

# Carotenoid News

Vol. 14, No. 2  
August 2004

## FROM THE EDITOR

*"Tout progrès scientifique est un progrès de méthode"*

The words of the French philosopher and mathematician, René Descartes (1596-1650) are as true today as when he wrote them in *The Discourse on Method*. Last year we celebrated a centennial of chromatography, the analytical method originally created to separate carotenoids, which generated countless developments in many areas of chemistry, biology and medicine. In recent years, molecular biologists developed new techniques of gene array analysis, which promise real progress in our understanding of carotenoid function by the investigation of multiple gene expression and the resulting subtle changes in cell metabolism. Sometimes a serendipitous discovery of a new method promotes the development of science, and a new idea in science induces the search for new methods. This interlocking chain of innovation carries us into a brilliant future, promising longer, healthier and happier life for all humanity.

The current issue of the Carotenoid News exemplifies the latest developments in carotenoid science and methodology, from an extensive list of recent publications, through polemics about Raman spectroscopy, to the description of meetings full of presentations and reports of the latest findings.

Maria S. Sapuntzakis (Chicago, IL)

## News from the CARIG Steering Committee

The annual meeting of the CARIG Steering Committee was held April 17 in Washington, DC during EB 2004. The Committee thanked departing chair, Dale Cooper, for his outstanding leadership. Treasurer Neal Craft completed his 3-year term and also left the Committee. We are grateful to both Dale and Neal for their dedicated work in organizing the CARIG Conferences, including the necessary fundraising. The Committee welcomed new members, Alexandrine During, Harold Furr, and Elizabeth Johnson. The Committee also thanked the organizers of this year's CARIG Conference, John Landrum, Julie Mares, and Sherry Tanumihardjo, for their contributions to a very successful meeting. (Please see the report below.) Wendy White accepted the Chair for the coming year and Harold Furr agreed to be the new Treasurer. The current membership of the CARIG Steering Committee includes:

Wendy White (Chair) – Iowa State University  
Harold Furr (Treasurer) – Craft Technologies, Inc.  
Alexandrine During – USDA Human Nutrition Research Center, Beltsville  
Elizabeth Johnson – Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University  
John Landrum – Florida International University  
Julie Mares – University of Wisconsin, Madison  
Cheryl Rock – University of California, San Diego  
Noel Solomons (Fundraising) – Centre for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM), Guatemala  
Maria Stacewicz-Sapuntzakis (Newsletter Editor and member *ex officio*) – University of Illinois, Chicago  
Sherry Tanumihardjo – University of Wisconsin, Madison  
The next issue of Carotenoid News will include the agenda for CARIG Conference at EB 2005 in San Diego, CA.

## CARIG 2004 CONFERENCE REPORT

There was an overflow attendance for this year's CARIG conference on April 17, 2004 in Washington, DC. that focused on

the three carotenoids, Lutein, Zeaxanthin, and Lycopene. Dr. Steven Schwartz, Ohio State University, gave the James Allen Olson Memorial Perspectives on Carotenoids lecture, "Food Matrix Effects on Carotenoid Absorption." Dr. Phyllis Bowen (University of Illinois, Chicago), Dr. Karin Wertz (DSM), and Dr. Klaus Kraemer (BASF) presented results from their research programs updating the attendees on some of their latest research. Current progress on the study of factors that control absorption of lutein and zeaxanthin were presented by Dr. John Landrum (Florida International University), Dr. Sherry Tanumihardjo (University of Wisconsin, Madison), Dr. Wolfgang Schalch, (DSM), and Dr. Elizabeth Johnson (Tufts University, USDA Human Nutrition Research Center on Aging) and Dr. Janet Novotny of the USDA Laboratories at Beltsville, Maryland. Dr. Julie Mares capped off the morning's discussions and provided those in attendance with a perspective on the emerging understanding of the importance of these three carotenoids and their significance to human health.

## CARIG Travel Awards

CARIG will award one or more \$500 grants for travel to Experimental Biology 2005 in San Diego, CA. The award will be based on the scientific merit of EB'05 abstracts submitted by graduate students who have not yet received a Ph.D. degree. Please send a copy of your abstract (on the EB 2005 form) and a letter that briefly describes the significance of the research and your role in the research to: Wendy S. White, Ph.D., Department of Food Science and Human Nutrition, 1127 Human Nutritional Sciences Building, Iowa State University, Ames, IA 50011-1120; FAX: 515 294-5390; E-mail (preferred): [wswwhite@iastate.edu](mailto:wswwhite@iastate.edu). **The deadline for submitted abstracts is March 1, 2005.**

## UPCOMING EVENTS

**November 15-17, 2004**

**XXII International Vitamin A Consultative Group Meeting, Lima, Peru.** Vitamin A and the Common Agenda for Micronutrients. **Contact:** ILSI Human Nutrition Institute, One Thomas Circle, NW, 9th floor, Washington, DC 20005-5802, **tel:** 202-659-9024, **fax:** 202-659-3617, **E-mail:** [hni@ilsi.org](mailto:hni@ilsi.org), **website:** [www.ivacg.ilsa.org](http://www.ivacg.ilsa.org)

**November 15-18, 2004**

**6th World Congress on the Processing Tomato, Melbourne, Australia.** **Contact website:** [www.worldtomatocongress.com.au](http://www.worldtomatocongress.com.au)  
**tel:** +61 35825 46 33

**December 9-12, 2004**

**Carotenoids and Dietary Lipids in Health and Disease, Kraków, Poland.** DLARFID (Dietary Lipids as Risks Factors in Development) International Conference organized by EU Commission. **Contact:** Dr. Aldona Dembińska-Kieć, Department of Clinical Biochemistry, Jagiellonian University, Medical College, Kopernika 15a, 31-501 Kraków, Poland. **fax:** +48 12 421-40-73, **E-mail:** [dlarfid@dlarfid.org](mailto:dlarfid@dlarfid.org), **website:** [www.dlarfid.org/conference](http://www.dlarfid.org/conference) [see program below]

**March 31- April 6, 2005**

**Experimental Biology 2005 and XXXV International Congress of Physiological Sciences, San Diego, CA**

**Contact:** EB2005, FASEB Office of Scientific Meetings & Conferences, 9650 Rockville Pike, Bethesda MD 20814-3998, **website:** [www.faseb.org/meetings/eb2005](http://www.faseb.org/meetings/eb2005)

**July 17-22, 2005**

**14<sup>th</sup> International Symposium on Carotenoids, Edinburgh, Scotland.** **Contact :** Prof. Andrew J. Young, Liverpool John Moores University **tel:** +44 151 231 3575, **fax:** +44 151 708 9867, **E-mail:** [a.j.young@livjm.ac.uk](mailto:a.j.young@livjm.ac.uk), **web:** [www.carotenoidsociety.org](http://www.carotenoidsociety.org)

## **DLARFID CONFERENCE PROGRAM: CAROTENOIDS AND DIETARY LIPIDS IN HEALTH AND DISEASE**

**Thursday, December 9th**

### **Opening Session and First Plenary Lecture**

6:15pm *Gerard Ailhaud* (France). Fatty acid composition of fats as an early determinant of childhood obesity.

**Friday, December 10th**

### **Session I: LIPIDS AND CAROTENOIDS. SOURCES, METABOLISM AND MECHANISMS OF GENOMIC AND NON-GENOMIC ACTIONS**

8:45am *Gerard Southon* (UK). Carotenoid bioavailability: vs complex foods and individual supplements response.

9:30am *Christian A. Drevon* (Norway). Fatty acids and expression of adipokines.

10:15am *Livar Froyland* (Norway). Structurally different marine oils in health and disease.

11:00am *Ruan Elliott* (UK). Mechanisms of genomic and non-genomic actions of lipids and carotenoids.

11:40am *Johannes von Lintig* (Germany). Carotenoid metabolism and its impact on animal physiology.

12:20pm *Saverio Cinti* (Italy). Morphology of tissues from animals treated with  $\beta$ -carotene.

### **Session IIa: CAROTENOIDS AND LIPIDS IN CELL PROLIFERATION AND DIFFERENTIATION (NORMAL CELLS)**

2:00pm *Andreu Palou* (Spain). Gene regulation by  $\beta$ -carotene in ferrets.

2:40pm *Gerd Schmitz* (Germany). Influence of  $\beta$ -carotene and nuclear receptor ligands on barrier tissues and monocyte/macrophage system.

3:20pm *Andreu Palou* (Spain). Vitamin A as a regulator of adipogenesis and adipocyte metabolism-derived medical complications.

4:15pm *Jan Nedergaard* (Sweden). Carotenoid effects on brown and white adipose tissue differentiation.

4:45pm *Lise Madsen* (Norway/Denmark). Regulation of adipocyte differentiation and function by polyunsaturated fatty acid.

5:15pm *Karsten Kristiansen* (Denmark). Molecular mechanisms controlling white versus brown adipocyte differentiation.

