Compendium of LifePak Studies & Abstracts
Pharmanex Research Institute, Provo, UT

The studies below appear in this compendium in the following order:


Updated January 2014
**Study Reference:**


Abstract provided below for your convenience can be found online at the following URL:

**ABSTRACT**

**Background:**
Cigarette smoking is well-known to associate with accelerated skin aging as well as cardiovascular disease and lung cancer, in large part due to oxidative stress. Because metabolites are downstream of genetic variation, as well as transcriptional changes and post-translational modifications of proteins, they are the most proximal reporters of disease states or reversal of disease states.

**Methods:**
In this study, we explore the potential effects of commonly available oral supplements (containing antioxidants, vitamins and omega-3 fatty acids) on the metabolomes of smokers (n = 11) compared to non-smokers (n = 17). At baseline and after 12 weeks of supplementation, metabolomic analysis was performed on serum by liquid and gas chromatography with mass spectroscopy (LC-MS and GC-MS). Furthermore, clinical parameters of skin aging, including cutometry as assessed by three dermatologist raters blinded to subjects’ age and smoking status, were measured.

**Results:**
Long-chain fatty acids, including palmitate and oleate, decreased in smokers by 0.76-fold (P = 0.0045) and 0.72-fold (P = 0.0112), respectively. These changes were not observed in non-smokers. Furthermore, age and smoking status showed increased glow (P = 0.004) and a decrease in fine wrinkling (P = 0.038). Cutometry showed an increase in skin elasticity in smokers (P = 0.049) but not in non-smokers. Complexion analysis software (VISIA) revealed decreases in the number of ultraviolet spots (P = 0.031), and cutometry showed increased elasticity (P = 0.05) in smokers but not non-smokers.

**Conclusions:**
Additional future work may shed light on the specific mechanisms by which long-chain fatty acids can lead to increased glow, improved elasticity measures and decreased fine wrinkling in smokers’ skin. Our study provides a novel, medicine-focused application of available metabolomic technology to identify changes in sera of human subjects with oxidative stress, and suggests that oral supplementation (in particular, commonly available antioxidants, vitamins and omega-3 fatty acids) affects these individuals in a way that is unique (compared to non-smokers) on a broad level.
Study reference:


Abstract provided below for your convenience can be found online at the following URL:

http://www.fasebj.org/cgi/content/meeting_abstract/24/1_MeetingAbstracts/805.4?sid=5612edc2-ca96-42b2-9849-924abbea8f00

**ABSTRACT**

*Cordyceps sinensis* and its fermentation product, CordyMax (CM), are used for anti-aging and health preservation. We have reported CM’s anti-fatigue and endurance enhancement functions in multi-sport endurance Caucasians athletes (*Chin J Clin Pharmacy* 2007, 5:16). LifePak (LP) is enriched in carotenoids and antioxidant nutrients to improve antioxidant capability in young athletes (*FASEB J* 2009, 23:1007.3). We further tested the improvement of exercise metabolism and antioxidant capacity with CM and LP in a self-controlled trial in young Chinese elite athletes (n=25, 18.1 yrs on average) recruited from China bicycle teams. They received CM 3.18 g/day and LP 2 sachets/day for 6 wks. All subjects remained on the same diet plan and training intensity during the study. Before and after the treatment, subjects performed graded exercise test on cycle ergometer and photonic assays for antioxidant scores. At a work load of 100W, 150W or 200W, RER were reduced significantly by 27%, 31%, & 36% (p<0.001), and blood lactate by 14%, 31% & 33%, and also reduced by 24% & 20% immediately and 3 min after exercise (p<0.05). VO2 peak, HR and O2 pulse were not changed in these elite athletes. Carotenoids antioxidant scores were increased by 21% (p=0.02). In conclusion, our data suggest benefits of CM-LP in improving antioxidant capacity and exercise metabolisms to enhance endurance capability and reducing risks of exercise injury in Chinese elite athletes.
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

Abstract provided below for your convenience can be found online at the following URL: [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](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:


Abstract provided below for your convenience can be found online at the following URL: http://www.fasebj.org/cgi/content/meeting_abstract/21/5/A709?maxtoshow=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.
Objective: To observe the variety of carotenoids level in the body through the detection of skin carotenoids.

Methods: 120 adult subjects were paired according to age, gender and the level of skin carotenoids at entry, and divided randomly into treatment and control groups. The treatment subjects took 12.6 mg β-carotene per day for 8 weeks. At the beginning, 4th and 8th week, the skin carotenoids were detected with resonance Raman spectroscopy. Meanwhile, 25h-dietary questionnaires for all participants were carried out.

