum corneum layers of human skin and are believed to confer antioxidant and photo-protective benefits to the skin (Alaluf et al., *J Nutr* 132:399, 2002; Stahl et al., *J Nutr* 131:1449, 2001).

Carotenoid molecules have characteristic long chains of conjugated double-bonds, which generate strong and unique Raman signals. The dietary carotenoids alpha-carotene, beta-carotene, lycopene, lutein and zeaxanthin can all produce strong Raman spectroscopy signals at 511 nm when excited with 473 nm laser light. The BioPhotonic properties of carotenoids are highly specific and with little or no interference from any other biomolecules present in human skin, such as melanin, vitamin E or skin lipids. As a result, Raman spectroscopy allows for a non-invasive, rapid, accurate, and safe assessment of carotenoid levels in the skin. Research suggests that

skin carotenoid levels correlate with levels of carotenoids in the diet and blood (Hata et. Al., *J Invest Dermatology* 115:441, 2000). Reviews of the application of Raman spectroscopy to measure skin carotenoids were published by Hata et al. (*J Invest Dermatology* 115:441, 2000) and Ermakov et al. (*Optics Letters* 26:1179, 2001).

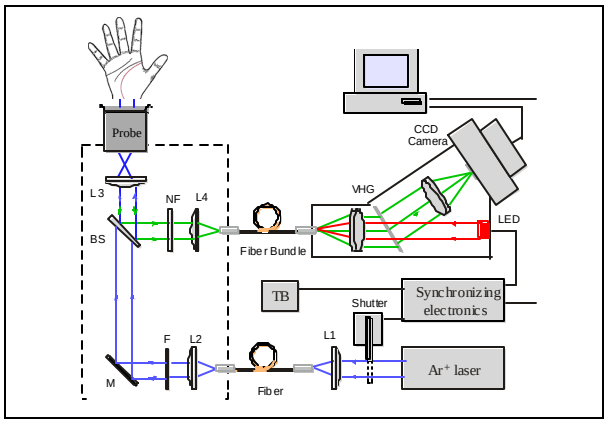
The present study used questionnaires to examine in a large number of subjects whether or not there are relationships of skin carotenoid readings with age, gender, race/ethnicity, body mass index (BMI), smoking, usage of dietary supplements (i.e., LifePak®), and consumption of fruits and vegetables. This information is important to help validate the Pharmanex® BioPhotonic Scanner as a novel, non-invasive optical tool for *in vivo* dietary assessment.

Materials and Methods

A total of 1,375 employees of Nu Skin Enterprises® and their family and friends were recruited. No groups were excluded from the study. Subjects participating in this study were instructed to complete a computer-administered questionnaire to assess demographical, dietary and lifestyle variables. The questionnaire contained a food frequency query asking subjects to record their consumption of foods containing more than 1 mg of total carotenoids per serving according to the USDA carotenoids database. Subjects then underwent the measurement of carotenoid levels in the skin on the palm of the hand using the Pharmanex® BioPhotonic Scanner at the Pharmanex Research Institute in Provo, Utah.

Figure 1 shows a schematic drawing of the Pharmanex® BioPhotonic Scanner describing the components and its operation.

Figure 1: Pharmanex® BioPhotonic Scanner

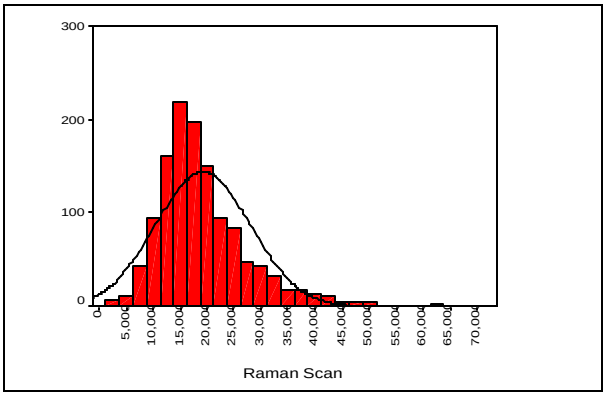


Body fat percentage was measured with a near-infrared device (Futrex-5000/XL, Futrex Inc., Galthersburg, MD). Subjects were asked for voluntary participation in a home urine malondialdehyde (MDA) test (Free Radical Test™, Vespro Life Sciences, Lenexa, KS) using the first morning void, and to report the test results (one of four color-matched outcomes) to the study coordinator. BioPhotonic responses were correlated with the information gathered in the questionnaire and the urinary MDA test to accomplish the study objectives. Information obtained from the subject questionnaire and the carotenoid measurements was tabulated. Correlations (positive or negative) between the individual items on the questionnaire and the carotenoid levels were examined and presented graphically. Statistical significance was examined using appropriate tests (t-test, correlation analysis).

Results and Discussion

All 1,375 of the recruited subjects participated in the study, which was completed within eight weeks. The Pharmanex® BioPhotonic Scanner was able to accommodate more than 300 subjects per day, owing to the short time required to obtain the measurement (less than one minute). The overall histogram of all study subjects is presented in Figure 2.

Figure 2: Histogram of Study Subjects



The overall mean BioPhotonic response was 19,072 with a standard deviation of 8,828 units. The lowest measurement was 1,556 units and the highest measurement was 73,416 units, while the majority of subjects (68%) fell between 10,244 and 27,900 units.

General Demographics

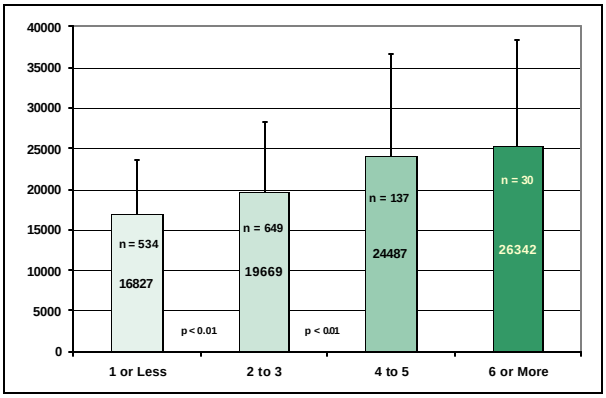
There were small, non-significant differences between women (19,244, n=666) and men (18,937, n=704), which can be explained by a slightly higher

reported consumption of fruits and vegetables in women (2.34 servings/day) compared to men (2.03 servings/day). There were no significant differences in the BioPhotonic responses between the different age groups. Among race and ethnic groups, Asian subjects measured significantly higher than white-Caucasian, Hispanic and African-American subjects. Again, this is can probably be explained with the higher reported consumption of fruits and vegetables of Asians (2.59 servings/day) compared to white-Caucasians (2.15 servings/day). Overall, this study showed that demographic variables do not influence the BioPhotonic measurements, and that any observed differences can be explained by different dietary habits.

Fruit and Vegetable Consumption

As expected, there was a pronounced, positive relationship between self-reported fruit and vegetable intake (a dietary source of antioxidants and carotenoids) and the BioPhotonic measurments as follows (see Figure 3): one or less servings/day: 16,827 ± 6,725; two to three servings/day: 19,669 ± 8,557; four to five servings/day 23,997 ± 12,648; and six or more servings/day: 25,377 ± 12,953 units. These data will help validate the BioPhotonic skin carotenoid measurements as a convenient marker of fruit and vegetable intake.

Figure 3: Scanner Readings vs. Fruit & Vegetable Intake



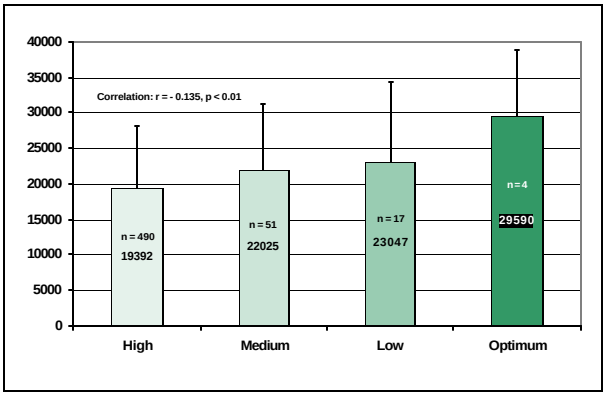
Carotenoid Consumption

Analysis of the self-reported consumption of carotenoid-rich foods resulted in a similar relationship as observed with fruits and vegetable intake, although overall carotenoid consumption was probably overestimated. Subjects consuming 15 or less mg carotenoids daily scored lower (16,440 ± 6,876 units, n=541) than those with 15-30 mg/day (20,097 ± 8,879 units, n=516, $p<0.05$), who in turn scored lower than subjects reporting more than 30 mg/day (21,889 ± 10,376 units, n=318, $p<0.05$).

Urinary Free Radical Acitivity

Of the 1,375 subjects, 562 completed and reported the results for the urinary MDA test, and the majority (490 subjects) reported high MDA levels (test scores were: "optimum," "low," "medium," and "high" free radical activity). Nevertheless, there appears to be a consistent and inverse relationship between free radical activity (urinary MDA) and the BioPhotonic measurement of skin carotenoids as shown in Figure 4.

Figure 4: Scanner Readings vs. Urinary MDA



This result was expected, because carotenoids are singlet oxygen quenchers and therefore an important part of the body's antioxidant network. Further studies using more sophisticated tests of free radical activity and antioxidant status are ongoing to further validate this relationship.