5:45pm *Aldona Dembinska-Kiec & Mariusz Ratajczak* (Poland).  $\beta$ -Carotene and fatty acids as regulators of umbilical blood progenitor cell differentiation

**Saturday, December 11th**

### **Session IIb: CAROTENOIDS AND LIPIDS IN CELL PROLIFERATION AND DIFFERENTIATION (CANCER CELLS)**

8:45am *Paola Paozza* (Italy). Redox regulation of cell growth by carotenoids against skin damage from sunlight.

9:15am *Regina Goralczyk* (Switzerland).  $\beta$ -Carotene interaction with NNK or cigarette smoke in the A/J-mouse lung cancer model: effects on tumor formation,  $\beta$ -carotene metabolism, RAR $\beta$  and CYP450 gene regulation.

9:45am *Jaap Keijer* (Netherlands). Uptake and molecular effects of  $\beta$ -carotene in human colon and lung cell lines.

10:15am *Piotr Laidler* (Poland). The effect of beta-carotene and fatty acids on proliferation and apoptosis of human melanoma and prostate cancer cells.

11:00am *Aleksander Skotnicki & Tomasz Sacha* (Poland). The effect of carotenoids/retinoids on proliferation, differentiation and apoptotic potential of human myeloid leukemia cells.

11:30am *Xiang-Dong Wang* (USA). Carotenoids, gene regulation and lung cancer prevention.

12:00pm *John Bertram* (USA). Differential regulation of connexin 43 expression by carotenoids and retinoids.

12:30pm *Aldona Dembinska-Kiec* (Poland).  $\beta$ -Carotene, fatty acids and angiogenesis.

### **Chaired Poster Session**

#### **Session III: CAROTENOIDS - THE BIOPHYSICAL ASPECTS**

2:00pm *Kazimierz Strzalka* (Poland). Introduction.

2:05pm *George Britton* (UK). A different perspective on what does the carotenoid have to say.

2:20pm *Wilhelm Stahl* (Germany). Carotenoids in nutritional protection.

2:40pm *T. George Truscott* (UK). Are dietary carotenoids beneficial?

Reactions of carotenoids with oxy-radicals and singlet oxygen.

3:00pm *Wieslaw Gruszecki & Kazimierz Strzalka* (Poland). Carotenoids as modulators of membrane physical properties.

3:40pm *Tadeusz Sarna* (Poland). Model and cellular in vitro study of antioxidant and photoprotective properties of macular pigments.

### **Short presentations**

**Sunday, December 12th**

### **Session IV: DIETARY CAROTENOIDS AND LIPIDS: LESSON LEARNED FROM EPIDEMIOLOGICAL AND GENETIC STUDIES**

8:30am *Paul A. Grimaldi* (France). Roles of PPAR delta in lipid absorption and metabolism. A new target for the treatment of type 2 diabetes.

8:55am *Joachim Thiery* (Germany). Oxidized sterols, cholesterol precursors and phytosterols in patients with coronary artery disease.

9:25am *Prakash B. Bhosale* (USA). Retina and carotenoids.

9:55am *Ralph Ruehl* (Hungary/Germany). Liver catabolism of xenobiotics and  $\beta$ -carotene.

10:20am *Maria Stacewicz-Sapuntzakis & Phyllis E. Bowen* (USA). Role of lycopene and tomato products in prostate health.

### **RECENT / FORTHCOMING PUBLICATIONS**

**Sight and Life Newsletter 1/2004 and 2/2004**, publication of the Task Force SIGHT AND LIFE, PO Box 2116, 4002 Basel, Switzerland, **tel:** 41-61-688-7494, **fax:** 41-61-688-1910, **website:** [www.sightandlife.org](http://www.sightandlife.org) See especially:

Vol1/2004: Erhardt, J.G. Printed version of the new vitamin A table, pp 25-34 [extensive list of foods with their vitamin A content, calculated using the new conversion factors].

Vol 2/2004: Vuong, L.T. & Burgess, M.L. Green rice--a hidden source of pro-vitamin A and micronutrients for rice-consuming populations, pp 10-12.

**Phytochemicals in Nutrition and Health.** Ed. Meskin M.S., Bidlack W.R., Davies A., CRC Press, Boca Raton, FL.

Burri B.J. Lycopene and Human Health.

Landrum J.T., Bone R.A. & Herrero C. Astaxanthin,  $\beta$ -Cryptoxanthin, Lutein and Zeaxanthin.

**Lycopene and the Prevention of Chronic Diseases.** Nutrition & Health Conference Report. Vol.1, No1. Major Findings from five international conferences (1997-2001). Caledonian Science Press, 2002

**Lycopene** (Scientific Review) prepared by Roche Vitamins Inc. 2003 ([www.nutraaccess.com](http://www.nutraaccess.com)), including a mini CD-ROM.

### **Alphabetical Listing of Recent Publications**

Prepared by Dr. Harold Furr, Craft Technologies, Inc.

More extensive list may be found at [www.carotenoidsociety.org](http://www.carotenoidsociety.org)

Anonymous (2003) Lycopene. Monograph. Altern.Med.Rev. 8: 336-342.

Aoi, W., Naito, Y., Sakuma, K., Kuchide, M., Tokuda, H., Maoka, T., Toyokuni, S., Oka, S., Yasuhara, M. & Yoshikawa, T. (2003) Astaxanthin limits exercise-induced skeletal and cardiac muscle damage in mice. Antioxid.Redox.Signal. 5: 139-144.

Assuncao, R. B. & Mercadante, A. Z. (2003) Carotenoids and ascorbic acid composition from commercial products of cashew apple (*Anacardium occidentale* L.). J.Food Composition and Analysis 16: 647-657.

Astley, S. B., Elliott, R. M., Archer, D. B. & Southon, S. (2004) Evidence that dietary supplementation with carotenoids and carotenoid-rich foods modulates the DNA damage: repair balance in human lymphocytes. Br.J.Nutr. 91: 63-72.

Astley, S. B., Hughes, D. A., Wright, A. J., Elliott, R. M. & Southon, S. (2004) DNA damage and susceptibility to oxidative damage in lymphocytes: effects of carotenoids in vitro and in vivo. Br.J.Nutr. 91: 53-61.

Baroli, I., Do, A. D., Yamane, T. & Niyogi, K. K. (2003) Zeaxanthin accumulation in the absence of a functional xanthophyll cycle protects *Chlamydomonas reinhardtii* from photooxidative stress. Plant Cell 15: 992-1008.

Bergheim, I., Parlesak, A., Dierks, C., Bode, J. C. & Bode, C. (2003) Nutritional deficiencies in German middle-class male alcohol consumers: relation to dietary intake and severity of liver disease. Eur.J.Clin.Nutr. 57: 431-438.

Berry, A., Janssens, D., Humbelin, M., et al. (2003) *Paracoccus zeaxanthinifaciens* sp. nov., a zeaxanthin-producing bacterium. Int.J.Syst.Evol.Microbiol. 53: 231-238.

Bhosale, P., Ermakov, I. V., Ermakova, M. R., Gellermann, W. & Bernstein, P. S. (2004) Resonance Raman quantification of nutritionally important carotenoids in fruits, vegetables, and their juices in comparison to HPLC analysis. J.Agric.Food Chem. 52: 3281-3285.