Results: After supplementation (LifePak), the value of the skin carotenoids went up 23.3% at the 4th week and 44% at the 8th week over the beginning of the treatment group, and there was a very significant difference comparing with the control group (P < 0.001). The intakes of food and dietary β-carotene were not significantly different between two study groups during study.

Conclusion: The increase of skin carotenoids in treatment group may relate to the supplement of β-carotenoid. Raman scattering method, as a non-invasive method, is useful to reflect the level of carotenoids in human body through the measurement of carotenoids in skin.
Study reference:

No online abstract available

ABSTRACT

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

Abstract provided below for your convenience can be found online at the following URL:  

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 ($r^2=0.704$, $P<0.001$). Non-smokers and subjects with less sun-light exposure had significantly higher scores than those for cigarette smokers/former smokers and people with high exposure to sun light ($P<0.001$). The higher the BMI is, the lower the scores are ($P<0.001$), indicating diluted fat-soluble carotenoids in the body as a function of increases in body fat mass. We also found that The more daily consumption of fruits-vegetables and dietary supplements, the higher the scores are ($P<0.01$). Daily LifePak intake resulted in increases in the scores by 24% after 4 weeks of LifePak and by 44% after 8 weeks ($P<0.001$).

Conclusions- Biophotonic scanner scores reflect steady state levels of antioxidant carotenoids in human’s skin. Fruits-vegetables intakes and LifePak supplementations increase body’s antioxidant capacity, but smoking and sun-light exposure reduce it.
A Double Blind Placebo Study showing the Effect of lifepak nano supplementation on Skin Carotenoid Scores
February 2006

Carsten R. Smidt, Ph. D., FACN, Angela Mastaloudis, Ph. D., Stephen Poole, B.S.
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 monomolecular 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

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

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 Biophotic Scanner.
References


Brown, MJ, Ferruzzi, MG, Nguyen, ML, Cooper, DA, Eldridge, AL, Schwartz, SJ and White, WS Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection AJCN 2004;80:396-403.


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 µmol/L), serum ß-carotene (0.56 ± 0.3 to 1.26 ± 0.6 µmol/L), lutein (0.29 ± 0.1 to 0.34 ± 0.1 µmol/L), lycopene (0.94 ± 0.3 to 1.31 ± 0.4 µmol/L), ascorbate (50 ± 23 to 85 ± 29 µmol/L), α-tocopherol (34 ± 9.4 to 54 ± 13 µmol/L) and skin carotenoids (18353 ± 4827 to 25358 ± 5229 units). Only serum ascorbate changed in P (53 ± 24 to 67 ± 39 µmol/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.
Clinical Screening Study: Use of the Pharmanex® BioPhotonic Scanner to Assess Skin Carotenoids as a Marker of Antioxidant Status

Carsten R. Smidt, Ph.D., FACN

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 of 1,375 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 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 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 measurements 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.

**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 Activity**

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

**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.*

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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ABSTRACT

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

**Supported by Pharmanex, LLC, Provo, UT.**
Effect of LifePak<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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.

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.*

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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EFFECTS OF LIFEPAK® SUPPLEMENTATION ON ANTIOXIDANT STATUS AND LDL-OXIDATION IN HEALTHY NON-SMOKERS

C. R. Smidt,1 PhD, R. J. Seidehamel,2 PhD, S. Devaraj,3 PhD, I. Jialal,3 MD, PhD
1 Pharmanex Research Institute, Provo, UT; 2 GFI Pharmaceutical Services, Inc., Evansville, IN; 3 University of California at Davis Medical Center, Sacramento, CA

ABSTRACT

Background: Although there is substantial evidence for antioxidant effects of individual nutrients, such as vitamins C and E, little research has been done to show the antioxidant efficacy of nutritionally complete micronutrient supplements.

Objective: This study examined whether a comprehensive multivitamin/mineral supplement (MVMS; LifePak®) improves antioxidant status and resistance to LDL oxidation in healthy non-smokers.

Methods: In this double-blind, randomized crossover study, 50 subjects received MVMS and placebo (PL) for 6 weeks with a 6-week washout period. MVMS provided vitamin C, vitamin E, carotenoids and flavonoids among other nutrients.

Results: MVMS significantly increased serum concentrations of ascorbic acid (from 68.1 to 94.3 µmol/L), β-carotene (from 335 to 716 nmol/L), α-carotene (from 77 to 592 nmol/L), and vitamin E (α-tocopherol, from 20.0 to 36.9 µmol/L), with no changes in PL treatment. MVMS significantly decreased LDL oxidizability, as the lag time was prolonged (by 17 %), and oxidation rate was reduced without changes with PL treatment.

