“The greatest reward for doing is the opportunity to do more.”
Jonas Salk, medical researcher (1914-1995)

This statement by the famous creator of successful polio vaccine could summarize lifelong attitude of the late Professor Phyllis Bowen, whose life and work are celebrated in this newsletter. Dr. Bowen, one of the CARIG founders, was a “prime mover” who encouraged this editor to undertake and continue the preparation of Carotenoid News for 25 years. She offered valuable advice, necessary corrections, and even helped with the mailing of up to 500 copies in the days before the internet publication.

The previous issue of the newsletter (August 2017) announced the next CARIG Symposium at incorrect time and place due to the editor’s ignorance of a change in the American Society for Nutrition plans. From now, ASN has its own, stand-alone annual meeting. This year Nutrition 2018 will convene on June 9-12 in Boston. CARIG, being a RIS of ASN, will continue having our Symposium on Friday before the ASN meeting, while oral and poster presentations will take place during the Nutrition 2018. These developments necessitate change in publication dates of the CARIG newsletter, and it also seems like an appropriate time to change the editorship of Carotenoid & Retinoid News. This editor would like to thank all collaborators, members of CARIG and readers for their help, patience and companionship on our long journey in the realm of carotenoid and retinoid research.

Maria Stacewicz-Sapuntzakis

In Memoriam
Phyllis Elaine Bowen (1940 - 2018)
Phyllis E. Bowen, Professor Emerita of the Kinesiology and Nutrition Department at the University of Illinois at Chicago (UIC), passed away on March 19, 2018. She obtained her M.S. and Ph.D. degrees in Nutrition from Cornell University, Ithaca, NY. In 1976 Dr. Bowen became an Assistant Professor of the Department of Human Nutrition and Foods at the Virginia Polytechnic Institute and State University, and in 1983 she joined the faculty of the Department of Nutrition and Medical Dietetics at UIC. Dr. Bowen had wide research interests that included, among others, the metabolism of cholesterol, pharmacokinetics of carotenoids, dietary effects on DNA oxidative damage, and the effects of carotenoids on cell proliferation in various cancers. Her specialty was conducting meticulous human feeding studies with strictly controlled diets, many including supplementary doses of carotenoids, and also whole food intervention studies with tomato products. Dr. Bowen has an impressive number (over 100) of peer-reviewed publications and even more presentations at various scientific conferences, as well as invited lectures at many national and international meetings. Her oratory skills were often used in popular media presentations and interviews (in press, radio, TV). She was very active in many professional organizations, including the American Dietetic Association, American Society for Nutrition, International Carotenoid Society (2018 Fellow), and was a founding member of Carotenoid Research Interactive Group and Functional Foods for Health Program. Dr. Bowen held numerous posts at UIC, as an Associate Head (1988-94) and Head (1994-96) of Human Nutrition Department, and as a member or chair of various education, executive, faculty and student affairs committees. She was a dedicated teacher, earning many teaching excellence awards and universally admired by her undergraduate and graduate students. She directed 44 M.S. degree recipients, three Ph.D. dissertations and four postdoctoral students, in addition to serving on innumerable thesis committees. The title of Professor Emerita (since 2009) did not make her rest on well-deserved laurels. She continued to volunteer, serve, and lead both in academia and her community (Oak Brook, Illinois) where everybody knew and loved her. Despite her busy schedule she still found time for more accomplishments in singing, gardening and painting. She is already missed by all her friends, colleagues and acquaintances.

CARIG Travel Awards
CARIG will award at least two monetary prizes, based on a poster competition to be held in conjunction with the CARIG Reception at Nutrition 2018 on Friday, June 8, 2018, in Hynes Convention Center, Room 313. Graduate students and postdoctoral trainees are eligible. Posters must
address carotenoid and/or vitamin A research. For those assigned an oral presentation rather than a poster at Nutrition 2018, printed copies of your slides with a print copy of your abstract and a small banner may be used for the CARIG poster competition. No advance registration is required to participate in the poster competition. Contact: Elizabeth Johnson (Elizabeth.Johnson@tufts.edu).