Blades, B. L., Dufficy, L., Englberger, L., Daniells, J. W., Coyne, T.,

- Hamill, S. & Wills, R. B. (2003) Bananas and plantains as a source of provitamin A. *Asia.Pac.J.Clin.Nutr.* 12: S36.
- Bogers, R. P., Dagnelie, P. C., Westerterp, K. R., Kester, A. D., van Klaveren, J. D., Bast, A. & van den Brandt, P. A. (2003) Using a correction factor to correct for overreporting in a food-frequency questionnaire does not improve biomarker-assessed validity of estimates for fruit and vegetable consumption. *J.Nutr.* 133: 1213-1219.
- Bone, R. A., Landrum, J. T., Guerra, L. H. & Ruiz, C. A. (2003) Lutein and zeaxanthin dietary supplements raise macular pigment density and serum concentrations of these carotenoids in humans. *J.Nutr.* 133: 992-998.
- Borowska, J., Kowalska, M., Czaplicki, S. & Zadernowski, R. (2003) Effect of hydrothermal processing on carrot carotenoids changes and interactions with dietary fiber. *Nahrung.* 47: 46-48.
- Botsoglou, N., Papageorgiou, G., Nikolakakis, I., Florou-Paneri, P., Giannenas, I., Dots, V. & Sinapis, E. (2004) Effect of dietary dried tomato pulp on oxidative stability of Japanese quail meat. *J.Agric.Food Chem.* 52: 2982-2988.
- Breithaupt, D. E. (2004) Identification and quantification of astaxanthin esters in shrimp (*Pandalus borealis*) and in a microalga (*Haematococcus pluvialis*) by LC-MS using negative ion atmospheric pressure chemical ionization. *J.Agric.Food Chem.* 52: 3870-3875.
- Breithaupt, D. E., Weller, P., Wolters, M. & Hahn, A. (2004) Comparison of plasma responses in human subjects after the ingestion of 3R,3R'-zeaxanthin dipalmitate from wolfberry (*Lycium barbarum*) and non-esterified 3R,3R'-zeaxanthin using chiral HPLC. *Br.J.Nutr.* 91: 707-713.
- Briviba, K., Schnabele, K., Rechkemmer, G. & Bub, A. (2004) Supplementation of a diet low in carotenoids with tomato or carrot juice does not affect lipid peroxidation in plasma and feces of healthy men. *J.Nutr.* 134: 1081-1083.
- Broekmans, W. M., Kloppe-Ketelaars, I. A., Weststrate, J. A., et al. (2003) Decreased carotenoid concentrations due to dietary sucrose polyesters do not affect possible markers of disease risk in humans. *J.Nutr.* 133: 720-726.
- Brown, M. J., Ferruzzi, M. G., Nguyen, M. L., Cooper, D. A., Eldridge, A. L., Schwartz, S. J. & White, W. S. (2004) Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection. *Am.J.Clin.Nutr.* 80: 396-403.
- Browning, D. F., Whitworth, D. E. & Hodgson, D. A. (2003) Light-induced carotenogenesis in *Myxococcus xanthus*: functional characterization of the ECF sigma factor CarQ and antisigma factor CarR. *Mol.Microbiol.* 48: 237-251.
- Calucci, L., Pinzino, C., Zandomenighi, M., Capocchi, A., Ghiringhelli, S., Saviozzi, F., Tozzi, S. & Gallechi, L. (2003) Effects of gamma-irradiation on the free radical and antioxidant contents in nine aromatic herbs and spices. *J.Agric.Food Chem.* 51: 927-934.
- Cantrell, A., McGarvey, D. J., Truscott, T. G., Rancan, F. & Bohm, F. (2003) Singlet oxygen quenching by dietary carotenoids in a model membrane environment. *Arch.Biochem.Biophys.* 412: 47-54.
- Castelletti, S., Morosinotto, T., Robert, B., Caffari, S., Bassi, R. & Croce, R. (2003) Recombinant Lhca2 and Lhca3 subunits of the photosystem I antenna system. *Biochemistry* 42: 4226-4234.
- Chang, C. W., Chu, G., Hinz, B. J. & Greve, M. D. (2003) Current use of dietary supplementation in patients with age-related macular degeneration. *Can.J.Ophthalmol.* 38: 27-32.
- Cheng, L. (2003) Xanthophyll cycle pool size and composition in relation to the nitrogen content of apple leaves. *J.Exp.Bot.* 54: 385-393.
- Christen, W. G., Manson, J. E., Glynn, R. J., Gaziano, J. M., Sperduto, R. D., Buring, J. E. & Hennekens, C. H. (2003) A randomized trial of  $\beta$ -carotene and age-related cataract in US physicians. *Arch.Ophthalmol.* 121: 372-378.
- Cortes, C., Esteve, M. J., Frigola, A. & Torregrosa, F. (2004) Identification and quantification of carotenoids including geometrical isomers in fruit and vegetable juices by LC with UV-diode array detection. *J.Agric.Food Chem.* 52: 2203-2212.
- Croce, R., Muller, M. G., Caffari, S., Bassi, R. & Holzwarth, A. R. (2003) Energy transfer pathways in the minor antenna complex CP29 of Photosystem II: A femtosecond study of carotenoid to chlorophyll transfer on mutant and WT complexes. *Biophys.J.* 84: 2517-2532.
- Darlington, S., Williams, G., Neale, R., Frost, C. & Green, A. (2003) A randomized controlled trial to assess sunscreen application and  $\beta$ -carotene supplementation in the prevention of solar keratoses. *Arch.Dermatol.* 139: 451-455.
- de Sa, M. C. & Rodriguez-Amaya, D. B. (2004) Optimization of HPLC quantification of carotenoids in cooked green vegetables -- Comparison of analytical and calculated data. *J.Food Composition and Analysis* 17: 37-51.
- De-Oliveira, A. C., Silva, I. B., Manhaes-Rocha, D. A. & Paumgarten, F. J. (2003) Induction of liver monooxygenases by annatto and bixin in female rats. *Braz.J.Med.Biol.Res.* 36: 113-118.
- Diwadar-Navsariwala, V., Novotny, J. A., Gustin, D. M., Sosman, J. A., Rodvold, K. A., Crowell, J. A., Stacewicz-Sapuntzakis, M. & Bowen, P. E. (2003) A physiological pharmacokinetic model describing the disposition of lycopene in healthy men. *J.Lipid Res.* 44: 1927-1939.
- Dixon, L. B., Zimmerman, T. P., Kahle, L. L. & Subar, A. F. (2003) Adding carotenoids to the NCI Diet History Questionnaire database. *J.Food Composition and Analysis* 16: 269-280.
- Djuric, Z., Uhley, V. E., Naegeli, L., Lababidi, S., Macha, S. & Heilbrun, L. K. (2003) Plasma carotenoids, tocopherols, and antioxidant capacity in a 12-week intervention study to reduce fat and/or energy intakes. *Nutrition.* 19: 244-249.
- Dragnev, K. H., Stover, D. & Dmitrovsky, E. (2003) Lung cancer prevention: the guidelines. *Chest* 123: 60S-71S.
- Drisko, J. A., Chapman, J. & Hunter, V. J. (2003) The use of antioxidants with first-line chemotherapy in two cases of ovarian cancer. *J.Am.Coll.Nutr.* 22: 118-123.
- El-Agamey, A. & McGarvey, D. J. (2003) Evidence for a lack of reactivity of carotenoid addition radicals towards oxygen: a laser flash photolysis study of the reactions of carotenoids with acylperoxy radicals in polar and non-polar solvents. *J.Am.Chem.Soc.* 125: 3330-3340.
- Engelhardt, H. (2004) One century of liquid chromatography. From Tswett's columns to modern high speed and high performance separations. *J.Chromatogr.B.Analyt.Technol.Biomed.Life Sci.* 800: 3-6.
- Englberger, L., Aalbersberg, W., Fitzgerald, M. H., Marks, G. C. & Chand, K. (2003) Provitamin A carotenoid content of different cultivars of edible pandanus fruit. *J.Food Composition and Analysis* 16: 237-247.
- Englberger, L., Darnton-Hill, I., Coyne, T., Fitzgerald, M. H. & Marks, G. C. (2003) Carotenoid-rich bananas: a potential food source for alleviating vitamin A deficiency. *Food Nutr.Bull.* 24: 303-318.
- Englberger, L., Schierle, J., Marks, G. C. & Fitzgerald, M. H. (2003) Micronesian banana, taro, and other foods: newly recognized sources of provitamin A and other carotenoids. *J.Food Composition and Analysis* 16: 3-19.
- Epstein, K. R. (2003) The role of carotenoids on the risk of lung cancer. *Semin.Oncol.* 30: 86-93.
- Ervin, R. B., Wright, J. D., Wang, C. Y. & Kennedy-Stephenson, J. (2004) Dietary intake of selected vitamins for the United States population: 1999-2000. *Advance Data from Vital and Health Statistics* no. 339: 1-4.
- Faivre, B., Gregoire, A., Preault, M., Cezilly, F. & Sorci, G. (2003) Immune activation rapidly mirrored in a secondary sexual trait. *Science* 300: 103.
- Fawzi, W. W., Msamanga, G. I., Wei, R., Spiegelman, D., Antelman, G., Villamor, E., Manji, K. & Hunter, D. (2003) Effect of providing vitamin supplements to human immunodeficiency virus-infected, lactating mothers on the child's morbidity and CD4+ cell counts. *Clin.Infect.Dis.* 36: 1053-1062.
- Felicissimo, M. P., Bittencourt, C., Houssiau, L. & Pireaux, J. J. (2004) Time-of-flight secondary ion mass spectrometry and X-ray photoelectron spectroscopy analyses of *Bixa orellana* seeds. *J.Agric.Food Chem.* 52: 1810-1814.
- Ferreira, A. L., Yeum, K. J., Russell, R. M., Krinsky, N. I. & Tang, G. (2004) Enzymatic and oxidative metabolites of lycopene. *J.Nutr.Biochem.* 15: 493-502. [corrected version]
- Fitze, P. S., Kolliker, M. & Richner, H. (2003) Effects of common origin and common environment on nestling plumage coloration in the great tit (*Parus major*). *Evolution Int.J.Org.Evolution* 57: 144-150.
- Fleischmann, P., Watanabe, N. & Winterhalter, P. (2003) Enzymatic carotenoid cleavage in star fruit (*Averrhoa carambola*). *Phytochemistry* 63: 131-137.
- Furutani, Y., Sudo, Y., Kamo, N. & Kandori, H. (2003) FTIR spectroscopy of the complex between pharaonis phorbodopsin and its transducer protein. *Biochemistry* 42: 4837-4842.
- Gallagher, C. E., Cervantes-Cervantes, M. & Wurtzel, E. T. (2003) Surrogate biochemistry: use of *Escherichia coli* to identify plant cDNAs that impact metabolic engineering of carotenoid accumulation. *Appl.Microbiol.Biotechnol.* 60: 713-719.
- Garcia, A. L., Ruhl, R., Herz, U., Koebrick, C., Schweigert, F. J. & Worm, M. (2003) Retinoid- and carotenoid-enriched diets influence the ontogenesis of the immune system in mice. *Immunology* 110: 180-187.
- Garcia-Closas, R., Berenguer, A., Jose Tormo, M., et al. (2004) Dietary sources of vitamin C, vitamin E and specific carotenoids in Spain. *Br.J.Nutr.* 91: 1005-1011.
- Garcia-Plazaola, J. I., Hernandez, A., Artetxe, U. & Becerril, J. M. (2004) Regulation of the xanthophyll cycle pool size in duckweed (*Lemna minor*) plants. *Physiol.Plant.* 116: 121-126.
- Gastaldelli, M., Canino, G., Croce, R. & Bassi, R. (2003) Xanthophyll binding sites of the CP29 (Lhcb4) subunit of higher plant photosystem II investigated by domain swapping and mutation analysis. *J.Biol.Chem.* 278: 19190-19198.
- Golbeck, J. H. (2003) The binding of cofactors to photosystem I analyzed by spectroscopic and mutagenic methods. *Annu.Rev.Biophys.Biomol.Struct.* 32: 237-256.
- Gomez-Aracena, J., Bogers, R., van't Veer, P., Gomez-Gracia, E., Garcia-Rodriguez, A., Wedel, H. & Fernandez-Crehuet Navajas, J. (2003) Vegetable consumption and carotenoids in plasma and adipose tissue in Malaga, Spain. *Int.J.Vitam.Nutr.Res.* 73: 24-31.
- Gotto, A. M. (2003) Antioxidants, statins, and atherosclerosis. *J.Am.Coll.Cardiol.* 41: 1205-1210.
- Granot, E. & Kohen, R. (2004) Oxidative stress in abetalipoproteinemia patients receiving long-term vitamin E and vitamin A supplementation. *Am.J.Clin.Nutr.* 79: 226-230.