Conclusions: MVMS significantly increased serum antioxidants and decreased LDL oxidizability. Results suggest that MVMS supplementation may have cardiovascular and antioxidant benefits in healthy non-smokers.

Keywords: Multivitamin, Antioxidants, Atherosclerosis, Cardiovascular Disease, Carotenoids, α-Carotene, β-Carotene, α-Tocopherol, Vitamin C
INTRODUCTION

Aerobic metabolism and environmental factors can generate a variety of reactive oxygen species (ROS), which can damage DNA, as well as structural and functional proteins, carbohydrates, and lipids (1,2). ROS have been implicated in the pathogenesis of many chronic diseases, including atherosclerosis (3-6). Some of the health-promoting effects of antioxidant nutrients, such as the vitamins C and E, carotenoids, and flavonoids, are thought to be based on their ability to quench ROS (6-8).

Numerous clinical studies have demonstrated the health benefits of individual antioxidant nutrients, and more recently, a few human studies have addressed the effects of limited combinations of antioxidant nutrients (9-11). Studying the effects of more complete antioxidant nutrient combinations is a worthwhile endeavor, because most of these nutrients closely interact with each other and with the body’s intrinsic antioxidant systems to provide protection from free radical damage (12-15). Consequently, it is safe to assume that the best overall protection from free radical damage and pathologic conditions may be achieved by consuming diets and supplements rich in a variety of antioxidant nutrients (7).

However, little human research has been done on dietary supplements combining more than three antioxidant nutrients. An extensive on-line literature search in the Medline database (1966 - present) yielded no human prospective studies testing combinations of vitamins C and E, selenium, carotenoids and flavonoids. Girodon et al. tested a combination of vitamins C and E, β-carotene, zinc and selenium in amounts close to the RDA in French hospitalized elderly, and showed improved antioxidant status (9). Likewise, Preziosi et al. supplemented 201 middle-aged healthy subjects in a multi-center study with a similar antioxidant combination, and showed improvements in biochemical indicators of antioxidant status (10). Jialal and Grundy supplemented men with a high-dose combination of α-tocopherol, β-carotene and vitamin C versus α-tocopherol alone for three months and observed similar reductions of LDL oxidizability in both groups, concluding that the addition of β-carotene and vitamin C did not enhance α-tocopherol’s efficacy (16).

The present study was designed to evaluate the antioxidant effects of a commercially available, comprehensive multivitamin/mineral supplement with above-RDA amounts of vitamins C and E (i.e., 500 mg and 300 IU, respectively) and other antioxidant nutrients, such as carotenoids, flavonoids, the trace element cofactors for antioxidant enzymes (Cu, Zn, Mn, and Se) and all other essential micronutrients. The present study determined the effects of this micronutrient supplement on antioxidant status and resistance against LDL oxidation in healthy adult non-smokers.

METHODS AND MATERIALS

Subjects. Included in this study were 50 healthy male and female subjects between 18 and 65 years of age recruited by GFI Pharmaceutical Services, Inc. in the Evansville, Indiana, area.
The number of subjects was estimated using statistical power calculations based on data from existing vitamin E supplementation studies. All subjects were healthy non-smokers consuming typical U.S. diets with less than five daily servings of fruits and vegetables. Good health was determined by physical exam, medical history and clinical laboratory evaluation. Except for the test product, subjects did not take any dietary supplements or drugs with potential antioxidant or oxidant effects three months prior to or during the study. Subjects also did not consume more than one serving of alcoholic beverages daily on average and had no evidence or history of substance abuse. They were instructed and willing to maintain their usual dietary and exercise habits throughout the duration of the study. Female subjects were not pregnant or nursing and had to use adequate birth control precautions, and postmenopausal women receiving hormone replacement therapy were instructed to remain at the same dosage throughout the study.

**Treatments.** This study was carried out as a double-blind, randomized cross-over design with two six-week treatment periods and a six-week washout period in between. The study protocol, informed consent forms and final report were reviewed and approved by an Institutional Review Board (Ohio Valley IRB). The 50 subjects were randomly assigned to receive either a commercially available comprehensive multi-vitamin/mineral supplement (MVMS; LifePak®, Pharmanex, Provo, Utah, U.S.A.) or an identically appearing placebo (PL). Both treatment and placebo supplements were manufactured under Good Manufacturing Practices. The composition of MVMS is shown in Table 1. After the washout period, treatments were reversed for each subject. The study was conducted during the months of December-April. Subjects were instructed to take one packet (containing 3 capsules) of MVMS or PL twice daily, i.e., with their morning and evening meals. Safety was assessed by physical examination, clinical laboratory evaluation (blood chemistry, hematology and urinalysis) and by monitoring of adverse reactions. The conduct of the study at the contract research site was monitored by an independent clinical study monitor.