UPCOMING EVENTS

June 8, 2018
CARIG Annual Conference, Boston, MA. Carotenoids and Retinoids throughout the Lifespan. Contact: Elizabeth Johnson, CARIG RIS Chair, Email: Elizabeth.Johnson@tufts.edu [see program below].

June 9-12, 2018
Nutrition 2018, Boston, MA. Hynes Convention Center, website: www.nutrition.org/N18

June 10-15, 2018
4th International FASEB Conference on Retinoids. Steamboat Springs, CO. Website: www.faseb.org/SRC

June 16-22, 2018

July 11-13, 2018

Carotenoid and Retinoid Interactive Group (CARIG) Symposium at Nutrition 2018

Carotenoids & Retinoids throughout the Lifespan
Hynes Convention Center, Room 311
1:00 - 5:00 PM
1:00-1:15 PM Introduction. Chair: Elizabeth J. Johnson (Tufts University)
1:15-2:00 PM James A. Olson Lecture: Vitamin A, Carotenoids, Health and Survival by Person, Place and Time. Keith P. West (John Hopkins Bloomberg School of Public Health)
2:00-2:30 PM Infants: Vitamin A and related compounds in maternal-infant pairs, breast milk and formula; serum retinol in women of childbearing age in US. Corrine Hanson (University of Nebraska Medical Center)
3:00-3:30 PM Children: Biofortification of food and possible vitamin A toxicity. Sherry A. Tanumihardjo (University of Wisconsin-Madison)
3:30-4:00 PM Young Adults: Lutein and zeaxanthin status and auditory thresholds young adults. Billy R. Hammond (University of Georgia)
4:00-4:30 PM Oldest of the Old: Metabolomics, brain carotenoids and pathology and cognition in centenarians. Jirayu Tanprasertsuk (Tufts University)
4:30 - 5:00 PM Discussion
6:00 - 8:00 PM CARIG reception (Room 313)

FORTHCOMING / RECENT PUBLICATIONS

SIGHT AND LIFE Magazine 31 (2) 2017. PO Box 2116, 4002 Basel, Switzerland, tel: 41-61-815-8756, website: www.sightandlife.org
See especially:


Topical Collection "Marine Carotenoids"
Topical Collection of Marine Drugs journal will be dedicated to marine carotenoids and focused on the benefits of carotenoids for human beings. For a better understanding of the physiological effects of marine carotenoids, this Collection should include the most recent developments in the sources, analysis, chemistry, and biochemistry of marine carotenoids. (Collection editors: Dr. Takashi Maoka and Dr. Tatsuya Sugawara). Website: www.mdpi.com/journal/marinedrugs/special_issues/marine_carotenoids_collection
FEATURED ARTICLE

Effect of thermal treatment and light irradiation on the stability of lycopene with high Z-isomers content

The stability of lycopene with high Z-isomers content during thermal treatment and light irradiation was investigated. Purified (all-E)-lycopene was thermally isomerized to the Z-isomers in dichloromethane (CH₂Cl₂) at 50°C for 24 h. The total content of the Z-isomers of lycopene reached 56.1%. The mixture of lycopene isomers was stored in the dark at 4°, 25°, and 40°C for 30 days, and under light irradiation using a fluorescent light at 4°C for 336 h. The degradation rate of lycopene during thermal treatment rose with increasing temperature and the activation energy for decomposition of the mixture of lycopene isomers was calculated to be 71.0 kJ/mol. The degradation rate of lycopene isomers was almost the same under thermal treatment. On the other hand, during light irradiation, isomerization was promoted rather than decomposition, i.e. (9Z)- and (13Z)-lycopene converted to the (all-E)-isomer.