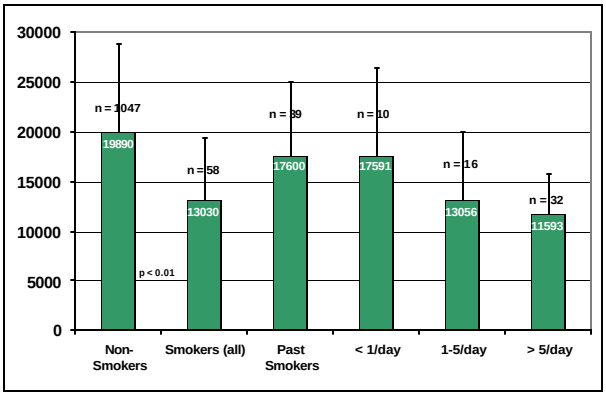
Smoking

Tobacco smoke is a potent cause of free radical damage and smoking is clearly associated with increased oxidative stress and decreased markers of antioxidant defense (Lesgards JF et al., *Environ Health Perspect* 110:479-86, 2002).

Figure 5 shows the BioPhotonic responses related to smoking status. Smokers scored 34.5% lower than non-smokers, and BioPhotonic skin responses were lowest in those who smoked the most, i.e., more than five cigarettes daily. Fruit and vegetable consumption was similar across smoking categories, except that those who smoked more than five cigarettes daily did report significantly lower fruit and vegetable consumption than those who smoked less than one cigarette daily. These results help validate

the BioPhotonic skin carotenoids measurements as a marker of the antioxidant health status (Walmsley CM et al., *Public Health Nutr* 2:199-208, 1999).

Figure 5: Smoking



Body Composition

Previous studies have shown inverse relationships between body mass index (BMI) or body fat content and serum or plasma carotenoid concentrations (Reitman A et al., *Isr Med Assoc J* 4:590-3, 2002; Neuhouser ML et al., *J Nutr* 131:2184-91, 2001). This is believed to be due to a dilution effect of adipose tissue serving as a storage site for carotenoids. The same relationship was observed in the present study. BioPhotonic skin carotenoid responses in the palm declined with increasing BMI as follows: BMI < 25 = 21,347 ± 9,661 (n=564); BMI 25-29.9 = 18,549 ± 7,319 (n=378, $p<0.05$), and BMI > 30 = 15,432 ± 6,621 (n=184, $p<0.05$). The same relationship was observed when body fat was estimated using a near-infrared device: Body fat < 15% = 21,320 ± 9,394 (n=165); body fat 15-24.9% = 19,985 ± 8,665 (n=422, $p=0.08$); body fat 25-34.9% = 18,998 ± 8,762 (n=460, $p=0.09$); and body fat >35% = 15,925 ± 7,496 (n=90, $p<0.01$). Self-reported fruit and vegetable consumption was similar across all BMI and percent body fat groups. These findings compare well with established analysis methods and help substantiate the BioPhotonic skin carotenoid measurements as an assessment tool of carotenoid status.

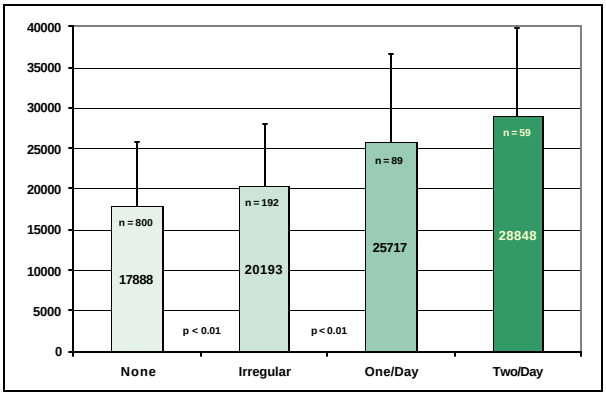
Sunlight Exposure

Reported sunlight exposure was inversely related to the BioPhotonic skin measurements as follows: Low exposure = 20,085 ± 9,776 (n=550); moderate exposure = 18,660 ± 8,069 (n=716, $p<0.01$); and high exposure = 16,446 ± 7,508 (n=96, $p<0.05$). This was observed despite a significantly higher consumption of fruits and vegetables by subjects with high sunlight exposure (2.46 ± 1.33 servings/day, $p<0.05$) compared to those with low and moderate exposure (2.14 ± 1.22 and 2.17 ± 1.18 servings/day, respectively). This suggests that carotenoid antioxidants help protect the skin from UV-light induced free radical damage and are consumed in the process.

LifePak® Supplement Usage

Antioxidant supplements can improve antioxidant status and this has been shown as well for LifePak® (Pharmanex LLC, Provo, UT) in earlier clinical studies showing increases in serum antioxidant concentrations and improved resistance to *ex vivo* LDL oxidizability (Smidt et al., *FASEB J* 13:A546, 1999). The present study shows a pronounced and positive relationship between LifePak® supplementation and BioPhotonic skin carotenoid measurements as shown in Figure 6.*

Figure 6: Scanner Readings vs. LifePak® Usage



Subjects habitually consuming LifePak® at the recommended dosage (two packets daily) measured 61% higher than those not using LifePak® ($p<0.001$). LifePak® users had about the same BioPhotonic skin measurements as people who reported eating more than five servings of fruits and vegetables daily. Fruit and vegetable consumption was similar across LifePak® usage categories, except for irregular LifePak® users who reported less intake (2.04 ± 1.09 servings/day) than people using one packet of LifePak® daily (2.39 ± 1.24 servings/day, $p<0.05$). However, this difference is small and not considered a confounding variable based on the magnitude of the effect of fruit and vegetable consumption observed in this study. These results suggest that LifePak® antioxidants and carotenoids are

* These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease.

bioavailable and support the body's antioxidant network. LifePak® contains more than 40 antioxidant nutrients, including 15 mg/day of mixed carotenoids (6 mg beta-carotene, 5 mg lycopene, 2 mg alpha-carotene and 2 mg lutein) (formulas vary by market).*

Conclusions

This is the first large screening study to help validate and gain field experience with the Pharmanex® BioPhotonic Scanner. The study confirmed and helped validate all expectations for this health assessment instrument. Specifically, measurements appear not to be confounded by general demographic variables, such as age, sex and race/ethnicity, and they show the expected relationships with body composition, oxidative stress (urinary MDA, smoking) and dietary habits (fruit and vegetable consumption and LifePak® usage). Further studies are being conducted at Pharmanex and in academic institutions to confirm these and other conclusions.

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Effect of LifePak® Supplementation on Antioxidant Status Using BioPhotonic Raman Spectroscopy

Carsten R. Smidt, Ph.D., FACN

A new, non-invasive BioPhotonic Raman spectroscopy method was used to assess the antioxidant efficacy of the multi-nutrient supplement LifePak® in 25 healthy volunteers for 12 weeks. Raman spectroscopy measures skin (palm) carotenoids as an important indicator of the antioxidant health status. BioPhotonic skin carotenoid readings increased significantly from a baseline of 18,828 units to 32,175 units at the end of the study. Although some individual variability was observed, all subjects experienced an increase in BioPhotonic skin response. Fruit and vegetable consumption was monitored during the study and found to be unchanged throughout. These results suggest that LifePak® supplementation leads to significant strengthening of the body's antioxidant health status as indicated by the BioPhotonic measurement of skin carotenoids.*

Introduction

Recent advancements in laser technology have offered new opportunities for the health care industry. The application of BioPhotonic technology increasingly enables non-invasive biological measurements. Raman spectroscopy is a powerful laser spectroscopy that detects the characteristic vibrational/rotational energy levels of a molecule. Inelastically scattered light ("Raman" scattering) originates when energy is exchanged between incident light photons and the scattering molecules, resulting in a characteristic red shift when comparing the incoming with the scattered photon. Raman spectroscopy generates a spectral fingerprint, which depends on a molecule's unique vibrational energy scheme. Since Raman scattering is linear, the intensity of a Raman spectroscopy measurement is directly proportional to the amount of molecules.

Recently, Raman spectroscopy has been applied to the measurement of carotenoids present in the *stratum corneum* layers of human skin (Hata et al., *J Invest Dermatology* 115:441, 2000; and Ermakov et al., Optics Letters 26:1179, 2001). Carotenoids play an important role in human health (Gerster, *Int J Vitam Nutr Res* 63:93, 1993), and are believed to confer antioxidant and photo-protective benefits to the skin (Alaluf et al., *J Nutr* 132:399, 2002; Stahl et al., *J Nutr* 131:1449, 2001). Raman spectroscopy allows for a non-invasive, rapid, accurate, and safe assessment of carotenoid levels in the skin. Research suggests that skin carotenoid levels correlate with levels of carotenoids in the diet and blood (Hata et. al., *J Invest Dermatology* 115:441, 2000).

Carotenoids scavenge singlet oxygen and are an important part of the body's antioxidant defense system (Omaye ST et al., *J Am Coll Nutr* 15:469-74, 1996; Handelman GJ, *Nutrition* 17:818-22, 2001). Serum carotenoid concentrations as well as BioPhotonically measured skin carotenoid responses are affected by oxidative stress, smoking, sunlight exposure and fruit and vegetable consumption as demonstrated by a large Pharmanex study concluded recently to assess these relationships in 1,375 subjects (unpublished results, Pharmanex, LLC, Provo, UT). Therefore, the skin carotenoid's BioPhotonic response appears to be a convenient and suitable indicator of the body's antioxidant status.