**Measurements.** At the beginning and end of each treatment period, serum and plasma samples were collected after an overnight fasting period, and assayed to determine antioxidant status and LDL oxidizability. A separate serum sample for ascorbate analysis was collected and preserved using a 1:1 dilution with freshly prepared meta-phosphoric acid, 10%. All serum and plasma samples were stored at -70 °C. At the end of the clinical treatments, sera and plasma samples were shipped overnight on dry ice to the analytical laboratories. Serum ascorbate was determined using a Cobas Fara II spectrofluorometric analyzer (Roche Diagnostic System, Inc., Branchburg, NJ) by a colorimetric, ferrozine-based reaction after TCA protein precipitation (modified McGown et al. 1982 method). Serum uric acid was determined using the Cobas Fara II analyzer by an enzymatic colorimetric method based on uricase, peroxidase and 4-aminoantipyrine (Sigma Chemical Co., St. Louis, MO). Serum selenium and iron were determined using atomic absorption spectrophotometry. Serum lipid-soluble antioxidants (β- and α-carotene, α-tocopherol, retinol and retinyl palmitate) were analyzed by a modified C$_18$ reversed phase HPLC method of Epler et al. (17) using isocratic mobile phase, diode array detection for retinol and carotenoids and fluorescence detection for the tocopherols. Internal standards (NIST) were used for quantitation, after ethanolic protein denaturation and hexane extraction. LDL oxidizability was measured at the Center for Human Nutrition, University of Texas Southwestern Medical Center, Dallas Texas, as described by Marangon et al. (19). Briefly, LDL were isolated by ultra-centrifugation, passed through a Biorad® column and protein was estimated by the Lowry method. Thereafter, copper-
catalyzed LDL oxidation was carried out with 100 µg/mL LDL protein and 2.5 µM copper at 37 °C for a five-hour period. Conjugated diene formation was monitored in ten-minute intervals at 234 nm. From these data, the lag phase and the oxidation rate were calculated. Clinical laboratory

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Six capsules (= 1 daily supply in 2 packets) provide:</strong></td>
</tr>
<tr>
<td><strong>Amount</strong></td>
</tr>
<tr>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>Vitamin A (retinyl palmitate), 5,000 IU</td>
</tr>
<tr>
<td>β-Carotene (from palm fruit extract and Dunaliella salina), 10,000 IU</td>
</tr>
<tr>
<td>Vitamin C (calcium ascorbate)</td>
</tr>
<tr>
<td>Vitamin D₃ (cholecalciferol), 400 IU</td>
</tr>
<tr>
<td>Vitamin E (d-α-tocopherol (92% succinate, 8% non-esterified), 300 IU</td>
</tr>
<tr>
<td>d-β/γ/δ-tocopherol and tocotrienols (non-esterified)</td>
</tr>
<tr>
<td>Thiamin (mononitrate)</td>
</tr>
<tr>
<td>Riboflavin</td>
</tr>
<tr>
<td>Niacin (niacin, niacinamide)</td>
</tr>
<tr>
<td>Vitamin B₆ (pyridoxine hydrochloride)</td>
</tr>
<tr>
<td>Folate (folic acid)</td>
</tr>
<tr>
<td>Vitamin B₁₂ (cyanocobalamin)</td>
</tr>
<tr>
<td>Biotin</td>
</tr>
<tr>
<td>Pantothenic Acid (D-calcium pantothenate)</td>
</tr>
<tr>
<td>Vitamin K₁ (phylloquinone)</td>
</tr>
<tr>
<td>Calcium (carbonate, citrate, propionate, glycine chelate)</td>
</tr>
<tr>
<td>Magnesium (aspartate, oxide, glycine chelate)</td>
</tr>
<tr>
<td>Iron (glycine chelate)</td>
</tr>
<tr>
<td>Iodine (potassium iodide)</td>
</tr>
<tr>
<td>Zinc (glycine chelate)</td>
</tr>
<tr>
<td>Copper (glycine chelate)</td>
</tr>
<tr>
<td>Manganese (glycine chelate)</td>
</tr>
<tr>
<td>Selenium (L-selenomethionine, sodium selenite)</td>
</tr>
<tr>
<td>Chromium (glycine niacin chelate)</td>
</tr>
<tr>
<td>Molybdenum (glycine chelate)</td>
</tr>
<tr>
<td>Vanadium (vanadyl sulfate)</td>
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<tr>
<td>Silicon (sodium metasilicate)</td>
</tr>
<tr>
<td>Boron (citrate)</td>
</tr>
<tr>
<td>α-Carotene (from palm fruit extract)</td>
</tr>
<tr>
<td>Lutein (from marigold flower extract)</td>
</tr>
<tr>
<td>Lycopene (from tomato extract)</td>
</tr>
<tr>
<td>α-Lipoic Acid</td>
</tr>
<tr>
<td>Broccoli and Cabbage Extracts (20:1, with glucosinolates)</td>
</tr>
<tr>
<td>Quercetin</td>
</tr>
<tr>
<td>Citrus Bioflavonoids (hesperidin, naringenin)</td>
</tr>
<tr>
<td>Grape Seed Extract (min. 92% polyphenols)</td>
</tr>
<tr>
<td>Curcumin (from turmeric extract, 95%)</td>
</tr>
<tr>
<td>Soy Isoflavones (from soy isoflavone extract, 40%)</td>
</tr>
</tbody>
</table>