Murakami K et al. Food Chem 250 (June 2018)
DOI:10.1016/j.foodchem.2018.01.062

NEWS AND VIEWS

Transcription factor ISX mediates the cross talk between diet and immunity

The intestinal epithelium is a major site for the conversion of dietary β-carotene to retinaldehyde by the enzyme BCO1. The majority of retinaldehyde is further metabolized to retinol (vitamin A), esterified and packaged into triacylglycerol-rich chylomicrons for bodily distribution. Some serve on-site for the synthesis of retinoic acid, a hormone-like compound, which exerts pleiotropic and dominant effects on gastrointestinal immunity. We report here that the intestine-specific homeobox protein ISX is critical to control the metabolic flow of β-carotene through this important branching point of vitamin A metabolism. This transcription factor rules the expression of retinoic acid-inducible target genes such as Aldh1a2, Dhrs3, and Ccr9. The β-carotene-inducible disruption of retinoid homeostasis affected gut-homing and differentiation of lymphocytes and displayed morphologically in large lymphoid follicles along the intestine. Furthermore, it was associated with an infiltration of the pancreas by gut-derived lymphocytes that manifested as a pancreatic insulitis with β-islet cell destruction and systemic glucose intolerance. Thus, our study identifies an important molecular interlink between diet and immunity and indicates that vitamin A homeostasis must be tightly controlled by ISX to maintain immunity and tolerance at the intestinal barrier.


Colonic mucosal bacteria are associated with variability in serum carotenoid concentrations

Relatively high serum carotenoid levels are associated with reduced risks of chronic diseases, but inter-individual variability in serum carotenoid concentrations is modestly explained by diet. The bacterial community in the colon could contribute to the bioaccessibility of carotenoids by completing digestion of plant cell walls and by modulating intestinal permeability. The objective of this study was to evaluate whether colonic bacterial composition is associated with serum and colon carotenoid concentrations. The study was a randomized dietary intervention trial in healthy individuals who were at increased risk of colon cancer. Colon mucosal biopsy samples were obtained before and after 6 months of intervention without prior preparation of the bowels. Participants were recruited from Ann Arbor, MI, and nearby areas from July 2007 to November 2010. Biopsy data were available from 88 participants at baseline and 82 participants after 6 months. Study participants were randomized to counseling for either a Mediterranean diet or a Healthy Eating diet for 6 months. At baseline, bacterial communities in biopsy samples from study participants in the highest vs the lowest tertile of total serum carotenoid levels differed by several parameters. Linear discriminant analysis effect size identified 11 operational taxonomic units that were significantly associated with higher serum carotenoid levels. In linear regression analyses, three of these accounted for an additional 12% of the variance in serum total carotenoid concentrations after including BMI, smoking, and dietary intakes in the model. These factors together explained 36% of the inter-individual variance in serum total carotenoid concentrations. However, the bacterial community in the colonic mucosa was resistant to change after dietary intervention with either a Mediterranean diet or Healthy Eating diet, each of which doubled fruit and vegetable intakes. In conclusion, the colonic mucosal bacterial community was associated with serum carotenoid concentrations at baseline but was not appreciably changed by dietary intervention.

Assessment of skin carotenoid status through reflection spectroscopy as a measure of fruit and vegetable consumption

We evaluated the feasibility, reliability and validity of reflection spectroscopy (RS) to assess skin carotenoids in a racially diverse sample. Study 1 was a cross-sectional study of corner store customers (n=479) in Eastern North Carolina, USA, who completed the NCI Fruit and Vegetable Screener as well as RS measures. Feasibility was assessed by examining the time it took to complete three RS measures, reliability was assessed by examining the variation between three RS measures, and validity was examined by correlation with self-reported fruit and vegetable consumption. In Study 2, validity was assessed in a smaller sample (n=30) by examining associations between RS measures and dietary carotenoids, fruits and vegetables as calculated from a validated FFQ and plasma carotenoids. It took on average 94 seconds to complete three RS readings per person. The average variation between three readings for each participant was 6.8%. In Study 2, in models adjusted for age, race and sex, there were statistically significant associations between RS measures and FFQ-estimated carotenoid intake (P<0.0001); FFQ-estimated fruit and vegetable consumption (P<0.01); and plasma carotenoids (P<0.0001). RS is a potentially improved method to approximate fruit and vegetable consumption among diverse participants. RS is portable and easy to use in field-based public health nutrition settings. More research is needed to investigate validity and sensitivity in diverse populations.