- Groussard, C., Machefer, G., Rannou, F., Faure, H., Zouhal, H., Sergent, O., Chevanne, M., Cillard, J. & Gratas-Delamarche, A. (2003) Physical fitness and plasma non-enzymatic antioxidant status at rest and after a Wingate test. *Can.J.Appl.Physiol.* 28: 79-92.
- Guil-Guerrero, J. L., Rebolloso-Fuentes, M. M. & Torija Isasa, M. E. (2003) Fatty acids and carotenoids from stinging nettle (*Urtica dioica* L.). *J.Food Composition and Analysis* 16: 111-119.
- Halsted, C. H. (2003) Dietary supplements and functional foods: Two sides of a coin? *Am.J.Clin.Nutr.* 77: 1001S-1007S.
- Hartmann, D., Thurnmann, P. A., Spitzer, V., Schalch, W., Manner, B. & Cohn, W. (2004) Plasma kinetics of zeaxanthin and 3'-dehydro-lutein after multiple oral doses of synthetic zeaxanthin. *Am.J.Clin.Nutr.* 79: 410-417.
- Heinrich, E. & Getoff, N. (2003) Mitomycin C-activity effected by vitamins B1, C, E and  $\beta$ -carotene under irradiation with gamma-rays. *Z.Naturforsch.[C]*. 58: 244-248.
- Herron, K. L. & Fernandez, M. L. (2004) Are the current dietary guidelines regarding egg consumption appropriate? *J.Nutr.* 134: 187-190.
- Hwang, E.S., Bowen, P.E. (2004) Effect of lycopene on lipid peroxidation and oxidative DNA damage in LNCaP human prostate cancer cells. *Food Sci.Biotechnol.* 13: 297-301.
- Ito, Y., Wakai, K., Suzuki, K., Tamakoshi, A., et al. (2003) Serum carotenoids and mortality from lung cancer: a case-control study nested in the Japan Collaborative Cohort (JACC) study. *Cancer Sci.* 94: 57-63.
- Jin, E., Feth, B. & Melis, A. (2003) A mutant of the green alga *Dunaliella salina* constitutively accumulates zeaxanthin under all growth conditions. *Biotechnol.Bioeng.* 81: 115-124.
- Jones, C. M., Mes, P. & Myers, J. R. (2003) Characterization and inheritance of the Anthocyanin fruit (Aft) tomato. *J.Hered.* 94: 449-456.
- Kant, A. K. (2003) Interaction of body mass index and attempt to lose weight in a national sample of US adults: association with reported food and nutrient intake, and biomarkers. *Eur.J.Clin.Nutr.* 57: 249-259.
- Kerfeld, C. A., Sawaya, M. R., Brahmamand, V., Cascio, D., Ho, K. K., Trevithick-Sutton, C. C., Krogmann, D. W. & Yeates, T. O. (2003) The crystal structure of a cyanobacterial water-soluble carotenoid binding protein. *Structure.(Camb).* 11: 55-65.
- Kim, S., Park, J. & Hwang, I. K. (2004) Composition of main carotenoids in Korean red pepper (*Capsicum annuum*, L.) and changes of pigment stability during the drying and storage process. *J.Food Sci.* 69: 39-44.
- Kobayashi, M., Sasaki, S. & Tsugane, S. (2003) Validity of a self-administered food frequency questionnaire used in the 5-year follow-up survey of the JPHC Study Cohort I to assess carotenoids and vitamin C intake: comparison with dietary records and blood levels. *J.Epidemiol.* 13: S82-S91.
- Kostecka-Gugala, A., Latowski, D. & Strzalka, K. (2003) Thermotropic phase behaviour of alpha-dipalmitoylphosphatidylcholine multibilayers is influenced to various extents by carotenoids containing different structural features—evidence from differential scanning calorimetry. *Biochim.Biophys.Acta* 1609: 193-202.
- Kumanyika, S. K., Mauger, D., Mitchell, D. C., Phillips, B., Smicklas-Wright, H. & Palmer, J. R. (2003) Relative validity of food frequency questionnaire nutrient estimates in the Black Women's Health Study. *Ann.Epidemiol.* 13: 111-118.
- Lakshmi, K. V., Poluektov, O. G., Reifler, M. J., Wagner, A. M., Thurnauer, M. C. & Brudvig, G. W. (2003) Pulsed high-frequency EPR study on the location of carotenoid and chlorophyll cation radicals in photosystem II. *J.Am.Chem.Soc.* 125: 5005-5014.
- Lavy, A., Naveh Y., Coleman R., Mokady S. & Werman M.J. (2003) Dietary *Dunaliella bardawil*, a  $\beta$ -carotene-rich alga, protects against acetic acid-induced small bowel inflammation in rats. *Inflam. Bow. Dise.* 9:372-379.
- Lavy A., Karban A., Suissa A., Yassin K., Hermesh I., & Ben-Amotz A.,(2004) Natural  $\beta$ -carotene for the prevention of post-ERCP pancreatitis. *Pancreas* 29:45-50
- Leathers, T. D. (2003) Bioconversions of maize residues to value-added coproducts using yeast-like fungi. *FEM.Yeast.Res.* 3: 133-140.
- Lesellier, E., West, C. & Tchaplai, A. (2003) Advantages of the use of monolithic stationary phases for modelling the retention in sub/supercritical chromatography. Application to cis/trans- $\beta$ -carotene separation. *J.Chromatogr.A.* 1018: 225-232.
- Li, Z., Hu, C. Y., Mo, B. Q., Xu, J. D. & Zhao, Y. (2003) [Effect of  $\beta$ -carotene on gene expression of breast cancer cells]. *Ai.Zheng.* 22: 380-384.
- Limpens, J., van Weerden, W. M., Kramer, K., Pallapies, D., Obermuller-Jevic, U. C. & Schroder, F. H. (2004) Re: Prostate carcinogenesis in N-methyl-N-nitrosourea (NMU)-testosterone-treated rats fed tomato powder, lycopene, or energy-restricted diets. *J.Natl.Cancer Inst.* 96: 554; author reply 55
- Liu, C., Russell, R. M. & Wang, X. D. (2004)  $\alpha$ -Tocopherol and ascorbic acid decrease the production of  $\beta$ -apo-carotenals and increase the formation of retinoids from  $\beta$ -carotene in the lung tissues of cigarette smoke-exposed ferrets in vitro. *J.Nutr.* 134: 426-430.
- Liu, C. S., Glahn, R. P. & Liu, R. H. (2004) Assessment of carotenoid bioavailability of whole foods using a Caco-2 cell culture model coupled with an in vitro digestion. *J.Agric.Food Chem.* 52: 4330-4337.