Results of the Pharmanex population study also showed that subjects consuming the antioxidant multi-nutrient supplement LifePak® had significantly higher skin carotenoid levels than subjects not taking antioxidant supplements (unpublished results, Pharmanex, LLC, Provo, UT). The present study was conducted to determine if there is a causal relationship between skin carotenoids and LifePak® supplementation.*

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Materials and Methods

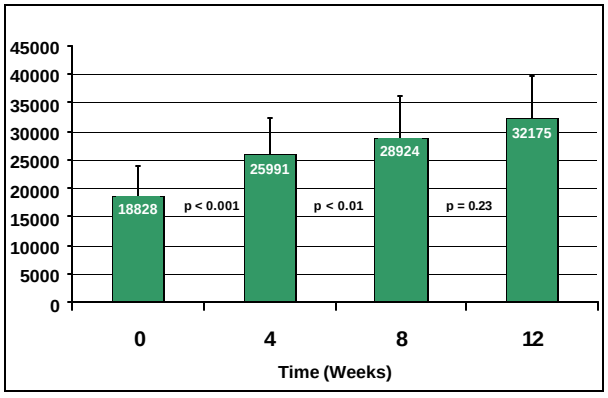
Twenty-five healthy non-smokers between the ages of 18 and 65 years were recruited for this study. Subjects were excluded from the study if they had used antioxidant supplements within the prior three months. Subjects participating in this study were instructed to complete a computer-administered questionnaire to assess demographical, dietary and lifestyle variables. The questionnaire contained a food frequency query asking subjects to record their consumption of foods containing more than 1 mg of total carotenoids per serving according to the USDA carotenoids database. Subjects then underwent the measurement of carotenoid levels in the skin on the palm of the hand using a BioPhotonic Scanner at the University of Utah, Salt Lake City, UT, in the laser laboratory of Werner Gellermann, Ph.D., Department of Physics. On the same day of this initial baseline measurement, subjects were supplemented with LifePak® for 12 weeks at the recommended dosage of two packets daily. The skin carotenoid's BioPhotonic response was measured at 4, 8 and 12 weeks of the study. Statistical significance was examined using appropriate tests (t-test).

Results

A total of 25 subjects met all study criteria and participated in the study. Retained subjects numbers at 4, 8 and 12 weeks were 22, 17 and 12, respectively. Reasons for dropouts were related to scheduling and compliance problems. No serious adverse reactions were reported. Fruit and vegetable consumption was monitored throughout the study and remained constant at about 2.2 servings daily.

The effects of 12 weeks of LifePak® supplementation on biophotonically measured skin carotenoids are shown in Figure 1.

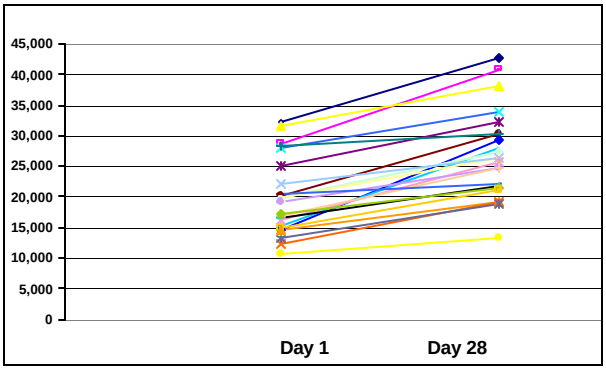
Figure 1: Changes in Biophotonic Response



The supplement increased mean scores by 38% ($p<0.001$) from baseline to week 4, by another 11% by week 8 ($p<0.01$), and by another 11% by week 12 (n.s.). Changes at any time point were significant compared to baseline ($p<0.001$).*

Figure 2 shows the individual changes observed from baseline to day 28 of the study. All subjects experienced increases in skin carotenoids. The mean increase was 7,687 ± 3,212 units (mean ± std. dev.), ranging from 1,500 to 14,600 units. The mean increase for the entire 12-week study period (n=12) was 10,750 ± 5,865 units, ranging from 1,900 to 20,300 units.*

Figure 2: Individual Changes, Days 1-28



Discussion

Antioxidant supplements can improve antioxidant status and this has been shown as well for LifePak® (Pharmanex LLC, Provo, UT) in earlier clinical studies showing increases in serum antioxidant concentrations and improved resistance to *ex vivo* LDL oxidizability (Smidt et al., *FASEB J* 13:A546, 1999). The observed increases in skin carotenoids with LifePak® supplementation in present study at unchanged intake of fruits and vegetables confirm our hypothesis of a cause and effect relationship that was suggested in our earlier population study. LifePak® is a complete vitamin/mineral supplement with additional antioxidant nutrients and provides a total of 15 mg carotenoids daily, as 6 mg beta-carotene, 2 mg alpha-carotene, 5 mg lycopene and 2 mg lutein. The present study not only confirms our earlier studies that the carotenoids of LifePak® appear in blood serum (Smidt et al., *FASEB J* 13:A546, 1999), but also shows that the supplement's carotenoids are delivered to the skin as an important site of action.*

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The individual variability reported in this study (Figure 2) can be attributed to several factors. Carotenoid absorption may vary due to meal size, the amount of fat consumed with the supplement and perhaps genetic predisposition (Kostic D et al., *Am J Clin Nutr* 62:604-10, 1995; Omaye ST et al., *J Am Coll Nutr* 15:469-74, 1996). It is possible that the low responders in our study did not take the supplement as directed, i.e., with meals, or that the meals were low-fat or fat-free meals. Dietary fat is needed to enable bile secretion and subsequent chylomicron formation in the intestine, which are necessary for carotenoid absorption. In addition, individually different levels of oxidative stress, which again depend on genetic and environmental factors, may have affected skin carotenoid levels.

Conclusions

These results suggest that LifePak® supplementation leads to significant strengthening of the body's antioxidant health status as indicated by BioPhotonic measurement of skin carotenoids.*

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A Double Blind Placebo Study showing the Effect of lifepak nano supplementation on Skin Carotenoid Scores February 2006

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Pharmanex Research Institute, Provo, UT.

Skin Carotenoid Scores (SCS) are a biomarker of overall antioxidant status. This study used Raman Spectroscopy to examine the ability of lifepak nano to increase SCS as compared to a placebo. Lifepak nano increased SCS in all subjects, and was especially effective in raising scores that were above 30,000 Raman Intensity Counts at baseline. The findings of this study suggest excellent bioavailability of lifepak nano's unique CR-6 Liponutrient delivery system including cutting edge nano-carotenoids in fish oil.

Introduction

Carotenoids are an important group of phytonutrients which have been designated as the best biological marker of fruit and vegetable consumption, as well as the best indicator of over all antioxidant status (Institute of Medicine, 2000; Svilaas, 2004). Epidemiological and clinical studies substantiate the protective effects of carotenoids in many areas of health, including cardiovascular, skin and eye health (Smidt, 2005a). As an integral part of the antioxidant network, carotenoids preserve other antioxidants from free radical destruction, thus strengthening the entire antioxidant defense system.

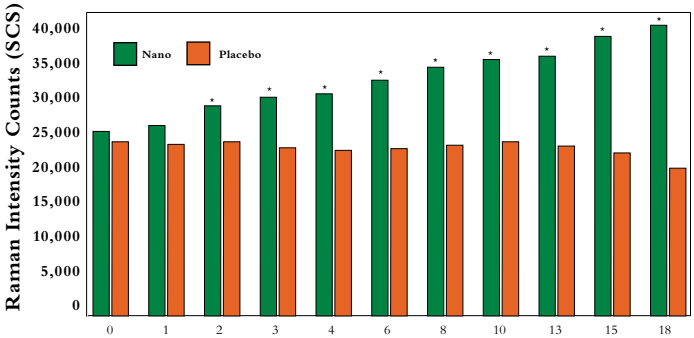
Because of their central role to health, and their ability to accurately predict overall antioxidant status, many techniques have been developed to assess carotenoid levels in human tissues. Previously, blood serum carotenoids have been viewed as the gold standard to assess tissue carotenoid concentrations; recently however, the well established method of Raman Spectroscopy (RS) has been adapted to accurately and non-invasively measure carotenoid concentrations in living human skin. Skin carotenoid concentrations are reported as Raman Intensity Counts and commonly referred to as Skin Carotenoid Score (SCS). The higher the score, the higher the concentration of carotenoid molecules detected at the site of measurement (Ermakov, 2001; Ermakov, 2004; Hata, 2000; Zidichouski, 2004).

This study used Raman Spectroscopy to assess changes in Skin Carotenoid Scores (SCS) over eighteen weeks of supplementation with lifepak nano or placebo. Lifepak nano is a comprehensive dietary supplement containing vitamins, minerals, and over sixty antioxidants, including a unique delivery system known as CR-6 Liponutrient softgels containing fish oils (omega-3s) and highly absorbable carotenoids which have been enhanced through the application of nanotechnology. Nanotechnology is the highly sophisticated technique of manipulating single molecules (no larger than 100 nanometers), to enhance their functional behavior. The application of nanotechnology employed by Pharmanex is a mono-molecular encapsulation in which individual nutrients are embedded into single cyclodextrin molecules.

Rationale

Pharmanex has conducted multiple studies to show the ability of various antioxidant preparations to increase SCS, as a biomarker of over-all antioxidant status (Smidt, 2002; Smidt, 2005b). As a follow-up to these studies, this study seeks to confirm that the novel preparation enhanced by nanotechnology (lifepak nano), increases SCS as anticipated. Previous nanoized nutrients have shown superior absorption over non-nano preparations (Craft, 2005). The purpose of this study is to demonstrate lifepak nano's ability to increase SCS. Traditional carotenoids are poorly absorbed, and large scale surveys show average diets do not contain sufficient levels of these important antioxidants (Brown, 2004; Block, 1991). Lifepak nano's unique CR-6 Liponutrient delivery system will help to offset low consumption of fruits and vegetables and poor absorption, thus providing more protective benefits.

Figure 1: Average SCS Increase with lifepak nano



* p<0.05 compared to Week 0

This study was conducted by Pharmanex scientists. The study authors, Carsten R. Smidt and Angela Mastaloudis, are employees of Pharmanex, a division of Nu Skin Enterprises, Inc. Pharmanex produces and distributes dietary supplement products, including lifepak nano.