¹U.S. Food and Drug Administration, Daily Values for nutrition labeling.

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evaluations were performed by a CLIA approved clinical laboratory at GFI Pharmaceutical Services, Inc. Total fruit and vegetable consumption was assessed by a dietician using a 7-day food diary recorded by the subjects during the 3rd week of each treatment period. Intakes of specific fruits and vegetables were not assessed.

**Statistical analyses.** Subject demographics and baseline characteristics were tabulated and compared between the treatment groups using two-sample t-tests and Fisher's Exact tests. Changes in antioxidant status and LDL-oxidation variables were compared between the product groups using ANOVA for a two-period crossover design. The statistical model contained the main effect of sequence, subject within sequence, period and product. Sequence effects were tested using the subject within sequence as the error term. The period and product effects were tested using the residual error. The analysis was conducted on the raw day-0 to day-42 change and the percent change from day 0 to day 42. Summary statistics of changes in vital signs from screening to endpoint were tabulated. Changes in quantitative clinical laboratory variables were computed and the product groups were compared using the previously described two-period crossover ANOVA. Results are expressed as means ± SD. All data listings and statistical summations were performed using the Statistical Analysis System (SAS®), version 6.12.

**RESULTS**

Four out of the 50 subjects enrolled in this study dropped out. Reasons for dropout were adverse effects (hives, itching) due to PL (1 subject), pregnancy (1 subject), address change (1 subject) and non-compliance (1 subject). A total of 46 subjects were available for the baseline to endpoint analyses. The mean age of the 46 subjects that completed the study was 40.0 years (range: 20 - 65), and 72 % of the subjects were female. The mean body weight (± SD) for all subjects was 77.4 ± 19.2 kg. Subject demographics, as well as mean body weight and body mass index (BMI) for each treatment sequence are listed in Table 2.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sequence 1 (n = 24)</th>
<th>Sequence 2 (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>9 (38 %)</td>
<td>4 (18 %)</td>
</tr>
<tr>
<td></td>
<td>15 (62 %)</td>
<td>18 (82 %)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>39.1 ± 12.0</td>
<td>40.9 ± 11.4</td>
</tr>
<tr>
<td>Range (y)</td>
<td>25 - 65</td>
<td>20 - 62</td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>76.3 ± 19.1</td>
<td>78.7 ± 19.8</td>
</tr>
<tr>
<td>Body Mass Index (kg/m^2)</td>
<td>26.6 ± 4.8</td>
<td>27.3 ± 6.5</td>
</tr>
</tbody>
</table>

1 means ± SD.
Body weight and BMI were not significantly different between treatment sequences. Systolic and diastolic blood pressure, other vital signs, and all blood chemistry, hematology and urinalysis measurements were normal at study initiation and remained normal throughout the study. Mean compliance in terms of product accountability for MVMS and PL were 94.7 ± 4.6 % and 92.8 ± 5.6 %, respectively. This difference was statistically significant (p = 0.012). No severe adverse events were reported during the study. The only event judged to be possibly related to product was hives and itching after five days of receiving PL product. The average fruit and vegetable consumption obtained from 7-day food diaries recorded during the third week of each treatment period remained unchanged between treatment periods (p = 0.82) and between treatments (MVMS: 2.7 ± 0.9; PL: 2.7 ± 1.1; p = 0.80). Results confirmed that the 6-week washout and treatment periods in this cross-over study appeared to be sufficiently long to ensure complete return to baseline concentrations of all variables measured in this study, except α-carotene which showed a small but significant carry-over effect.