- Lockwood, S. F., O'Malley, S. & Mosher, G. L. (2003) Improved aqueous solubility of crystalline astaxanthin (3,3'-dihydroxy- $\beta$ ,  $\beta$ -carotene-4,4'-dione) by Captisol (sulfobutyl ether  $\beta$ -cyclodextrin). *J.Pharm.Sci.* 92: 922-926.
- Ma, Y. Z., Holt, N. E., Li, X. P., Niyogi, K. K. & Fleming, G. R. (2003) Evidence for direct carotenoid involvement in the regulation of photosynthetic light harvesting. *Proc.Natl.Acad.Sci.U.S.A.* 100: 4377-4382.
- Maharshak, N., Shapiro, J. & Trau, H. (2003) Carotenoderma—a review of the current literature. *Int.J.Dermatol.* 42: 178-181.
- Maoka, T., Akimoto, N., Fujiwara, Y. & Hashimoto, K. (2004) Structure of new carotenoids with the 6-oxo- $\kappa$ -end group from the fruits of paprika, *Capsicum annuum*. *J.Nat.Prod.* 67: 115-117.
- Marin, A., Ferreres, F., Tomas-Barberan, F. A. & Gil, M. I. (2004) Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuum* L.). *J.Agric.Food Chem.* 52: 3861-3869.
- Matsubara, S., Morosinotto, T., Bassi, R., Christian, A. L., Fischer-Schliebs, E., Luttg, U., Orthen, B., Franco, A. C., Scarano, F. R., Forster, B., Pogson, B. J. & Osmond, C. B. (2003) Occurrence of the lutein-epoxide cycle in mistletoes of the *Loranthaceae* and *Viscaceae*. *Planta* 217: 868-879.
- Matsui, K., Takaichi, S. & Nakamura, M. (2003) Morphological and biochemical changes in carotenoid granules in the ventral skin during growth of the Japanese newt *Cynops pyrrhogaster*. *Zoolog.Sci.* 20: 435-440.
- Mayne, S. T. (2003) Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. *J.Nutr.* 133: 933S-940S.
- Mendes-Pinto, M. M., Ferreira, A. C., Oliveira, M. B. & Guedes De Pinho, P. (2004) Evaluation of some carotenoids in grapes by reversed- and normal-phase LC: A qualitative analysis. *J.Agric.Food Chem.* 52: 3182-3188.
- Minoggio, M., Bramati, L., Simonetti, P., et al. (2003) Polyphenol pattern and antioxidant activity of different tomato lines and cultivars. *Ann.Nutr.Metab.* 47: 64-69.
- Moldrem, K. L., Li, J., Simon, P. W. & Tanumihardjo, S. A. (2004) Lutein and  $\beta$ -carotene from lutein-containing yellow carrots are bioavailable in humans. *Am.J.Clin.Nutr.* 80: 131-136.
- Muhlhofer, A., Buhler-Ritter, B., Frank, J., Zoller, W. G., Merkle, P., Bosse, A., Heinrich, F. & Biesalski, H. K. (2003) Carotenoids are decreased in biopsies from colorectal adenomas. *Clin.Nutr.* 22: 65-70.
- Munne-Bosch, S. & Penuelas, J. (2003) Photo- and antioxidative protection, and a role for salicylic acid during drought and recovery in field-grown *Phillyrea angustifolia* plants. *Planta* 217: 758-766.
- Nagler, L. G., Lankin, V. Z., Kozachenko, A. I. & Gurevich, S. M. (2003) Rate of free-radical oxidation of C18 diene and triene fatty acids in aqueous micellar solutions and effectiveness of  $\beta$ -carotene as an inhibitor of their oxidation. *Biochemistry (Mosc).* 68: 203-208.
- Nakashima, Y., Kusakabe, T., Kusakabe, R., Terakita, A., Shichida, Y. & Tsuda, M. (2003) Origin of the vertebrate visual cycle: genes encoding retinal photoisomerase and two putative visual cycle proteins are expressed in whole brain of a primitive chordate. *J.Comp.Neurol.* 460: 180-190.
- Navarrete-Bolanos, J. L., Jimenez-Islas, H., Botello-Alvarez, E., Rico-Martinez, R. & Paredes-Lopez, O. (2004) Improving xanthophyll extraction from marigold flower using cellulolytic enzymes. *J.Agric.Food Chem.* 52: 3394-3398.
- Nelson, J. L., DeMichele, S. J., Pacht, E. R. & Wennberg, A. K. (2003) Effect of enteral feeding with eicosapentaenoic acid,  $\gamma$ -linolenic acid, and antioxidants on antioxidant status in patients with acute respiratory distress syndrome. *J.Parenter.Enteral.Nutr.* 27: 98-104.
- Neuhouser, M. L., Patterson, R. E., Thornquist, M. D., Omenn, G. S., King, I. B. & Goodman, G. E. (2003) Fruits and vegetables are associated with lower lung cancer risk only in the placebo arm of the  $\beta$ -Carotene and Retinol Efficacy Trial (CARET). *Cancer Epidemiol.Biomarkers.Prev.* 12: 350-358.
- Nilsson, S. E., Sundelin, S. P., Wihlmark, U. & Brunk, U. T. (2003) Aging of cultured retinal pigment epithelial cells: oxidative reactions, lipofuscin formation and blue light damage. *Doc.Ophthalmol.* 106: 13-16.
- Nkondjock, A. & Ghadirian, P. (2004) Intake of specific carotenoids and essential fatty acids and breast cancer risk in Montreal, Canada. *Am.J.Clin.Nutr.* 79: 857-864.
- Novotny, J. A., Britz, S., Caulfield, F., Beecher, G. & Clevidence, B. (2003) Intrinsic labeling of plants for bioavailability studies. *Adv.Exp.Med.Biol.* 537: 131-139.
- Ozturk, Y., Buyukgebiz, B., Arslan, N., Ozer, E. & Lebe, B. (2003) Serum vitamin A and total carotene concentrations in well-nourished children with *Helicobacter pylori* infection. *J.Pediatr.Gastroenterol.Nutr.* 36: 502-504.
- Pinta, V., Ouchane, S., Picaud, M., Takaichi, S., Astier, C. & Reiss-Husson, F. (2003) Characterization of unusual hydroxy- and ketocarotenoids in *Rubrivivax gelatinosus*: involvement of enzyme CrtF or CrtA. *Arch.Microbiol.* 179: 354-362.
- Pott, I., Breithaupt, D. E. & Carle, R. (2003) Detection of unusual carotenoid esters in fresh mango (*Mangifera indica* L. cv. 'Kent'). *Phytochemistry* 64: 825-829.
- Prache, S., Priolo, A. & Grolier, P. (2003) Persistence of carotenoid pigments in the blood of concentrate-finished grazing sheep: its significance for the traceability of grass-feeding. *J. Anim. Sci.* 81: 360-367.