Methods and Materials

Fifty two subjects between 18 and 65 years of age qualified for study participation. Food frequency and health history questionnaires were used to screen for inclusion criteria, and the Pharmanex BioPhotonic Scanner was used to assess skin carotenoid levels. Individuals taking antioxidant supplements, having high exposure to sunlight or tanning bed use, pregnant women, or individuals using sunless tanning products were excluded from the study. All subjects were healthy non-smokers consuming typical U.S. diets containing less than five daily servings of fruits and vegetables, and had baseline Skin Carotenoid Scores between 13,000 and 35,000 Raman Intensity Counts. Participants were instructed to maintain their usual dietary and exercise habits throughout the duration of the study. Subjects (n=52) meeting study criteria were randomly assigned in a double blind manner to one of two groups—lifepak nano (n=27) or placebo (n=25). Additional subject statistics are available in table 1.

Table 1: Subject Statistics

	Lifepak nano	Placebo
Subject per group	(N=20)	(N=22)
Age	40 ± 8.6	35 ± 9.7
Height (cm)	175.3 ± 10.2	172.7 ± 12.7
Weight (kg)	77.6 ± 19.5	80.3 ± 21.3
BMI	26 ± 7	27 ± 5
Baseline	25,599 ± 6,199	23,459 ± 5,686
F/V Intake (servings/day)	2.58 ± .581	2.57 ± .660

Treatments

Study participants were randomly assigned to receive either lifepak nano or a placebo. Subjects were instructed to take lifepak nano or placebo twice daily, i.e., with their morning and evening meals. Additional nutritional information of the study treatments are available below, and a full list of lifepak nano nutrients is available at www.lifepaknano.com.

lifepak nano

Lifepak nano is a comprehensive dietary supplement containing all essential micro and macronutrients including vitamins, minerals, broad spectrum antioxidants to support the entire antioxidant network. A delivery of CR-6 Liponutrient softgel capsules with fish oil and nanoized carotenoids enhances the absorption of nutrients that are known to have poor absorption. Carotenoids cling together in the digestive tract causing poor bioavailability; however nano-encapsulated carotenoids allow for complete molecular dispersion, thus increasing the surface area of these important nutrients and maximizing their contact with the absorptive lining of the digestive tract.

Each serving of lifepak nano contains five capsules (dry ingredients) and two CR-6 Liponutrient softgel capsules (liquid ingredients, including omega-3 fatty acids and nanoized carotenoids.

Placebo

The placebo taken twice daily included: two powder capsules; and two softgel capsules with omega-3 fatty acids (containing no carotenoid antioxidant).

Statistical Analyses

Data are expressed as the mean ± SD (n=42 subjects). Analysis of variance was used to detect statistically significant between and within subject effects. An unpaired t-test was used to analyze differences between sexes with regard to subject characteristics (i.e. age, height, weight). Statistics were calculated using The SPSS System (SPSS Inc. Chicago, IL).

Results and Discussion

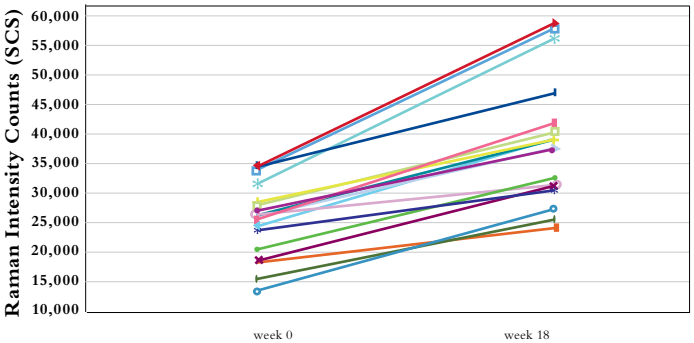
Forty-two subjects completed the study. A summary of subject characteristics are described in table 1. In the lifepak nano group seven subjects were disqualified for non-compliance, and three subjects were dropped from the placebo group also due to non-compliance. All participants in the lifepak nano group experienced dramatic increases in Skin Carotenoid Scores (figure 2). Consumption of lifepak nano increased Skin Carotenoid Scores much faster than expected, showing statistical significance within 2 weeks (figure 1). Skin Carotenoid Scores of the lifepak nano group increased an average of 17,757 +/- 10,113 Raman Intensity Counts in eighteen weeks compared to placebo. Which had no effect on Scanner score at any time (figure 1).

Throughout the eighteen weeks there was a continual increase in scanner score of the lifepak nano group, with no apparent plateau effect (figure 1).

Conclusion

Our findings indicate that lifepak nano effectively increases SCS, a biomarker of overall antioxidant status. All subjects in the lifepak nano group showed dramatic increases in Skin Carotenoid Scores (figure 2), with no signs of plateauing even at the end of the study (figure 1). Carotenoid antioxidant levels increased faster than expected, showing statistically significant increases in two weeks for the lifepak nano group, with no change in the placebo group. While lifepak nano is effective for all subjects, it was especially effective for subjects with scores that were above 30,000 Raman Intensity Counts at baseline (data not shown). We conclude that lifepak nano delivers significant benefit by increasing tissue antioxidants as measured by Biophotoic Scanner.

Figure 2: Individual Subject Response to lifepak nano



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Zidichouski J.A., Poole S.J., Gellermann W, and Smidt C.R., Clinical Validation of a Novel Raman Spectroscopic Technology to Non-Invasively Assess Carotenoid Status in Humans. J. Am. Coll. Nutr. 23(5): 468, 2004

Clinical Study: Effect of G3 and Other Juices on Antioxidant Network Status As Measured by Raman Spectroscopy October 2005

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Biophotonic Raman spectroscopy method was used to show the effect of Pharmanex® brand G3 and other juices on overall antioxidant network status. A total of 34 subjects (n=34) entered into this clinical study. Results confirmed that G3 significantly increases skin carotenoid score (~40%) after eight weeks of regular consumption. Furthermore, G3 increased skin-carotenoid score ~375% as compared to Tahitian Noni® and Xango™ (mangosteen) juices.

Introduction

The human body is continuously exposed to a variety of destructive oxidants including byproducts of energy metabolism, pollution, cigarette smoke and ultraviolet sunlight. To offset damage by these oxidants, the body has a number of natural antioxidant defense mechanisms. These include both intrinsic antioxidant enzymes, such as superoxide dismutase and catalase, and extrinsic antioxidant nutrients including vitamins E and C and carotenoids. However, when exposure to metabolic and environmental sources of oxidants exceeds that of the body's antioxidant defense system, a state of oxidative stress develops (Halliwell, B 1991).

There is a strong body of evidence that high intakes of fruits and vegetables rich in antioxidants have far reaching cell protective benefits, promote cardiovascular health, normal insulin metabolism, cognitive function, eye health and general overall health and well being. Science has also suggested that antioxidants and carotenoids help protect against oxidative stress (Liu, C. S. 2004). Thus, a combined diet of antioxidant-containing supplements, and fruits and vegetables, may help tip the balance away from pro-oxidants in favor of antioxidants. Carotenoids are some of the most abundant antioxidant nutrients present in fruits and vegetables. It has been theorized that carotenoids may be responsible for many of the protective effects of a diet high in fruits and vegetables in promoting cardiovascular health, eye health, and overall cell protection (During, A. 2004). In addition to providing antioxidant protection, these essential nutrients are involved in intercellular communication (Deming, 2002) and some serve as important precursors to vitamin A (Bernstein, 1998).

Recent technology, in the form of the BioPhotonic Scanner (Pharmanex), allows for the measurement of carotenoids as an indicator of overall antioxidant status in the skin, through raman spectroscopy (Svilaas, 2004). Advantages of measuring carotenoids in the skin are that it provides a good representation of systemic carotenoid status, habitual dietary fruit and vegetable intake and overall antioxidant status (Smidt, C.R., 2004). Primary carotenoids detected are: lycopene, β-carotene, α-carotene, α-cryptoxanthin, lutein, phytoene and phytofluene, with lycopene and β-carotene present in the highest amounts (Gellermann, 2002). This measurement technique is fast, painless, and cost effective, making it ideal for the assessment of antioxidant status in humans. Furthermore, carotenoid levels measured in the skin by raman spectroscopy technology have been demonstrated to be directly related to both self-reported fruit and vegetable consumption and antioxidant supplementation (Smidt, 2004).

Pharmanex provides supplements, including G3 that provide antioxidants, including carotenoids, shown to be beneficial for overall health and well-being.

Rationale

The purpose of this study was to assess antioxidant benefits, and to determine bioavailability of carotenoids, as a biomarker of overall antioxidant protection from three different branded fruit juice products.

The bioavailability of carotenoids, a biomarker of antioxidant status, was compared in 31 subjects (n=31) individuals consuming one of three different fruit juices for eight weeks (Figure 1). Bioavailability and antioxidant status was assessed using the BioPhotonic Scanner.

Figure 1: Subject Statistics

	G3 (n=11)	Noni® (n=10)	Xango™ (n=10)
Age (years)	30 ± 10	30 ± 7	35 ± 9
Height (cm)	172 ± 10	178 ± 10	179 ± 10
Weight (kg)	69 ± 15	80 ± 18	77 ± 16
BMI-Body Mass Index	23 ± 3	25 ± 5	24 ± 5
Baseline Score	24,273 ± 4,962	22,200 ± 4,685	20,492 ± 6,994
f/v intake (serv/day)	1.7 ± 1.5	2.5 ± 1.2	2.4 ±1.3

This study was conducted by Pharmanex scientists. The study authors, Carsten R. Smidt and Angela Mastaloudis, are employees of Pharmanex, a division of Nu Skin Enterprises, Inc. Pharmanex produces and distributes dietary supplement products, including g3.

Test Procedure

We measured the efficacy of three different juice mixtures in increasing skin carotenoid levels over an eight-week period as an indication of carotenoid bioavailability, as a biomarker of overall antioxidant protection.