### Table 3

Changes from Baseline to Endpoint in Levels of Serum Antioxidants and Iron (means ± SD; n = 46)

<table>
<thead>
<tr>
<th>Variable</th>
<th>MVMS</th>
<th>PL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Endpoint</td>
</tr>
<tr>
<td>Vitamin E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Tocopherol (µmol/L)</td>
<td>20.0 ± 8.5</td>
<td>36.9 ± 13.0c</td>
</tr>
<tr>
<td>(µmol/mmol serum lipid)</td>
<td>3.3 ± 0.85</td>
<td>6.2 ± 1.48c</td>
</tr>
<tr>
<td>Vitamin C (ascorbate, µmol/L)</td>
<td>68.1 ± 24.8</td>
<td>94.3 ± 26.4c</td>
</tr>
<tr>
<td>Carotenoids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Carotene (nmol/L)</td>
<td>335 ± 198</td>
<td>716 ± 429c</td>
</tr>
<tr>
<td>(nmol/mmol serum lipid)</td>
<td>62.7 ± 35.0</td>
<td>140.2 ± 100.1c</td>
</tr>
<tr>
<td>α-Carotene (nmol/L)</td>
<td>76.6 ± 81.7</td>
<td>592 ± 365c</td>
</tr>
<tr>
<td>(nmol/mmol serum lipid)</td>
<td>13.9 ± 14.2</td>
<td>113.0 ± 72.6c</td>
</tr>
<tr>
<td>Vitamin A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinol (µmol/L)</td>
<td>2.09 ± 0.61</td>
<td>2.18 ± 0.48</td>
</tr>
<tr>
<td>Retinyl palmitate (nmol/L)</td>
<td>22.8 ± 31.5</td>
<td>91.9 ± 166.8a</td>
</tr>
<tr>
<td>Selenium (µmol/L)</td>
<td>2.10 ± 0.41</td>
<td>2.47 ± 0.75b</td>
</tr>
<tr>
<td>Iron (total, µmol/L)</td>
<td>22.8 ± 6.6</td>
<td>21.5 ± 8.3</td>
</tr>
<tr>
<td>Uric Acid (µmol/L)</td>
<td>287 ± 85</td>
<td>285 ± 912</td>
</tr>
</tbody>
</table>

1-calculated as: serum concentration / (mmol/L total cholesterol + mmol/L total triacylglycerols)

a*p ≤ 0.05, significant difference in change from baseline to endpoint in supplement compared to placebo.
b*p ≤ 0.01, significant difference in change from baseline to endpoint in supplement compared to placebo.
c*p ≤ 0.001, significant difference in change from baseline to endpoint in supplement compared to placebo.
d*p ≤ 0.05, significant difference between supplement and placebo at baseline.

Changes in serum antioxidant concentrations are shown in Table 3. The six-week treatment with MVMS caused a significant 84.5 % increase in serum α-tocopherol (p ≤ 0.001) concentrations. Serum-lipid-normalized α-tocopherol concentrations showed similar changes as
serum α-tocopherol. Serum ascorbate concentrations were significantly increased by 38 % (p ≤ 0.001) with MVMS without changes in the PL period. MVMS elevated serum retinyl palmitate four-fold (p ≤ 0.001), whereas retinol concentrations remained unchanged (p = 0.321). Among the serum carotenoids, β-carotene and α-carotene showed significant 2.1 and 7.7-fold increases (p ≤ 0.001). Serum total selenium concentrations increased significantly by 17 % (p = 0.007) with MVMS. No changes in any of these serum measurements were observed during the PL period.

Results of the copper-catalyzed ex vivo LDL oxidation assays are shown in Table 4. The lag time of conjugated diene formation was significantly prolonged by 17 % (p ≤ 0.001) with MVMS, whereas no changes were observed with PL. Likewise, the oxidation rate was significantly decreased by MVMS (p ≤ 0.001) without effects during the PL period. At the same time, serum total cholesterol, LDL cholesterol, HDL cholesterol and triacylglycerol concentrations remained normal and unchanged with both treatments during the study (Table 4).