Bell Jilcott Pitts S et al. Public Health Nutr DOI:10.1017/S1368980017001700430X (Feb 2018)

Efficacy of diacetate esters of macular carotenoids

The accumulation of the carotenoids lutein, zeaxanthin, and meso-zeaxanthin in the center of the human retina, and known as the macula lutea or macular pigment, is believed to protect the retina from age-related macular degeneration. Since the macular pigment is of dietary origin, supplements containing the relevant carotenoids are readily available. In this study, we compared the changes in macular pigment over a 24-week supplementation period for two groups of 24 subjects, each assigned to either of two supplement formulations, 20 mg/day of lutein or equivalent combination of diacetate esters of the macular carotenoids. The latter group responded with a larger increase (0.0666 ± 0.0481) in macular pigment optical density than the former group (0.0398 ± 0.0430), driven largely by the older subjects. The difference was statistically significant (p = 0.0287). There was a general trend towards smaller increases in macular pigment for those subjects whose baseline value was high. However, the trend was only significant (p<0.05) for subjects in the diacetate esters group. No differences in response could be attributed to the gender of the subjects. We also observed no indication that the use of statin drugs by a few of the older subjects influenced their responses.


Lutein deposition across brain following formula or breast feeding of infant rhesus macaques

Lutein, a yellow xanthophyll, selectively accumulates in primate retina and brain. Lutein may play a critical role in neural and retinal development, but few studies have investigated the impact of dietary source on its bioaccumulation in infants. We explored the bioaccumulation of lutein in infant rhesus macaques following breastfeeding or formula feeding. From birth to 6 months of age, male and female rhesus macaques (Macaca mulatta) were either breastfed (BF) (n = 8), fed a formula supplemented with lutein, zeaxanthin, β-carotene, and lycopene (237, 19.0, 74.2, and 338 nmol/kg, SF) (n = 8), or fed a formula with low amounts of these carotenoids (38.6, 2.3, 21.5, and 0 nmol/kg, UF) (n = 7). The concentrations of carotenoids in serum and tissues were analyzed by HPLC. At 6 months of age, the BF group exhibited significantly higher lutein concentrations in serum, all brain regions, macular and peripheral retina, adipose tissue, liver, and other tissues compared to both formula-fed groups (P < 0.001). Lutein concentrations were higher in the SF group than in the UF group in serum and all tissues, with the exception of macular retina. Lutein was differentially distributed across brain areas, with the highest concentrations in the occipital cortex, regardless of the diet. Zeaxanthin was present in all brain regions but only in the BF infants; it was present in both retinal regions in all groups but was significantly enhanced in BF infants compared to either formula group (P<0.001). β-Carotene accumulated across brain regions in all groups, but was not detected in retina. Although lycopene was found in many tissues of the SF group, it was not detected in the brain or retina. Carotenoid supplementation of infant formula significantly increased serum and tissue lutein concentrations compared to unsupplemented formula, but they were still well below those in BF infants. Regardless of diet, occipital cortex showed selectively higher lutein deposition than other brain regions, suggesting lutein role in visual processing in early life.