G3 gấc superfruit blend with lipocarotenes™

G3, a Pharmanex® product, is composed of four main fruit juices from concentrate: Gac (Mormordica cochinchinensis), Cili (rosa roxburghii tratt), Siberian pineapple (Hippophae rhamnoids, also known as Sea Buckthorn), and Chinese lycium, (Lycium barbarum, also known as wolfberry). G3 is composed of food-grade ingredients commonly found in the global food supply making it a safe, well-tolerated supplement. G3 is a food-grade product that is commercially available.

Xango™ Juice (mangosteen)

Xango™ is composed of mangosteen (Garcinia mangostana) from whole fruit juice, apple fruit juice, pear fruit juice, grape fruit juice, pear fruit puree, blueberry fruit juice, raspberry fruit juice, strawberry fruit juice, cranberry fruit juice, cherry fruit juice, citric acid, natural flavor, pectin, xanthan gum and sodium benzoate. Xango™ is a food-grade product that is commercially available.

Tahitian Noni® Juice

Tahitian Noni® is composed of reconstituted Morinda citrifolia (noni fruit) juice from pure noni puree from French Polynesia, natural grape juice concentrate, natural blueberry juice concentrate, and natural flavors. Tahitian Noni® is a food-grade product that is commercially available.

Randomization Criteria:

Subjects (n = 34) meeting study criteria were randomly assigned to one of three treatment groups—Noni,® Xango™ (mangosteen), or G3. (Figure 2).

Figure 2: Subjects Per Group

G3	Xango™	Tahitian Noni®
N = 12	N = 11	N = 11

Supplementation:

Twice per day, with breakfast and with dinner, for eight weeks, subjects consumed, based on their randomization category:

1) 3 fluid ounces G3
2) 3 fluid ounces Xango™ (mangosteen)
3) 3 fluid ounces Tahitian Noni®

Carotenoids and other fat soluble nutrients are not absorbed along the same pathway as other nutrients that are present in the diet. Fat-soluble nutrients are absorbed with fat, through the lymphatic system along with other hydrophobic or "water fearing" nutrients. This alternate form of absorption assists the body in absorbing material that is near incompatible with the watery environment found in cells of the body. The fat-soluble nutrients interact with the intestinal environment the same way that oil interacts with water. The interactions of the two would make it impossible for the body to absorb a significant amount without minimizing the interaction with water in the absorption pathway. In order to minimize the interaction with water in the absorption pathway, subjects were instructed to consume the juice with meals containing a moderate amount of fat in order to ensure sufficient carotenoid absorption. In the event subjects chose to consume the juice with a fat-free meal they were instructed to consume two Optimum Omega softgels containing a total of 360 mg EPA and 240 mg DHA fish oils along with the juice in order to ensure carotenoid bioavailability. (Weber F. 1983)

Diet

Subjects were asked to maintain their typical diet for the eight weeks of the study. In order to control for the effects of diet on their BioPhotonic Scanner score, subjects were encouraged to avoid making changes to their diet, especially the addition or omission of foods high in carotenoids, including colorful fruits and vegetables such as citrus fruits, leafy green vegetables, tomatoes and tomato products as well as squash. In addition, subjects were encouraged to maintain consistent intake of beverages including, but not limited to coffee, tea, red wine and other alcohol. Subjects were provided with instructions on how to fill out the questionnaire, including food descriptions and instructions on how to best estimate portion size. Dietary questions about fruits and vegetables intake have been validated previously (Smidt, CR 2004).

Subjects were scanned one week prior to supplementation, the first day of supplementation and then every other week for the duration of the study (eight weeks). A final scan was conducted on the last day of supplementation for a total of six scans.

Results

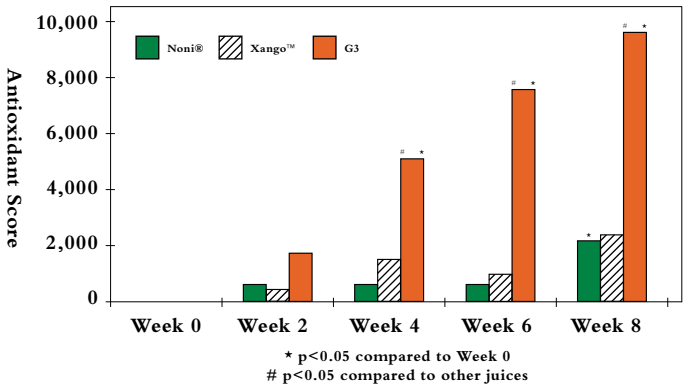
Thirty one subjects completed the study. One subject from the Tahitian Noni® group dropped out in the first week due to the taste of the juice. One subject from the Xango™ (mangosteen) group dropped after the baseline screening due to pregnancy. One subject dropped from the Xango™ group and did not give a reason.

Drinking G3 significantly increased Scanner scores within four weeks (Figure 3). Scores increased an average of 9273 ± 5081 Counts in eight weeks (Range 5,000-14,000 Counts). Furthermore, consumption of G3 led to higher Scanner scores than either Xango™ (mangosteen) or Tahitian Noni® at weeks four, six and eight.

No changes in the Noni® group were observed other than a moderate 2,400 ± 1,060 count increase at week 8 compared to the beginning of the study (Figure 3).

Consumption of Xango™ (mangosteen) had no effect on Scanner score at any time (Figure 3).

Figure 3: Change in Antioxidant Score



Discussion

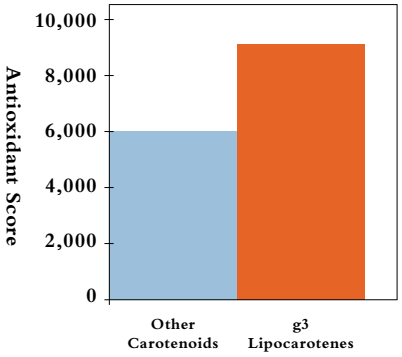
G3, consumed as recommended (three fluid oz twice a day) dramatically increased scanner scores in 10 out of 11 subjects. Average score increases were: 4818 ± 2786 (21%) after four weeks, 7455 ± 5392 (32%) after six weeks and 9273 ± 5081 Counts (40%) after eight weeks. There was no apparent plateau in the increase in Scanner scores in response to drinking G3 for eight weeks. Therefore, it may be theorized that if the study had been carried out for a longer duration, scores would have continued to increase indefinitely.

G3 increased Scanner scores 375% more than either Xango™ (mangosteen) or Noni® Juice.

Among the potent phytonutrients found in G3 are Lipocarotenes.™ A Lipocarotene™ is a matrix of beta-carotene and fatty acids that enables efficient absorption and transport of beta-carotene and other fat-soluble vitamins. Significant concentrations of long chain fatty acids (~7-10% by weight) are found in the seed membrane and pulp of the Gac fruit. This oil is also a rich source of vitamin E and essential fatty acids.

The Lipocarotene™ form of carotenoids in G3 are as much as 55% more bioavailable than those from other sources (Vuong 2002) (Figure 4)

Figure 4: Lipocarotene Carotenoids 55% more available



From this study, it can be concluded that G3 effectively increases skin-carotenoid levels, a biomarker of overall antioxidant protection.

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Compendium of Abstracts

Raman detection of carotenoids in tissues

Raman spectroscopy in the Eye

Study reference:
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.
http://www.ncbi.nlm.nih.gov/pubmed/18382494

ABSTRACT

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

Study reference:
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.
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.

Study reference:
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.
http://www.ncbi.nlm.nih.gov/pubmed/12359594

ABSTRACT

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

Study reference:
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

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

Study reference:
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 at: http://www.iupac.org/publications/pac/2002/pdf/7408x1419.pdf

ABSTRACT

The human macula uniquely concentrates extraordinarily high levels of two xanthophylls carotenoids, lutein and zeaxanthin. The function, metabolism, and physiology of these yellow pigments are incompletely understood, but they are likely to prevent age-related damage to the foveal region by virtue of their ability to act as free-radical quenching antioxidants and to absorb phototoxic blue light with high efficiency. A wealth of circumstantial evidence suggests that high macular levels of these two carotenoids may protect against agerelated 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.

Study reference:
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 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.

Study reference:
Ermakov IV, Ermakova MR, Gellermann W. Simple Raman instrument for in vivo detection of macular pigments. Appl Spectrosc 2005;59(7):861-7.

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

ABSTRACT

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

Study reference:
Ermakov I, Ermakova M, Gellermann W, Bernstein PS. Macular pigment Raman detector for clinical applications. J Biomed Opt 2004; 9(1):139–48.
http://www.ncbi.nlm.nih.gov/pubmed/14715066

ABSTRACT

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

Study reference:
Ermakov IG, McClane RW, Gellermann W. Resonant Raman detection of macular pigments in the living human retina. Optics Letters 2001;26(4):202–204.
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.

Study reference:
Gellermann, W., Bernstein PS. Noninvasive detection of macular pigments in the human eye. J Biomed Opt. 2004 Jan-Feb;9(1):75-85. Review.
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 autofluorescense techniques and put them in perspective with recently developed more molecule-specific Raman detection methods.

Study reference:
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.
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.

Study reference:
Gellermann, W., Ermakov, I,V., McClane, R.W. Raman imaging of human macular pigments. Optics Letters 2002; 27(1):833–835.
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.

Study reference:
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 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.