**TABLE 4**

Baseline-to-Endpoint Changes in LDL Oxidizability and Blood Lipids

(means ± SD; n = 46)

<table>
<thead>
<tr>
<th>Variable</th>
<th>MVMS</th>
<th>PL</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Endpoint</td>
</tr>
<tr>
<td>LDL oxidizability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lag time (min)</td>
<td>43.4 ± 6.3</td>
<td>50.9 ± 8.6</td>
</tr>
<tr>
<td>Ox. rate (µmol/min×g protein)</td>
<td>12.5 ± 1.8</td>
<td>11.4 ± 1.7</td>
</tr>
<tr>
<td>Blood Lipids (mmol/L)</td>
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<tr>
<td>Total cholesterol</td>
<td>4.74 ± 1.00</td>
<td>4.55 ± 0.85</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>2.83 ± 0.79</td>
<td>2.60 ± 0.79</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.50 ± 0.42</td>
<td>1.51 ± 0.46</td>
</tr>
<tr>
<td>Triacylglycerols</td>
<td>0.88 ± 0.42</td>
<td>0.97 ± 0.47</td>
</tr>
</tbody>
</table>

*p ≤ 0.001, significant difference in change from baseline to endpoint in supplement compared to placebo.

There were significant correlations of baseline-to-endpoint changes in serum-lipid-normalized concentrations of α-tocopherol (R = 0.56, p = 0.0002), α-carotene (R = 0.50, p = 0.0009) and β-carotene (R = 0.36, p = 0.0227), as well as serum ascorbate (R = 0.33, p = 0.0270) with the changes in lag time of LDL oxidizability. No significant correlations were observed of serum antioxidants and LDL oxidation rate.

**DISCUSSION**

To the best of our knowledge, this is the first published study that tested antioxidant effects of a nutritionally complete micronutrient supplement in healthy adults. MVMS provided RDA-based amounts of most vitamins and minerals, and higher amounts of those nutrients that have demonstrated clinical benefits at above-RDA dietary intakes, such as vitamins C and E.
addition, MVMS also included several non-essential antioxidant micronutrients in the carotenoid and flavonoid categories. The complete composition of MVMS is shown in Table 1.

**Serum Vitamin E.** Vitamin E supplementation has been shown to reduce the progression of atherosclerosis (20-22) and to inhibit LDL oxidizability (23). Whereas U.S. vitamin E intakes appear to be slightly below the RDA of 8-10 mg/d (12-15 IU/d) (24), current recommendations for optimum vitamin E daily intakes range from 100 to 250 IU/d (25,26). Daily amounts of 800 IU are considered safe for long-term supplementation (27,28). MVMS provided 201 mg (300 IU) of vitamin E from RRR-α-tocopherol and other natural tocopherols. Serum α-tocopherol baseline concentrations and the observed 84.5 % increase with MVMS in the present study (Table 3) are in agreement with the changes shown in other human studies using similar doses and duration (23-30).

**Serum Vitamin C.** Vitamin C supplementation above the current RDA of 60 mg shows promising effects in preventing cataract (31) and cardiovascular disease (32,33), and as adjunct therapy in diabetics (34). Recent studies suggest that the optimum dietary intake of vitamin C may be between 200 and 500 mg/d, and that intakes over 500 mg/d may have little or no additional health benefits (35,36). Serum vitamin C concentrations (Table 3) increased significantly with MVMS which provided 500 mg of ascorbate, even though serum baseline and PL ascorbate were at concentrations indicating above-RDA dietary intakes (35,37). Because baseline ascorbate concentrations already approached saturation concentrations (35,37), the observed increase with MVMS (by 26.2 µmol/L to 94.3 ± 26.4 µmol/L) is remarkable. With the observed mean consumption of fruits and vegetables of only 2.7 servings/day, and no data on individual fruit and vegetable intake, we can only speculate that the high vitamin C intakes may have come from popular fortified foods and beverages. In addition, a major portion of the fruit and vegetable intake by the study population could have come from citrus fruit, which is typically abundant during the time of year this study was conducted (December-April).

**Serum Carotenoids.** Many carotenoids promote immune function and may prevent cancer (38-40). Carotenoid intakes in the U.S. population are considered low and reflect low fruit and vegetable consumption (41). The amounts of carotenoids present in MVMS resemble dietary intakes from diets with the recommended five servings of fruits and vegetables (Table 1). The significant 114 % increase in serum β-carotene (Table 3) observed in the present study with 6 mg/d β-carotene confirms data reported in the previously published literature (9,11). The 7.7-fold increase in serum α-carotene concentration observed with MVMS supplementation (Table 3) providing 2 mg/d of α-carotene confirm existing data showing serum α-carotene responds well to dietary supplementation, even in the presence of large amounts of other carotenoids (11,42). Overall, the present study did not show strong evidence of potentially adverse interactions among the supplemented carotenoids, and thus confirmed similar conclusions by Mayne et al. (42).