- Prakash, P., Liu, C., Hu, K. Q., Krinsky, N. I., Russell, R. M. & Wang, X. D. (2004)  $\beta$ -Carotene and  $\beta$ -apo-14'-carotenoic acid prevent the reduction of retinoic acid receptor beta in benzo[a]pyrene-treated normal human bronchial epithelial cells. *J.Nutr.* 134: 667-673.
- Qian, P., Addlesee, H. A., Ruban, A. V., Wang, P., Bullough, P. A. & Hunter, C. N. (2003) A reaction center-light-harvesting 1 complex (RC-LH1) from a *Rhodospirillum rubrum* mutant with altered esterifying pigments: characterization by optical spectroscopy and cryo-electron microscopy. *J.Biol.Chem.* 278: 23678-23685.
- Qin, F., Shite, J. & Liang, C. S. (2003) Antioxidants attenuate myocyte apoptosis and improve cardiac function in CHF: association with changes in MAPK pathways. *Am.J.Physiol.Heart Circ.Physiol.* 285: H822-H832.
- Rahmani, B., Jampol, L. M. & Feder, R. S. (2003) Clinicopathologic reports, case reports, and small case series: peripheral pigmented corneal ring: a new finding in hypercarotenemia. *Arch.Ophthalmol.* 121: 403-407.
- Ramadan, M. F. & Morsel, J. T. (2003) Oil goldenberry (*Physalis peruviana* L.). *J.Agric.Food Chem.* 51: 969-974.
- Reifen, R., Haftel, L., Faulks, R., Southon, S., Kaplan, I. & Schwarz, B. (2003) Plasma and buccal mucosal cell response to short-term supplementation with all trans- $\beta$ -carotene and lycopene in human volunteers. *Int.J.Mol.Med.* 12: 989-993.
- Ribaya-Mercado, J. D., Solon, F. S., Fermin, L. S., Perfecto, C. S., Solon, J. A. A., Dolnikowski, G. G. & Russell, R. M. (2004) Dietary vitamin A intakes of Filipino elders with adequate or low liver vitamin A concentrations as assessed by the deuterated-retinol-dilution method: implications for dietary requirements. *Am.J.Clin.Nutr.* 79: 633-641.
- Richelle, M., Enslin, M., Hager, C., et al. (2004) Both free and esterified plant sterols reduce cholesterol absorption and the bioavailability of  $\beta$ -carotene and  $\alpha$ -tocopherol in normocholesterolemic humans. *Am.J.Clin.Nutr.* 80: 171-177.
- Rock, C. L. (2003) Carotenoid update. *J.Am.Diet.Assoc.* 103: 423-425.
- Schulpis, K. H., Tsakiris, S., Karikas, G. A., Moukas, M. & Behrakis, P. (2003) Effect of diet on plasma total antioxidant status in phenylketonuric patients. *Eur.J.Clin.Nutr.* 57: 383-387.
- Schupp, C., Olano-Martin, E., Gerth, C., Morrissey, B. M., Cross, C. E. & Werner, J. S. (2004) Lutein, zeaxanthin, macular pigment, and visual function in adult cystic fibrosis patients. *Am.J.Clin.Nutr.* 79: 1045-1052.
- Schwartz, S. H., Qin, X. & Zeevaert, J. A. (2003) Elucidation of the indirect pathway of abscisic acid biosynthesis by mutants, genes, and enzymes. *Plant Physiol.* 131: 1591-1601.
- Schweigert, F. J. (2003) Research note: changes in the concentration of  $\beta$ -carotene,  $\alpha$ -tocopherol and retinol in the bovine corpus luteum during the ovarian cycle. *Arch.Tierernahr.* 57: 307-310.
- Schweigert, F. J., Bathe, K., Chen, F., Buscher, U. & Dudenhausen, J. W. (2004) Effect of the stage of lactation in humans on carotenoid levels in milk, blood plasma and plasma lipoprotein fractions. *Eur.J.Nutr.* 43: 39-44.
- Schweigert, F. J., Klingner, J., Hurtienne, A. & Zunft, H. J. (2003) Vitamin A, carotenoid and vitamin E plasma concentrations in children from Laos in relation to sex and growth failure. *Nutr.J.* 2: 17.
- Shikany, J. M., Patterson, R. E., Agurs-Collins, T. & Anderson, G. (2003) Antioxidant supplement use in Women's Health Initiative participants. *Prev.Med.* 36: 379-387.
- Shivashankara, K. S., Isobe, S., Al-Haq, M. I., Takenaka, M. & Shiina, T. (2004) Fruit antioxidant activity, ascorbic acid, total phenol, quercetin, and carotene of Irwin mango fruits stored at low temperature after high electric field pretreatment. *J.Agric.Food Chem.* 52: 1281-1286.
- Simova, E. D., Frengova, G. I. & Beshkova, D. M. (2003) Effect of aeration on the production of carotenoid pigments by *Rhodotorula rubra-lactobacillus casei* subsp. *casei* co-cultures in whey ultrafiltrate. *Z.Naturforsch.[C].* 58: 225-229.
- Sreekala, C. & Raghava, S. (2003) Exploitation of heterosis for carotenoid content in African marigold (*Tagetes erecta* L.) and its correlation with esterase polymorphism. *Theor.Appl.Genet.* 106: 771-776.
- Stacewicz-Sapuntzakis, M. & Diwadkar-Navsariwala, V. (2004) Carotenoids. In: *Nutritional Ergogenic Aids* (Driskell, J. & Wolinsky, I. eds.), pp. 325-353. CRC Press, Boca Raton, FL.
- Stepoe, A., Perkins-Porras, L., McKay, C., Rink, E., Hilton, S. & Cappuccio, F. P. (2003) Behavioural counselling to increase consumption of fruit and vegetables in low income adults: randomised trial. *BMJ.* 326: 855.
- Stocker, P., Lesgards, J. F., Vidal, N., Chaher, F. & Prost, M. (2003) ESR study of a biological assay on whole blood: antioxidant efficiency of various vitamins. *Biochim.Biophys.Acta* 1621: 1-8.
- Stover, D. E. (2003) Prevention of lung cancer. *Compr.Ther.* 29: 28-34.
- Surles, R. L., Weng, N., Simon, P. W. & Tanumihardjo, S. A. (2004) Carotenoid profiles and consumer sensory evaluation of specialty carrots (*Daucus carota*, L.) of various colors. *J.Agric.Food Chem.* 52: 3417-3421.
- Suzuki, K., Ito, Y., Ochiai, J., et al. (2003) The relationship between smoking habits and serum levels of 8-OHdG, oxidized LDL antibodies, Mn-SOD and carotenoids in rural Japanese residents. *J.Epidemiol.* 13: 29-37.
- Svilaas, A., Sakhi, A. K., Andersen, L. F., Svilaas, T., Strom, E. C., Jacobs, D. R., Jr., Ose, L. & Blomhoff, R. (2004) Intakes of antioxidants in coffee, wine, and vegetables are correlated with plasma carotenoids in humans. *J.Nutr.* 134: 562-567.
- Takaichi, S., Oh-oka, H., Maoka, T., Jung, D. O. & Madigan, M. T. (2003) Novel carotenoid glucoside esters from alkaliphilic heliobacteria. *Arch.Microbiol.* 179: 95-100.
- Tang, G., Qin, J., Dolnikowski, G. G. & Russell, R. M. (2004) Short-term (intestinal) and long-term (post intestinal) conversion of  $\beta$ -carotene to retinol in adults using a stable isotope reference method. *Am.J.Clin.Nutr.* (In Press)
- Tangney, C. C., Bienias, J. L., Evans, D. A. & Morris, M. C. (2004) Reasonable estimates of serum vitamin E, vitamin C, and  $\beta$ -cryptoxanthin are obtained with a food frequency questionnaire in older black and white adults. *J.Nutr.* 134: 927-934.
- Telfer, A., Frolov, D., Barber, J., Robert, B. & Pascal, A. (2003) Oxidation of the two  $\beta$ -carotene molecules in the photosystem II reaction center. *Biochemistry* 42: 1008-1015.
- Tormo, M. J., Navarro, C., Chirlaque, M. et al. (2003) Physical sports activity during leisure time and dietary intake of foods and nutrients in a large Spanish cohort. *Int.J.Sport.Nutr.Exerc.Metab.* 13: 47-64.
- Torok, K., Jarai, D., Szalay, N., Biro, L. & Molnar, D. (2003) [Antioxidant vitamin intake in obese children]. *Orv.Hetil.* 144: 259-262.
- Traber, M. G. (2004) The ABCs of vitamin E and  $\beta$ -carotene absorption. *Am.J.Clin.Nutr.* 80: 3-4.
- Trekli, M. C., Riss, G., Goralczyk, R. & Tyrrell, R. M. (2003)  $\beta$ -Carotene suppresses UVA-induced HO-1 gene expression in cultured FEK4. *Free Radic.Biol.Med.* 34: 456-464.
- Trombino, S., Serini, S., Di Nicuolo, F., Celleno, L., Ando, S., Picci, N., Calviello, G. & Palozza, P. (2004) Antioxidant effect of ferulic acid in isolated membranes and intact cells: synergistic interactions with  $\alpha$ -tocopherol,  $\beta$ -carotene, and ascorbic acid. *J.Agric.Food Chem.* 52: 2411-2420.
- Uleckiene, S. & Domkiene, V. (2003) Investigation of ethyl alcohol and beta-carotene effect on two models of carcinogenesis. *Acta Biol.Hung.* 54: 89-93.
- Varadi, G., Polyanka, H., Darko, E. & Lehoczi, E. (2003) Atrazine resistance entails a limited xanthophyll cycle activity, a lower PSII efficiency and an altered pattern of excess excitation dissipation. *Physiol.Plant.* 118: 47-56.
- Velayos, A., Papp, T., Aguilar-Elena, R., Fuentes-Vicente, M., Eslava, A. P., Iturriaga, E. A. & Alvarez, M. I. (2003) Expression of the carG gene, encoding geranylgeranyl pyrophosphate synthase, is up-regulated by blue light in *Mucor circinelloides*. *Curr.Genet.* 43: 112-120.
- Wang, Z., Yin, S., Zhao, X., Russell, R. M. & Tang, G. (2004)  $\beta$ -Carotene-vitamin A equivalence in Chinese adults assessed by an isotope dilution technique. *Br.J.Nutr.* 91: 121-131.
- Weller, P. & Breithaupt, D. E. (2003) Identification and quantification of zeaxanthin esters in plants using LC-MS. *J.Agric.Food Chem.* 51: 7044-7049.
- West, C. E., Eilander, A. & van Lieshout, M. (2003) Reply to Russell et al. *J.Nutr.* 133: 2917.
- Willcox, J. K., Catignani, G. L. & Lazarus, S. (2003) Tomatoes and cardiovascular health. *Crit.Rev.Food Sci.Nutr.* 43: 1-18.
- Wisniewska, A., Draus, J. & Subczynski, W. K. (2003) Is a fluid-mosaic model of biological membranes fully relevant? Studies on lipid organization in model and biological membranes. *Cell Mol.Biol.Lett.* 8: 147-159.
- Wright, M. E., Mayne, S. T., Swanson, C. A., Sinha, R. & Alavanja, M. C. (2003) Dietary carotenoids, vegetables, and lung cancer risk in women: the Missouri women's health study (U.S.). *Cancer Causes.Control.* 14: 85-96.
- Xu, Y., Leo, M. A. & Lieber, C. S. (2003) Lycopene attenuates arachidonic acid toxicity in HepG2 cells overexpressing CYP2E1. *Biochem.Biophys.Res.Comm.* 303: 745-750.
- Yokota, T., Etoh, H., Oshima, S., Hayakawa, K. & Ishiguro, Y. (2003) Oxygenated lycopene and dehydrated lutein in tomato puree. *Biosci.Biotechnol.Biochem.* 67: 2644-2647.