Study reference:
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

BACKGROUND: It has been hypothesized that the macular carotenoid pigments lutein and zeaxanthin may protect against macular and retinal degenerations and dystrophies. OBJECTIVE: To test this hypothesis by objectively measuring lutein and zeaxanthin levels in a noninvasive manner in patients who have retinitis pigmentosa (RP), choroideremia (CHM), and Stargardt macular dystrophy and comparing them with an age-matched healthy control population. METHODS: Using resonance Raman spectroscopy, a novel objective noninvasive laser-optical technique, we measured macular carotenoid levels in 30 patients (54 eyes) who have RP, CHM, and Stargardt macular dystrophy and compared them with 76 age-matched subjects (129 eyes) who did not have macular pathologic conditions in a case-control study. RESULTS: As a group, patients with RP and CHM had the same macular carotenoid levels as age-matched healthy control subjects (P =.76, 2-way analysis of variance). Patients with Stargardt macular dystrophy tended to have levels of macular carotenoid pigments that, on average, were about 50% lower than healthy controls (P =.02,unpaired 2-tailed t test). CONCLUSIONS: The patients with RP and CHM had normal levels of macular carotenoids, suggesting that nutritional supplementation with macular carotenoids such as lutein, zeaxanthin, or both will be unlikely to affect the clinical course of RP and CHM. Although the number of patients with Stargardt macular dystrophy examined was limited, their macular carotenoid levels were usually lower than those of subjects of a similar age with no macular pathologic condition.

Raman Spectroscopy in the Skin

Study reference:
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;13(4).
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.

Study reference:
Darwin M, Schanzer S, Teichmann A, Blume-Peytavi U, Sterry W, Lademann J. [Functional food and bioavailability in the target organ skin.] Hautarzt. 2006;57(4):286-90. [Article in 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.

Study reference:
Ermakov IV, Ermakova MR, Gellermann W, Lademann J. Noninvasive selective detection of lycopene and beta-carotene in human skin using Raman spectroscopy. J Biomed Opt 2004;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.

Study reference:
Ermakov IV, Ermakova MR, McClane RW, Gellermann W. et al. Resonance Raman detection of carotenoid antioxidants in living human tissues. Optics Letters 2001;26(15):1179-1181.
http://www.ncbi.nlm.nih.gov/pubmed/18049555

ABSTARCT

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

Study reference:
Gellermann, W., Ermakov, I.V., Scholz, T.A. and Bernstein, P. S. Noninvasive laser Raman detection of carotenoid antioxidants in skin. Cosmetic Dermatology 2002;15(9):65-68.

ABSTRACT

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

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

ABSTRACT

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

Study reference:
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.

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

ABSTRACT

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

Study reference:

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.
http://apjcn.nhri.org.tw/server/APJCN/Volume15/vol15apcns/IUNS-APCNS2006_HEC.pdf

ABSTRACT

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

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

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

Study reference:
Peng, Y.M., Peng, Y.S., Lin, Y., Moon, T., Roe, D.J. and Ritenbaugh, C. (1995) Concentrations and plasma-tissue-diet relationships of carotenoids, retinoids, and tocopherols in humans. *Nutrition and Cancer* **23,** 233-246.
http://www.ncbi.nlm.nih.gov/pubmed/7603884

ABSTRACT

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

Study reference:
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 - Volume 37 - Issue 5 - p S385
http://journals.lww.com/acsm-msse/Fulltext/2005/05001/Assessment_Of_The_Relationship_Between_Oxidative.1991.aspx

ABSTRACT

Purpose: Increasing evidence from epidemiological studies suggest that measures of chronic inflammation are associated with an increased risk of cardiovascular disorders, including ischemic heart disease, stroke and other thromboembolism. Oxidative stress may play a role in the development of the inflamed state, and previous studies have shown a possible protective effect of high levels of cardiorespiratory fitness. The purpose of this study was to examine the relationship between oxidative stress, markers of inflammation and cardiorespiratory fitness (VO2max) in the obese. **Methods:** A total of four hundred eighty three obese individuals (mean age 43.34 ± 8.32) were assessed for VO_{2max}, oxidative stress as measured photometrically in the blood with a Free Radical Analysis System (FRAS), C-Reactive protein (CRP), which was used as a measure of inflammation and Body Defense Score (BDS), which was used as an indicator of antioxidant status and measured non-invasively using Raman Spectroscopy (Biophotonic Scanner, Pharmanex. Descriptive data is shown below in table 1. **Results:** Body weight was negatively correlated with FRAS ($r=-0.199$, $p=0.009$) and BDS ($r=-0.171$, $p=0.002$), but not CRP. Additionally CRP was positively correlated with FRAS ($r=0.551$, $p<0.001$) and negatively with VO_{2max} ($r=-0.260$, $p<0.001$). No other associations among these variables were observed. **Conclusions:** These data support the concept that obesity stimulates an inflammatory response. Part of the inflammatory response may be due to oxidative stress. The strong negative correlation between body weight and BDS suggests that a diet rich in fruits and vegetables or antioxidant supplementation may be particularly important in obese individuals. Increased fitness may also protect against inflammation in these individuals.

Work Supported by: Pharmanex.

Study reference:
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.

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 caretenoids were observed. This data suggests that weight loss significantly decreases the skin carotenoid level, but the decrease in the skin carotenoid level was due to factors other than changes in dietary carotenoids. Further studies are needed to examine the mechanisms responsible for the decreases in SCS due to weight loss in overweight and obese individuals.

Supported by grants from Kraft Foods, Inc (primary) and Pharmanex (secondary)

Study reference:
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.

http://www.fasebj.org/cgi/content/meeting_abstract/20/4/A145-a?maxtoshow=&hits=10&RESULTFORMAT=&author1=zukley&andorexacttitle=and&andorexacttitleabs=and&andorexactfulltext=and&searchid=1&FIRSTINDEX=0&sortspec=relevance&volume=20&fdate=7/1/2005&tdate=6/30/2008&resourcetype=HWCIT

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

Bioavailable carotenoids and other antioxidants were provided by LifePak.

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

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

ABSTRACT

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

Study reference:
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.
http://www.pharmanexusa.com/library/px/pdf/scanner_corr_eb2004_abstract.pdf

ABSTRACT

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

Supported by Pharmanex, LLC

Study reference:
Smidt, C.R., Burke, D.S. Nutritional Significance and Measurement of Carotenoids. *Current Topics in Nutraceutical Research*. 2004b, Vol. 2, No. 2, pp. 79-91.
http://ctnr.newcenturyhealthpublishers.com/about/issue_2_2.php#2

ABSTRACT

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

Study reference:
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.

ABSTRACT

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

Supported by Pharmanex, LLC, Provo, UT.

Study reference:
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.

ABSTRACT

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

Study reference:
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.
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 >or=48 hours. RESULTS: Consistent positive correlations were observed for each of 3 separate same-day correlation plots of total serum versus RS skin carotenoids. Overall estimate of the line of best fit from analysis of covariance, using all 3 samples (n = 1116), yielded a Pearson correlation of R = 0.81 (r(2) = 0.66; $p < 0.001$). Based on analysis of variance, RS skin carotenoid methodology exhibited 0.9% less variance over the 3 tests than serum carotenoids by the HPLC method ($p < 0.03$). CONCLUSIONS: RS accurately measures total carotenoids in human skin with less intra-individual variability than measurement of serum carotenoids by HPLC analysis. RS technology is a valid and reliable noninvasive method to rapidly assess carotenoid nutritional status in humans.

Study reference:
Carlson, J., Stavens, S., Holubkav, R., Zidichouski, J., Mastaloudis, A., Smidt, C.R. 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

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

Study reference:
Bernstein P.S., Yoshida, M.D., Katz, N.B., McClane, R.W., Gellermann, W., Raman detection of macular carotenoid pigments in intact human retina. Invest Ophthalmol Vis Sci. 1998 Oct;39(11):2003-11.
Full length article available 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.

Study reference:
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.

ABSTRACT

Purpose: To describe the cross-sectional association of body composition with carotenoid antioxidants in overweight individuals in 176 overweight (BMI $\geq$ 27 $kg.M^{-2}$) participants. .

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

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

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

Study reference:
Stavens, S., Carlson, J., Holubkav, R., Zidichouski, J., Mastaloudis, A., Smidt, C.R. 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.
Full article available at: http://www.nuskin.com/global/library/pdf/program_abstract_6694.pdf

ABSTRACT

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

Study reference:
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;13(4).
http://www.ncbi.nlm.nih.gov/pubmed/19021353

ABSTRACT

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

Study reference:
Rerksuppaphol S, Rerksuppaphol L. Effect of fruit and vegetable intake on skin carotenoid detected by non-invasive Raman spectroscopy. J Med Assoc Thai 2006;89(8):1206-12.
http://www.ncbi.nlm.nih.gov/pubmed/17048431

ABSTRACT

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

Study reference:
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]

Guo HW, Li H, Huang ZY, Xue K, Zhou X, Ma YY, Liu M, Zhu ZG, Li CL, Zhu JS. Examination of Carotenoids in Human Skin by Biophotonic Raman Spectroscopy Scanner. Journal of Environmental and Occupational Medicine 2006;23(3):204-206.

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

Study reference:
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.

ABSTRACT

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

Study reference:
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.
http://www.ncbi.nlm.nih.gov/pubmed/16409093

ABSTRACT

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

Presentation reference:
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.

ABSTRACT

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

Two studies were undertaken to determine the correlation of SC measurement by RS (excitation at 473 and detection at 511 nm on the palm of the right hand) with serum carotenoid levels (HPLC). First we compared intra-individual variability of 372 healthy subjects who donated 3 blood samples along with simultaneous SC measurement for each draw within an 8 day period [1] . Secondly, we measured (n = 286) blood carotenoids (α- and β-carotene, lutein, zeaxanthin, lycopene and α- and β-cryptoxanthin), and correlated them with SC measurements [2]. Measurement of other serum nutrients (**t**ocopherols and ascorbic acid) and markers of oxidative stress [serum oxygen radical absorbance capacity (ORAC) and urinary F_2-isoprostanes (F_2-IsoPs), malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS)] were measured. All blood samples were taken following at least an 8 hour fast. Comparison of SC levels with BMI was also examined.