**Serum Retinol and Selenium.** The present study confirms that in healthy, well-nourished subjects serum retinol concentrations remain largely unaffected by vitamin A supplementation due to tight homeostatic control. However, serum retinyl palmitate was increased significantly due to the vitamin A (1.5 mg) provided by MVMS as retinyl palmitate. MVMS provided 100 µg/d of
selenium, 50% from sodium selenite and 50% from L-selenomethionine. Compared with other selenium supplementation studies (9,43-45), baseline serum selenium concentrations in this study population were somewhat high. Nevertheless, MVMS increased serum selenium concentration by 18% (Table 3).

**LDL Oxidizability.** There is now substantial evidence available to support the hypothesis that oxidatively modified LDL play a major role in atherogenesis (6,23,46), and that antioxidant nutrients that inhibit LDL oxidation, such as vitamin E, also slow the progression of atherosclerosis (20-22). In an 8-week dose-response study of vitamin E supplementation and LDL oxidation in healthy men, Jialal et al. (23) found significant increases in the lag time of conjugated diene formation with 400 to 1200 IU/d vitamin E, but not with 200 IU/d. Kruger et al. (47) showed in a single-blind placebo-controlled study of hypercholesterolemic men that a combination of 87 IU/d vitamin E, 300 mg/d ascorbic acid, 8.85 mg/d β-carotene, and 90 µg/d selenium increased the lag time of LDL oxidation by 10.6% after 6 months of supplementation. The significant 17% increase in lag time and the 9% decrease in oxidation rate observed in the present 6-week study (Table 4) confirm that MVMS's antioxidant combination effectively improved the resistance of LDL against oxidative damage in healthy adults. Consequently, MVMS may have important cardiovascular health benefits.

LDL oxidizability was significantly correlated with lipid-normalized serum concentrations of α-tocopherol, α-carotene and β-carotene, as well as serum ascorbate. Judging from the degree of correlation, it appears that α-carotene may be a more important antioxidant than β-carotene or ascorbate in inhibiting LDL oxidation. A role for α-carotene in reducing LDL and plasma oxidizability was also suggested by others (48) and warrants further studies.

**Conclusions.** The present study showed that MVMS significantly increased antioxidant status as measured by serum antioxidant levels, and improved resistance against LDL oxidation. Therefore, MVMS may have cardiovascular benefits in healthy adults consuming typical U.S. diets. The findings also confirmed two widely held assumptions: first, that a more complex antioxidant nutrient combination from 12 different sources is efficacious, and second, that the tested combination is efficacious in the presence of a full spectrum of non-antioxidant nutrients of a nutritionally complete micronutrient supplement.

**REFERENCES**


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Study Reference:


No online abstract is available:

ABSTRACT

Objective: The present study investigated whether LifePak®, a comprehensive optimum potency multiple nutrient supplement, can improve antioxidant status, decrease free radical activity, and improve resistance to LDL-oxidation in healthy volunteers.

Design: One-hundred-fifty non-smokers, ages 18-65 years, were recruited in the Houston, Texas area. Subjects were not taking any other dietary supplements and consumed typical American diets with less than 5 servings of fruits and vegetables. Seventy-five subjects were supplemented with LifePak® and another 75 subjects received placebo (each in 2 packets of 3 capsules each daily) for 6 weeks.

Measurements: Blood and urine samples were taken at the start and end of the supplementation period and analyzed for serum antioxidant nutrients, such as vitamin C, vitamin E, carotenoids and vitamin A, as well as serum oxygen radical absorption capacity, and markers of free radical damage, including serum total alkenals, hydroperoxides, and urinary alkenals, 8-hydroxyguanosine, and 8-epi-PGF₂α. Plasma samples were prepared for LDL (low-density lipoprotein) oxidizability assays.

Results: LifePak® supplementation significantly improved antioxidant status as evidenced by increased serum levels of β-carotene (from 0.236±0.200 µg/ml to 0.461±0.245 µg/ml; p<0.001), α-carotene (from 62±54 ng/ml to 355±194 ng/ml; p<0.001), and vitamin E (α-tocopherol, from 8.94±2.06 µg/ml to 15.96±5.21 µg/ml, p<0.001), with no changes in the placebo group. LifePak® significantly increased total serum oxygen radical absorption capacity by 5.0% (p<0.05) and lowered serum total alkenals by 9.6% (p<0.001). LifePak® effectively improved LDL’s resistance to oxidation, as the lag time was prolonged by about 18% (from 46.0±10.2 min to 54.5±13.9 min; p<0.001).

Conclusions: LifePak® supplementation for 6 weeks significantly increased antioxidant status, reduced serum alkenals, and improved LDL resistance to oxidation in healthy non-smokers consuming typical U.S. diets.

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Updated January 2014