## TECHNICAL NOTES

### Letter to the Editor:

#### Raman Detection of Carotenoid Antioxidants in Human Tissue

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 protective role as well in the protection of skin from ultraviolet and short-wavelength visible radiation. Lutein and lycopene may also have protective functions 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 non-invasive technology for the detection of carotenoids in living human tissue, we started to look into resonance Raman spectroscopy (RRS) as a novel approach for non-invasive, laser-optical carotenoid detection. Initially, we applied this new method to the detection of macular carotenoid pigments (MP), and more recently to the detection of carotenoids present in human skin and mucosal tissues<sup>1,2</sup>.

In the February 2004 issue of *Carotenoid News*, Hammond and Wooten (H&W) offered a highly negative assessment of the RRS carotenoid detection method for the eye and skin<sup>3</sup>. Heavily involved with the development and promotion of a competing psychophysical carotenoid detection technology for the eye<sup>4</sup>, these authors raise doubts regarding the quantification of carotenoid levels using RRS. They allege a host of problems involving tissue composition, melanin and hemoglobin content, hydration, thickness, scattering, eye movement, lens density, and calibration, and they summarily dismiss the validity of the technology<sup>3</sup>. Unfortunately, all of their arguments are identical to ones that they have previously expressed in E-letters to various journals that published our Raman results. Moreover, H&W fail to mention any of the counter arguments we published in corresponding rebuttals. Therefore, we would like to address the major points, as much as possible, also in this forum<sup>5</sup>.

Raman spectroscopy is a sensitive and highly specific form of vibrational spectroscopy that has been used routinely used for a long time to identify and quantify Raman active chemical compounds. Carotenoids are especially suitable for Raman measurements since they can be excited with light overlapping their visible absorption bands. Under this excitation condition, they exhibit a very strong resonance Raman response, with an enhancement factor of about five orders of magnitude relative to non-resonant Raman spectroscopy<sup>6</sup>. This allows one to detect the characteristic vibrational energy levels of carotenoids through their corresponding spectral fingerprint signature even in complex biological systems, as in living human tissue. Any off-resonance Raman response from other molecules present in the sampling volume would be strongly suppressed under these conditions and would be buried in background noise. Also, the tissue environment of the carotenoid molecule has only a negligible effect on the vibrational energy, thus making the Raman signature virtually identical for the isolated carotenoid molecule, the molecule in a solution, or the molecule in a cell environment.

H&W raise concerns regarding the validation of RRS-detected carotenoid levels. Certainly, validation is an important aspect, and it is desirable to ultimately correlate optical RRS measurements directly with chemical "gold standard" HPLC measurements. However, these experiments are difficult to perform on a large group of healthy normal subjects in view of the extreme invasiveness of the HPLC method, which typically requires prohibitively large tissue samples. In spite of this obstacle, we have started a study funded by the National Cancer Institute that includes a comparison of carotenoid content in living and biopsied skin involving 100 subjects<sup>7</sup>.

In this study we will make use of a recently developed, less invasive HPLC protocol employing carotenoid measurements of small tissue samples (3 x 3 mm punch biopsies)<sup>8</sup>.

As indirect supporting evidence regarding validation, we can point to our Raman results already obtained with retinal tissue. We observed a near perfect linear correlation over a wide concentration range comparing MP Raman responses and MP levels determined by HPLC in human cadaver eyes and in living monkey eyes [see ref 1 and refs therein]. Furthermore, we just finished a study involving a group of 104 human volunteers, where we compared HPLC derived carotenoid levels of fasting serum with Raman skin levels measured in the inner palm. We obtained a highly significant correlation ( $p < 0.001$ ) with a correlation coefficient of 0.78<sup>9,10</sup>. The main result of this study is shown in Fig. 1. In yet another study, where we measured the Raman response in the palm of 1266 healthy volunteers, we found a wide (27-fold) variation of skin carotenoid levels throughout the population, a pronounced positive relationship between self-reported fruit and vegetable intake (a source of carotenoids) and skin Raman response, and 34% reduced skin carotenoid levels in smokers as compared to non-smokers<sup>11</sup>. All

these results, which are consistent with literature reports for serum data, would be impossible to obtain if the carotenoid Raman detection method were invalid for complex biological tissue.

H&W list a staggering number of factors supposedly interfering with the detection of carotenoid Raman detection in skin, and in particular they question the suitability of the inner palm stratum corneum layer for RRS measurements. However, a check of the literature reveals that superficial dermal layers are a good indicator for carotenoid status in skin, and that they have indeed been used by other optical tests for this purpose. One example is reflection spectrophotometry, which even though it is less specific than Raman spectroscopy and accordingly is not as precise, has nevertheless been successfully employed to monitor dermal carotenoid level changes upon supplementation<sup>12</sup>. Also, researchers found that dermal carotenoid levels measured at various sites with this method, including the palm of the hand, were highly correlated with serum carotenoid levels<sup>13</sup>.

Regarding RRS measurements of MP, the Raman method has already revealed new and exciting results, such as a decline of MP with age<sup>1</sup>. This finding agrees qualitatively with recent HPLC analyses of excised retinal tissues carried out in our group that showed a decrease of MP levels with age<sup>14</sup>, and it correlates functionally with the known increase of macular degeneration with age. Another finding is that macular Raman counts, which correspond linearly to MP optical density at this concentration range, are 32% lower in macular degeneration eyes versus age-matched controls (not 3 % as H&W claim)<sup>15</sup>. Another feature of RRS is the possibility to use this spectroscopy in an imaging mode<sup>16</sup>. Indeed, in first RRS imaging experiments with human subjects, we have recently been able to simultaneously quantify and spatially map the complete MP distribution, and in this way obtained not only absolute MP levels but also interesting insight into spatial asymmetries and spatial extent of the MP distribution. Since it is highly specific, detecting only the carotenoid molecules of interest, we believe that RRS imaging is a viable alternative to other objective and quantitative imaging technologies currently being explored, such as reflection and autofluorescence methods.

In summary, we strongly disagree with H&W's assertion that RRS is unsuitable for quantitative detection of carotenoids in living tissue. It is highly specific, sensitive, precise, and it allows one to rapidly assess dermal carotenoid content in large populations with excellent correlation to serum levels. Regarding MP measurements, it can quickly and objectively assess composite lutein and zeaxanthin scores in averaged integral measurements and it also has also good potential to map and quantify the complete MP distribution. To our knowledge there are no serious confounding factors for the technology, and it has exciting application potential. In the nutritional supplement industry, it is already being used as an objective, portable device for the monitoring of effect of carotenoid-containing supplements on skin tissue carotenoid levels ("Biophotonic Scanner"). In ophthalmology, it may become a fast screening method for MP levels; and in cancer epidemiology, it may serve as a noninvasive novel biomarker for fruit and vegetable intake, replacing costly plasma carotenoid measurements with inexpensive and rapid skin Raman measurements. Lastly, due to its capability of selectively detecting lycopene<sup>17</sup>, the technology may be useful to investigate a specific role of lycopene in the prevention of prostate cancer and other diseases.

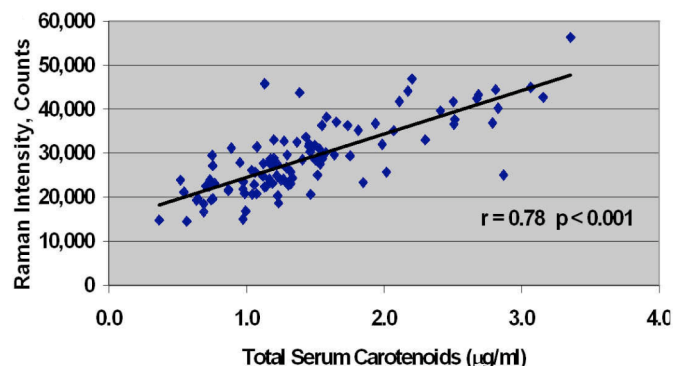


Fig. 1 Correlation of skin Raman intensity measured in the inner palm with serum carotenoids determined by HPLC, obtained for a group of 104 healthy male and female adults.