For the first study, consistent positive correlations were observed for each of three separate same-day measurements and correlations (Pearson's correlation of r = 0.82, 0.81, 0.80; $p<$ 0.001). Overall estimate of the line of best fit from ANCOVA using all three samples (n = 1,116) yielded a significant correlation (r = 0.81; $p < 0.001$). Based on ANOVA, SC by RS methodology exhibited 0.9% less variance over the three tests than serum carotenoids by the HPLC method ($p< 0.03$; $p< 0.001$). Similar correlation of SC and serum carotenoid levels was found in the second study (r=0.81). Regression analysis showed the strongest association of serum carotenoids was with β-carotene and skin carotenoids (r=0.76). SC measures were associated with serum levels of vitamins C (r = 0.37; $p < 0.001$) and E (alpha tocopherol); r = 0.34; $p < 0.001$) and inversely associated with urinary F_2-IsoPs (r = -0.19; p = 0.001), serum ORAC (r = -0.11, $p= 0.06$), urinary TBARS (r=-0.09, p=0.13) and MDA (r=0.10, p = 0.11). BMI was inversely correlated with SC.

SC is highly correlated with measurements made in human serum samples and less variable than serum carotenoids. Serum β-carotene is the carotenoid most highly correlated with SC. Additionally SC was modestly correlated with other key antioxidants (vitamins C and E), and inversely correlated with urinary (F_2-IsoPs) indicating the SC's potential to give insight on a person's overall antioxidant status. The inverse association of SC BMI warrant further investigation into influence i.e. oxidative stress, inflammation, etc on carotenoid status.

Acknowledgements

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

This work was supported in part by Pharmanex, UT.

Book Chapters/Book Segments

Book Reference

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. Carotenoids and Retinoids – Molecular Aspects and Health Issues. Champaign, IL: AOCS Press, 2005: Ch. 6, 86-114.

INTRODUCTION

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

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

Book Reference

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

Raman Spectroscopy Used to Measure Antioxidant Capacity

Noninvasive measurements of clinical parameters are always preferable to those requiring blood, urine, or tissue. Raman spectroscopy is just such a measurement technique and may become widely used in the future. Using a laser light that is pointed toward the fat pad of the palm, as the laser light penetrates the skin, the amount of carotenoids (all-trans-beta-carotene, lycopene, alpha-carotene, gamma-carotene, phytoene, phytofluene, sepapreno-betacarotene, 7,7'dihydro-betacarotene, astaxanthin, canthanxanthin, zeaxanthin, lutein, beta-apo8'carotenal, violaxanthins, and rhodoxanthin) is measured at the cellular level. Because all carotenoids have a carbon backbone with alternating carbon double and single bonds, the vibration of these bonds can be detected with Raman spectroscopy. Raman spectroscopy has been used to assess carotenoids in precancerous skin lesions as well as in the retina to assess early stages of macular degeneration (Ermakov et al., 2005). Caotenoids are powerful antioxidants, and because they are part of the "antioxidant network" a measure of their presence can give a good assessment of the antioxidant capacity of the cell. The Raman spectroscopy score also correlates inversely with urinary isoprostanes, a measure of oxidative stress (Carlson et al., 2006). Serum carotenoids significantly correlate with skin carotenoids, as measured using Raman spectroscopy and the Biophotonic Laser scanner (Smidt et al., 2004; Zidichouski et al., 2004). Serum carotenoids are a good measure of the absorptive capacity of the individual (see Chapter 3). Thus an individual with a diet high in fruits and vegetables and therefore large amounts of dietary carotenoids usually has a high carotenoid antioxidant score. The antioxidant score, or the numeric result from this scan, can be used to determine how well a person is processing carotenoid antioxidants and whether the antioxidants are reaching the cell where they exert their protective functions. The number, which seems to be in the range of 25,000 and higher in those with optimal health, increases with greater consumption of carotenoid-containing fruits and vegetables, consumption of carotenoid-containing nutritional supplements, smoking cessation, and loss of excess body fat (Carlson et al., 2006). The measurement is quick, easy, and inexpensive, making it a likely assessment tool for nutritional professionals in the future.

The following studies did not use the biophotonic scanner, however they establish carotenoid-status as an important biological marker

Study reference:
Buijsse B, Feskens EJ, Schlettwein-Gsell D, Ferry M, Kok FJ, Kromhout D, de Groot LC. Plasma carotene and alpha-tocopherol in relation to 10-y all-cause and cause-specific mortality in European elderly: the Survey in Europe on Nutrition and the Elderly, a Concerted Action (SENECA). Am J Clin Nutr. 2005 Oct;82(4):879-86.
Full length article available at: http://www.ajcn.org/cgi/reprint/82/4/879

ABSTRACT

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

Study reference:
Svilaas A, Sakhi AK, Andersen LF, Svilaas T, Strom EC, Jacobs DR Jr, Ose L, Blomhoff R. Intakes of antioxidants in coffee, wine, and vegetables are correlated with plasma carotenoids in humans. J Nutr. 2004 Mar;134(3):562-7.

Full length article available at: http://jn.nutrition.org/cgi/reprint/134/3/562

ABSTRACT

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

Note: The majority of studies cited in this document are hyper-linked to corresponding abstracts on the internet. Please see the references section at the end of this document in electronic format for easiest access. An electronic copy of this document can be obtained at www.pharmanexusa.com

Responses to Common Technical Questions Concerning the Pharmanex® BioPhotonic Scanner

Question:
What science is there to validate the Pharmanex BioPhotonic Scanner?

RESPONSE:
The Pharmanex BioPhotonic Scanner is backed by science.
The use of Raman spectroscopy for biological measurements is an established scientific discipline backed by years of research. The Pharmanex BioPhotonic Scanner is a patented application of Raman spectroscopy for the measurement of carotenoid antioxidant nutrients in living tissue for the improvement of nutrition. The use of biophotonics to assess biological molecules in living tissue is a distinct scientific discipline, and the Pharmanex BioPhotonic Scanner is an instrument that is based on this scientific discipline.

The use of Raman spectroscopy for the assessment of human tissue carotenoids has been validated by at least eight peer-reviewed studies conducted by third party entities unrelated to Pharmanex or the supplementation industry. (Bernstein, 1998, 2002; Ermakov, 2004a, 2004b; Gellermann, 2004, 2002; Hata, 2000; Zhao, 2003). Raman spectroscopy is an established and accepted detection method; a Medline search for the term *raman spectroscopy* yields over 4,400 articles. Most importantly, validation of Raman spectroscopy is recognized through the award of Nobel Prize to Sir CV Raman for its discovery in 1930.

Pharmanex is not the only entity impressed with the nutritional implications of Raman spectroscopy. In 2003 the National Cancer Institute (a division of the National Institutes of Health) awarded researchers at Yale University a $1 million grant to conduct a study using Raman detection of carotenoids as an objective measure of fruit and vegetable intake. Most large-scale nutritional studies rely on diet recall surveys, which are subject to errors of reporting fruit and vegetable consumption. (Mayne *et al*, 2003; YCC News Release, 2003).

Importantly, Pharmanex has validated the use of Raman spectroscopy for the measurement of carotenoids in four studies including a large-scale clinical screening study with 1,375 subjects that confirmed a correlation between antioxidant status and lifestyle parameters (Smidt, 2003). A second study established efficacy of LifePak® to improve the antioxidant status of subjects over a 12-week period (Smidt, 2002), and a third study established a highly significant correlation ($r=0.78$, $p < 0.001$) between serum carotenoid levels and skin carotenoid levels as assessed by the Pharmanex BioPhotonic scanner (Smidt, 2004a). A fourth study was presented at the 45th Annual Meeting of the American College of Nutrition in Long Beach, California. The 372-subject clinical study re-confirmed the excellent correlation between skin Scanner scores and blood carotenoids, the currently accepted gold standard in research. In addition, the study demonstrated that the Pharmanex® BioPhotonic Scanner measurement has less variability than blood carotenoids (measured by the conventional HPLC method). A fifth study was presented by Dr. James Rippe at the National Meeting of the American College of Sports Medicine in June 2004 (Indianapolis, IN). This study confirmed that in overweight and obese individuals the level of adipose tissue accumulation negatively influenced skin carotenoid levels, and thus antioxidant status. More studies are ongoing, and after peer review, will be published in scientific journals.

The Pharmanex BioPhotonic Scanner is consistently well received by experts in all areas of science. Pharmanex Scientists have presented the science behind LifePak® and the BioPhotonic scanner at a number of scientific meetings. In February 2003, Pharmanex scientists joined with Dr. James Rippe and the inventor of the biophotonic scanner, Dr. Werner Gellermann in a presentation at the New York Academy of Sciences. In February 2003 and 2004, Pharmanex Scientists attended scientific meetings on antioxidants organized by the Oxygen Club of California in Cadiz, Spain, and Santa Barbara, California. Dr. Lester

Packer (the "father of antioxidants") is the founder and honorary president of the Oxygen Club and chaired these meetings. Dr. Carsten Smidt presented a scanner study at the Santa Barbara meeting. In April 2003 and 2004, Dr. Carsten Smidt attended the Federation of American Societies for Experimental Biology (FASEB) meetings in San Diego and Washington DC, and presented the results of two different scanner studies. The FASEB meeting is attended by more than 10,000 scientists from around the world, but only a small percentage are selected to present. In January of 2004, Dr. Carsten Smidt attended and presented the scanner/serum correlation study at the prestigious Gordon Research Conference on Carotenoids in Ventura, California. Dr. Smidt also scanned 60 of the top antioxidant researchers in the world at this conference. In all instances the Pharmanex BioPhotonic Scanner has been very well received by the scientific community.