Plasma concentrations of lutein and zeaxanthin, macular pigment optical density, and their associations with cognitive performances among older adults

We investigated the cross-sectional associations between macular pigment optical density (MPOD), plasma lutein (L), and zeaxanthin (Z) concentrations and cognitive function in 184 older adults of the 3-City-Bordeaux cohort. MPOD was measured using the two-wavelength autofluorescence method with a modified scanning laser ophthalmoscope. Plasma L and Z (L+Z) concentrations were determined by HPLC and were considered either crude or expressed as a ratio of the concentration of plasma lipids (total cholesterol [TC] + triglycerides [TG]). Cognitive performances were assessed using the following four separate neuropsychological tests: the Mini-Mental State Examination (MMSE), the Isaacs Set Test (IST), the Benton Visual Retention Test (BVRT), and the sum of the three free recalls of the Free and Cued Selective Reminding Test (FCSRT). These test results were summarized by a composite global cognitive z-score. Higher MPOD at 0.5° was significantly associated with a higher composite z-score (β = 0.15, 95%CI 0.04-0.26), higher BVRT (β = 0.39, 95%CI 0.08-0.70), and higher IST (β = 1.16, 95%CI 0.11-2.22) performances. Higher plasma L+Z concentrations were significantly associated with higher IST scores (β = 0.97, 95%CI 0.01-1.94). Furthermore, a higher L+Z/TC+TG ratio was associated with a higher composite z-score (β = 0.12, 95%CI 0.01-0.23), along with higher IST (β = 1.02, 95%CI 0.002-2.04) and FCSRT (β = 1.55, 95%CI 0.41-2.69) performances. This analysis suggested that both higher MPOD and L+Z concentrations were significantly associated with higher cognitive performances. However, MPOD measurements have the advantage of being a fast and representative measure of long-term carotenoid intake.


Golden potato potential to improve vitamin A status in developing countries

Potato (Solanum tuberosum L.) is the third most widely consumed plant food by humans. Its tubers are rich in starch and vitamin C, but have low or null levels of essential nutrients such as provitamin A and vitamin E. Transformation of potato with a bacterial mini-pathway for β-carotene in a tuber-specific manner results in a “golden” potato (GP) tuber phenotype due to accumulation of provitamin A carotenoids (α- and β-carotene) and xanthophylls. Here, we investigated the bioaccessibility of carotenoids and α-tocopherol (αTC) in boiled wild type (wt) and golden tubers using in vitro digestion. Golden tubers contained up to 91 μg provitamin A carotenes/g DW, increased levels of xanthophylls, phytoene and phytofluene, as well as up to 78 μg vitamin E/g DW. Cubes from wild type and GP tubers were boiled and subjected to simulated digestion to estimate bioaccessibility of carotenoids and αTC. Retention in boiled GPs exceeded 80% for β-carotene (βC), α-carotene (αC), lutein, phytoene and αTC, but less than 50% for phytofluene. The efficiency of partitioning of total βC, αC, E-lutein, phytoene, phytofluene and αTC in the mixed micelle fraction during small intestinal digestion was influenced by genotype, tuber content and hydrophobicity. Apical uptake of the compounds that partitioned in mixed micelles by monolayers of human intestinal Caco-2 cells during incubation for 4h was 14-20% for provitamin A and xanthophylls, 43-45% for phytoene, 23-27% for phytofluene, and 53% for αTC. These results suggest that 150 g serving of boiled golden potatoes has the potential to contribute 42% and 23% of the daily requirement of retinol activity equivalents (RAE), as well as 34% and 17% of the daily vitamin E requirement for children and women of reproductive age, respectively.