1. For a recent review see: Gellermann W and Bernstein PS, Raman detection of human macular pigments, *J Biomed Optics* 9: 75-85, 2004.
2. Ermakov IV et al. (2001), Resonance Raman detection of carotenoid antioxidants in living human tissues, *Optics Lett* 26:1179-1181.
3. Hammond BR and Wooten BR, Raman spectroscopic detection of carotenoids in human skin and retina, *Carotenoid News*, February 2004 <http://www.carotenoidsociety.org/news/fnewsviews.html>
4. Snodderly DM, Land RI, Wooten BR, US Patent 6,315,412.
5. H&W's E-Letters and our responses regarding the RRS MP detection can be found at <http://www.iovs.org/cgi/eletters/39/11/2003> ;E-letters regarding RRS skin carotenoid detection are published in: *J Invest Dermatol* (2004) 122: 544-546, and 122: 546-548.
6. Koyama Y. Resonance Raman spectroscopy. In: Britton G, Liaaen-Jensen, Pfander H, (Eds). *Carotenoids, Vol 1B, Spectroscopy*, Basel, Birkhäuser, 1995: pp. 135-146.
7. The principal investigator of this project is Susan Mayne, Yale University School of Medicine.
8. Peng YM, et al. (1993), A nonsaponification method for the determination of carotenoids, retinoids, and tocopherols in solid human tissues, *Cancer Epidemiol* 2:139-144.
9. Zidichouski J et al. (2003), submitted for publication.
10. Smith CR and Burke DS (2004), Nutritional significance and measurement of carotenoids, *Current Topics in Nutraceutical Research* 2:79-91.
11. Gellermann W et al. (2003), Resonant Raman detectors for noninvasive assessment of carotenoid antioxidants in human tissue, *Proc. of Advanced Biomedical and Clinical Diagnostic Systems, International Society for Optical Engineering, SPIE, Vol. 4958, Bellingham, Washington*, pp 78-87.
12. Prince MR and Frisoli JK (1993),  $\beta$ -Carotene accumulation in skin, *Am J Clin Nutr* 57: 175-180.
13. Stahl W et al. (1998), Increased dermal carotenoid levels assessed by noninvasive reflection spectrometry correlate with serum levels in women ingesting Betatene, *J Nutr* 128: 903-907.
14. Da-You Zhao et al. (2004), unpublished results.
15. Bernstein et al. (2002), Resonance Raman measurement of macular carotenoids in normal subjects and in age-related macular degeneration patients, *Ophthalmology* 109: 1780-1787.
16. Ermakov IV et al. (2002), Raman imaging of human macular pigments, *Optics Letters* 27, 833-835.
17. Ermakov IV et al. (2004), Noninvasive selection of lycopene and  $\beta$ -carotene in human skin using Raman spectroscopy, *J Biomed Optics* 9: 332-338.

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### News and Views New Astaxanthin Producer

Fuji Chemical Industry Co., Ltd. formed a new US based subsidiary, FCI Health Science, Inc., located in Mount Laurel, NJ. The new company's mission is to market Fuji's ingredients to the nutritional, personal care and pharmaceutical industries. FCI has established marketing office on both the East and West Coast. The formation of FCI follows Fuji's acquisition of Swedish company, BioProcess AB (formerly AstaCarotene AB), and positions Fuji as the leading producer of astaxanthin from the microalgae, *Haematococcus pluvialis*.

*Nutrition Business Journal, March 18, 2004*

### The Mysteries of Animal Colors

Some researchers are exploring the mechanisms that variously give rise to the deep, dependable reds of birds like the cardinal or red-winged blackbird, and the shifting coloration of a house finch, whose feathers may be chili red one season and tamale yellow the next. "Some colors are more conspicuous in one lighting environment than another," said Dr. Geoffrey E. Hill, a professor of biology at Auburn University and author of "A Red Bird in a Brown Bag" (Oxford University Press, 2002). Many birds eat foods high in carotenoids, the antioxidant chemicals that make fruits and vegetables red, orange or yellow. But they are not just after a healthy dose of vitamins; they rely on the plant pigments to give flash to their feathers, often in just a few crucial spots – the showy shoulders of a zebra finch.

Some species like the cardinal are exquisitely efficient at extracting carotenoids from their food, and hence their plumage remains red, although it may lighten or darken depending on the relative abundance of berries. By contrast, the house finch changes color completely with shifts in pigment consumption. Dr. Hill has taken finches into captivity, fed them diets that were alternately low or high in carotenoids, and watched them switch like traffic lights from yellow to red and back again. Among wild finch populations in California, the birds may be scarlet in one neighborhood, orange in another, daisy yellow in the next, and bird watchers often are not aware that they are looking at the same species. But is redder always better? Dr. Hill and his colleagues are now trying to determine if there is some optimal carotenoidal complexion at which the birds are most attractive to each other.

*The New York Times, July 20, 2004*

### Order the Salad, but Don't Hold the Fat

People need to include moderate amounts of fat with their uncooked vegetables in order to absorb the beneficial nutrients, new research shows. U.S. investigators found that when people ate salads with fat-free dressing, their bodies did not absorb  $\alpha$ -carotene,  $\beta$ -carotene or lycopene -- substances known to protect against cancer and heart disease. In contrast, when people doused their salads with reduced-fat or regular dressing, their carotenoid levels went up substantially. These findings suggest that people should include small amounts of cheese, meat, or other sources of fat in their salads or opt for something other than non-fat dressing if they want to get the most out of their veggies, study author Dr. Wendy White told Reuters Health. And if people like to snack on carrot sticks in between meals, consider dipping them in ranch dressing, she added. However, she cautioned that fat intake is "really a balancing act," since many Americans currently consume too much fat in their diet. "We don't want them drowning their salad in full-fat salad dressing," said White, who is based at Iowa State University in Ames. "Fat is part of a healthy diet. The key is moderation."

Carotenoids are red, yellow and orange pigments found in fruits and vegetables. They act as antioxidants, which prevent disease-causing free radicals from damaging the DNA of cells. Long-term damage by free radicals leads to aging and chronic disease.

To investigate whether people need fat with their carotenoids to get their disease-fighting benefits, White and her colleagues asked 7 people to eat 3 different salads on 3 separate occasions. The salads contained spinach, romaine lettuce, cherry tomatoes and carrots, and were covered in non-fat dressing, reduced-fat dressing (with 6 g of fat) or full-fat dressing (28 g of fat). Reporting in the *American Journal of Clinical Nutrition*, White and her colleagues found that when people ate the non-fat salads, there was "virtually no absorption of  $\alpha$ -carotene,  $\beta$ -carotene or lycopene." However, when people used reduced-fat dressing on their salads, levels of these carotenoids increased in their bodies. Full-fat dressing increased carotenoids even further. In an interview, White explained that carotenoids are "fat-soluble," meaning they are essentially not absorbed by the body without the aid of fats. These findings may not apply to cooked vegetables, White noted, for research suggests that people absorb carotenoids more easily from cooked or processed vegetables. She added that, even without fat, vegetables still contribute other important substances, such as fiber, vitamin C and folate. But if you can sprinkle uncooked vegetables with a little bit of fat, that might not hurt, she said. "A few tablespoons of bacon bits are not going to ruin anyone's health."

*Reuters, July 27, 2004*

### Internet Addresses for Carotenoid Researchers

1. USDA Nutrient Database for Standard Reference (SR13) is a major source of food composition data for epidemiologists and nutritionists. Carotenoid Food Database contains best available estimates of carotenoid content in foods, also used in NDS-R: [www.nal.usda.gov/fnic/foodcomp/data/index.html](http://www.nal.usda.gov/fnic/foodcomp/data/index.html)

2. This list is intended to be an open forum for carotenoid researchers from around the world to discuss recent developments in this field: [CARIG.Forum@lists.unh.edu](mailto:CARIG.Forum@lists.unh.edu). To subscribe, send e-mail to: [listproc@lists.unh.edu](mailto:listproc@lists.unh.edu). In the body of the message write: subscribe CARIG Forum, your name.

3. International Carotenoid Society Webpage: [www.carotenoidsociety.org](http://www.carotenoidsociety.org) Anyone wishing to join the society and be listed in the web directory, please contact Andrew Young at [A.J.Young@livjm.ac.uk](mailto:A.J.Young@livjm.ac.uk)

4. LIPID BANK for Web. Carotenoid Section of Bioactive Lipid Database developed by Research Institute, International Medical Center of Japan, <http://lipidbank.jp>. Also available on International Carotenoid Society Webpage: [www.carotenoidsociety.org](http://www.carotenoidsociety.org) through Articles button.

5. Reference library prepared by LycoRed Natural Product; [www.lycopen.com-references](http://www.lycopen.com-references)

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Published twice a year, in February and August by **CAROTENOID RESEARCH INTERACTIVE GROUP (CARIG)**

**Chairperson:** Wendy White (Ames, IA)  
**Treasurer:** Harold Furr (Wilson, NC)  
**Editor:** Maria S. Sapuntzakis (Chicago, IL)  
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