Question:
If the BioPhotonic Scanner measures only carotenoids, how can it be used to infer the status of other nutrients?

RESPONSE:
Serum carotenoids correlate to overall antioxidant status
Carotenoid molecules are not regenerated like other antioxidants, and are degraded in the process of neutralizing free radicals or reactive oxygen species. A typical carotenoid molecule like lycopene or β-carotene is able to sustain more than 20 free radical hits by lipid radicals before it becomes completely destroyed (Tsuchiya, 1994). Lycopene and β-carotene are just two examples of antioxidants among hundreds of antioxidants that make up the *antioxidant network.* Carotenoids act sacrificially to protect other members of the antioxidant network (such as vitamins E and C) from having to sustain free radical hits; in this way carotenoids will support the entire antioxidant network consequently reducing the danger from oxidative stress (Packer, 1994; Packer and Coleman, 1999). Conversely, high levels of oxidative stress (e.g., with smoking) adversely affect the antioxidant network, and the resulting increased free radical activity leads to a depletion or reduction in tissue carotenoids (Smidt and Shieh, 2003; Gollnick and Siebenwirth, 2002, Dietrich, 2003).

A recent study conducted by Svilaas *et al.* established carotenoids as a reliable indicator of other dietary antioxidants. Svilaas and his colleagues assessed antioxidant intake from diets of more than 2,670 adults, and evaluated blood serum antioxidants of 61 individuals for seven consecutive days. Svilaas *et al.* found the ability of carotenoids to predict serum levels of other antioxidants was stronger than the predictive ability of alpha, beta, delta, and gamma-tocopherols as well as glutathione (Svilaas, 2004). Carotenoids are not only convenient biomarkers because they are accurate predictors of overall antioxidant status, but also they are Raman active and can be detected without the concerns of blood samples (Bernstein, 1998, 2002; Gellermann 2002a; Zhao, 2003, Ermakov, 2004b). Furthermore, carotenoids are delivered to tissues by the same mechanism as other fat-soluble antioxidants. This shared LDL delivery mode is the proposed mechanism to explain the correlation between tissue carotenoids and other fat-soluble antioxidants in multiple studies (Lasheras et al., 2002; Steinberg & Chait, 1998).

Serum carotenoids correlate to skin carotenoids
Skin carotenoids analyzed by HPLC were shown to correlate significantly to serum carotenoid levels (Peng, 1995). The significant correlation between skin biopsy-levels of carotenoids and serum carotenoid levels eliminates the need for routine skin removal. A recent study of 104 participants showed a highly significant correlation between serum total carotenoids and skin carotenoids as assessed by Raman Spectroscopy ($r = 0.78$, $p < 0.001$) (Smidt, 2004). A second study confirmed the correlation of the n=104; this second study included 372 participants. Three separate correlation plots were produced and all showed highly significant correlations (range .78 –.82, $p<.0001$) between total serum carotenoid level and Raman spectroscopy-derived skin carotenoid scores (Zidichouski, 2004). These data bridge the findings of Svilaas, and Peng, to validate Raman Spectroscopy as a method to assess skin carotenoid status as an indication of broad-spectrum antioxidant status, without the inconvenience of skin and blood samples.

Carotenoids accepted as indicator of fruit and vegetable intake
Carotenoids include more than 50 antioxidants widely distributed among fruits and vegetables. When ingested from dietary sources, the presence of carotenoids in living tissue is an indicator for the presence of

other important nutrients common to those dietary sources (Svilaas, 2004). Based on this correlation, the National Cancer Institute awarded a $1 million research-grant to Yale scientists to conduct a study using the Raman-detection of carotenoids as an objective measure of fruit and vegetable intake (Mayne *et al*, 2003; YCC News Release, 2003). The correlation between carotenoids and other nutrients applies to nutritional supplements only to the degree they deliver optimal amounts of all essential and generally beneficial nutrients. For this reason, LifePak® is formulated as a broad-spectrum multivitamin, mineral, antioxidant supplement, which contains nutrients in amounts similar to a diet rich in fruits and vegetables.

Question:
What aspects of health are positively affected by proper carotenoid nutrition?

RESPONSE:
Carotenoids have been shown in countless studies to support many areas of health
The scanner is not intended to diagnose, mitigate, treat, or cure any disease. Nonetheless, convincing evidence suggests that certain carotenoids have been linked to health benefits including reduced risk of age-related macular degeneration, cataracts, cardiovascular disease, and prostate cancer. A review article written by Pharmanex scientists will appear in the peer-reviewed journal *Current Trends in Nutraceutical Research*. The article includes a review of the role of carotenoids in human health and is summarized below (Smidt and Burke, 2004).

Eye Health
A number of studies support the protective role of carotenoids in the prevention of age-related eye diseases. For example, reduced risks of cataracts (Brown *et al.,* 1999; Chasan-Taber *et al.,* 1999) and age-related macular degeneration (Seddon, 1994; Bone, 2000, 2001, 2003; Landrum, 1997, 1996; Elless, 2000; Bernstein, 2002, Richer, 1999; Hammond, 1997) have been associated with high intakes of vegetables rich in the carotenoids lutein and zeaxanthin.

Cardiovascular Health
The carotenoids lutein and lycopene have been shown separately to support multiple aspects of cardiovascular health. Studies show positive health implications of lutein including decreased risk of mortality from cardiovascular disease (Kouris-Blazos, 2002), decreased progression of pre-atherosclerotic conditions (Dwyer, 2001), and other cardioprotective effects (Olmedilla, 2001; Cardinault, 2003).

The cardioprotective effects of lycopene have also been shown in multiple studies including reduced risk of myocardial infarction (Kohlmeier, 1997), lower risk of cardiovascular disease (Sesso, 2004), reduced LDL oxidation (Agarwal, 1998), and reduced production of LDL cholesterol (Fuhrman *et al.,* 1997).

Cancer
Epidemiological studies have shown that high intakes of tomatoes and tomato products, rich in lycopene, as well as high blood levels of lycopene are significantly associated with decreased prostate cancer risk (Deming, 2002; Giles, 1997; Giovanucci, 1995, 2002; Lu, 2001; Vogt, 2002). The finding of Kucuk *et al.* suggests that lycopene supplementation may decrease prostate cancer growth (Kucuk *et al.,* 2001). These effects may be attributed to lycopene's antioxidant and DNA protective properties (Riso *et al.,* 1999; Porrini and Riso, 2000).

Carotenoids may also play a role in cancer prevention because they can enhance gap junctional communication (GJC) between cells. (Krutovskikh *et al.,* 1995; Yamasaki *et al.,* 1995; Yamasaki, 1995; Dahl *et al.,* 1995; Trosko, 2003). Lycopene and □-carotene have been shown to enhance GJC significantly, and these effects are not related to their known antioxidant properties (Sies and Stahl, 1997; Stahl *et al.,* 1997; Zhang *et al.,* 1991). Thus, carotenoids may act via two distinct mechanisms of action to protect from cancer: as antioxidants to prevent mutagenic DNA alterations, and as promoters of GJC.

Oxidative Stress correlates to skin carotenoid concentration
Many of the above diseases are known to be linked to oxidative stress. A population study of 1,375 subjects was conducted at the Pharmanex Research Institute and found that individuals with high oxidative stress

generally have low skin carotenoid levels as measured by Raman spectroscopy, independent of subjects' dietary carotenoid consumption. This correlation was demonstrated by using Urinary MDA test, a proven model for oxidative stress (Smidt and Shieh, 2003).

Question:
Are other methods of testing more accurate at assessing antioxidant status?

RESPONSE:
Raman spectroscopy has been shown to be more reliable than other methods and more suitable for routine measurements reduced probability of error
Before Raman detection of carotenoids, established methods of measuring antioxidant status included the analysis of blood, urine, or tissue samples. These tests are expensive, invasive (e.g. *skin biopsies; blood draw*), have a higher probability of error (multiple steps involved in sample preparation and quantification all increase the magnitude of the overall error). Moreover, it may take weeks to receive the results. Also, the accuracy of tests using blood/serum or urine is placed in question due to possible effects of recent meals as the timing that the biological sample is taken (blood or urine) influences the outcome. A recent clinical study showed that Raman measurement of carotenoids in humans skin (palm of hand) correlates very highly with total serum carotenoid levels quantified from fasted serum (Smidt, Gellermann, & Zidichouski, 2004). The BioPhotonic Scanner is a great way to measure carotenoid levels safely and non-invasively at the site of action with the added advantage that it is well suited for routine measurements in large populations. The ability to assess carotenoid status through Raman spectroscopy will lead to further advances in nutritional and biological measurement.

Question:
How do Pharmanex formulations address the issue of excessively high levels of antioxidants acting as pro-oxidants?

RESPONSE:
Pharmanex products contain nutrient amounts proven to be safe in clinical studies

The levels of all nutrients found in Pharmanex products are based on well documented epidemiological, clinical, pre-clinical and safety studies. LifePak® is formulated to provide optimal nutrition with substantiated levels of nutrients that will not induce a pro-oxidative state. Included in the comprehensive blend of antioxidants is a balanced carotenoid combination in amounts similar to those provided by diets high in fruits and vegetables: 7.5 mg β-carotene, 5 mg lycopene, 2 mg α-carotene and 2 mg lutein. Each ingredient in LifePak® is present in amounts that are documented to be safe for long-term supplementation. Further, LifePak is safe when taken in conjunction with a diet high in fruits and vegetables. The daily amounts of all vitamins and minerals are well below the No-Observed Adverse Effect Levels (NOAEL) established by the Council for Responsible Nutrition (CRN) in 1997 and the Upper Limits (UL) established by the Food and Nutrition Board of the National Research Council.

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