Development of biofortified maize hybrids

In the traditional yellow maize, though it contains high kernel carotenoids, the concentration of provitamin A (proA) is quite low (<2 μg/g), compared to recommended level (15 μg/g). It also possesses poor endosperm protein quality due to low concentration of lysine and tryptophan. Natural variant of crtRB1 (β-carotene hydroxylase) and lcyE (lycopene-ε-cyclase) cause significant enhancement of proA concentration, while recessive allele opaque2 (o2) enhances the level of these amino acids. Development of biofortified maize enriched in proA, lysine and tryptophan, thus holds significance in alleviation of micronutrient malnutrition. In the present study, marker-assisted stacking of crtRB1, lcyE and o2 was undertaken in the genetic background of four maize hybrids (HQPM1, HQPM4, HQPM5, and HQPM7) popularly grown in India. HP704-22 and HP704-23 were used as donors, while four elite QPM parents viz., HKI161, HKI163, HKI193-1 and HKI193-2, were used as recipients. CrtRB1 showed severe segregation distortion, while lcyE segregated as expected. Recovery of recurrent parent genome (RPG) among selected backcross progenies ranged from 89 to 93%. Introgressed progenies possessed high concentration of proA (7.38-13.59 μg/g), compared
to 1.65-2.04 μg/g in the recurrent parents. The reconstituted hybrids showed an average of 4.5-fold increase in proA with a range of 9.25-12.88 μg/g, compared to original hybrids (2.14-2.48 μg/g). Similar plant, ear, and grain characteristics of improved versions of both inbreds and hybrids were observed when evaluated with their respective original versions. Mean lysine (0.334%) and tryptophan (0.080%) of the improved hybrids were at par with the original versions (lysine: 0.340%, tryptophan: 0.083%). Improved hybrids also possessed similar grain yield potential (6,301-8,545 kg/ha) with their original versions (6,135-8,479 kg/ha) evaluated at two locations. This is the first study of staking *crtRB1*, *lcyE*, and *o2*, favorable alleles in single genetic background. The improved inbreds can be effectively used as potential donor for independent and/or simultaneous introgression of *crtRB1*, *lcyE*, and *o2* in the future breeding program. These biofortified maize hybrids, rich in proA, lysine and tryptophan will hold great promise for nutritional security.


**Lycopene enrichment in tomato by novel genome editing**

Numerous studies have been focusing on breeding tomato plants with enhanced lycopene accumulation. In this study, we used a bidirectional strategy: promoting the biosynthesis of lycopene, while inhibiting its conversion to β- and α-carotene. The accumulation of lycopene was promoted by knocking down some genes associated with the carotenoid metabolic pathway. Finally, five genes were selected to be edited in genome by CRISPR/Cas9 system, using transformation mediated by *Agrobacterium tumefaciens*. Our findings indicated that CRISPR/Cas9 is a site-specific genome editing technology that allows highly efficient target mutagenesis in multiple genes of interest. Surprisingly, the lycopene content in tomato fruit subjected to genome editing was successfully increased about 5-fold. The homozygous mutations were stably transmitted to subsequent generations. Our results suggest that CRISPR/Cas9 system can be used for significantly improving lycopene content in tomato fruit with advantages such as high efficiency, rare off-target mutations, and stable heredity.


**9-cis-Epoxycarotenoid dioxygenase 3 regulates plant growth and enhances stress tolerance in rice**

Although abscisic acid (ABA) is an important hormone that regulates seed dormancy, stomatal closure, plant development, as well as responses to environmental stimuli, the physiological mechanisms of ABA response to multiple stress in rice remain poorly understood. In the ABA biosynthetic pathway, 9-cis-epoxycarotenoid dioxygenase (NCED) is the key rate-limiting enzyme. Here, we report important functions of *OsNCED3* in multi-abiotic stress tolerance in rice. The *OsNCED3* is constitutively expressed in various tissues under normal condition. Its expression is highly induced by NaCl, PEG (polyethylene glycol), and H2O2 stress, suggesting the roles for *OsNCED3* in response to the multi-abiotic stress tolerance in rice. Compared with wild-type plants, *nced3* mutants had earlier seed germination, longer post-germination seedling growth, increased sensitivity to water stress and H2O2 stress, increased stomata aperture under water stress and delayed leaf senescence. Further analysis found that *nced3* mutants contained lower ABA content compared with wild-type plants. The overexpression of *OsNCED3* in transgenic plants could enhance water stress tolerance, promote leaf senescence and increase ABA content. We conclude that *OsNCED3* mediates seed dormancy, plant growth, abiotic stress tolerance, and leaf senescence by regulating ABA biosynthesis in rice; and may provide a new strategy for improving the quality of crop.


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