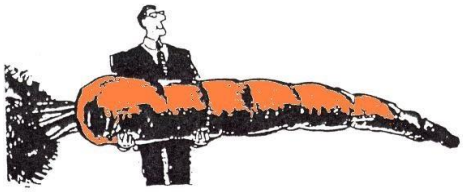


Carotenoid & Retinoid News

February 2017
Vol. 26, No. 1

From the Editor:

"I love to doubt as well as know."
Dante's Inferno, Canto XI, 93

The great Italian poet Dante Alighieri (1265-1321) perfectly expressed the attitude of a philosopher or scientist who loves to learn new things but is always skeptical. Indeed, that is the only way to approach the wealth of information and to progress in any area of scientific endeavor. As we get older, we develop so called crystallized intelligence, the ability to use the skills and knowledge acquired over a lifetime. How interesting is to find that the preservation of this cognitive function may be related to lutein intake and subsequent brain reserves. The localization of lutein in cell membranes, its antioxidant and anti-inflammatory actions may provide neuroprotection in specific regions of brain cortex that were found to be thicker in older adults with higher serum lutein, who also performed better on standard tests of crystallized intelligence (*Front Aging Neurosci 12/6/2016*). You will have plenty of opportunities to learn, doubt, and discuss recent developments in carotenoid and retinoid research during the exciting conferences this year, especially CARIG Annual Symposium at EB 2017 in Chicago and International Symposium on Carotenoids in Lucerne, Switzerland.

Maria S. Sapuntzakis (Chicago,IL)

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 Experimental Biology 2017 on Friday, April 21, 2017. 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 EB'2017, 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: Lisa Jahns (lisa.jahns@ars.usda.gov).

UPCOMING EVENTS

April 21, 2017
CARIG Annual Conference, Chicago, IL. Contact**:** Lisa Jahns, CARIG RIS Chair, **Email**: lisa.jahns@ars.usda.gov [more information below]

April 22-26, 2017
Experimental Biology 2017, Chicago, IL. Contact: EB2017, FASEB Office of Scientific Meetings & Conferences, 950 Rockville Pike, Bethesda, MD 20814-3998, **e-mail:** eb@faseb.org, **website:** www.experimentalbiology.org

June 21-23, 2017
Oxygen Club of California 2017 World Congress, Berlin, Germany. Metabolic Stress and Redox Regulation. **Website**: www.occ-2017.com

July 9-14, 2017
18th International Symposium on Carotenoids. Lucerne, Switzerland. www.icslucerne2017.org [abstract deadline March 31, 2017]. Research topics:
Nutrition and health
Chemistry: analytics and synthesis
Industrial production, extraction, synthesis
New methods in carotenoid research
Photochemistry and phytophysics
Carotenoids in the eye
Emerging carotenoid science
Apo-carotenoid and retinoid metabolism and function
Risk reduction of chronic disease
Plant biology and plant genetics
Carotenoid metabolism
Brain and cognition
Industrial production and commercial application
Food science and technology

October 9-12, 2017
17th International Nutrition & Diagnostics Conference, Prague, Czech Republic. Website: www.indc.cz

CARIG Events at Experimental Biology 2017
Friday, April 21, 1:00-5:00 PM
ASN CARIG Annual Symposium
Location: McCormick Place Convention Center
Room S105A
Co-Chairs: John W. Erdman, Jr, Nancy E. Moran

Symposium Title: Moving Towards Personalized Nutrition of Dietary Carotenoids: A Review of the Genetic and Non-genetic Factors Impacting Absorption, Metabolism, and Health

Symposium Background: Epidemiological studies continue to reveal associations between carotenoid exposure and health outcomes. In order to expand

our understanding of causal relationships between dietary carotenoid consumption and health, nutritional studies of carotenoids must be appropriately designed and account for the many dietary, physiologic, lifestyle, and genetic factors impacting carotenoid status and health effects.
Symposium Goal: To provide the scientific basis for dietary, physiologic, lifestyle, and genetic factors which influence carotenoid absorption, distribution, metabolism, and resultant bioactivity. In order to define a potential role for personalized carotenoid intake recommendations, we must comprehensively understand the major genetic and non-genetic factors influencing carotenoid "pharmacokinetics" and health effects. Recent evidence of the factors impacting absorption, distribution, metabolism and excretion (ADME) and resultant bioactivity of the major carotenoids will be presented. This symposium will not only be a valuable primer for investigators new to the carotenoid field, but will also integrate recent absorption, distribution, and metabolism findings, essential for designing nutritionally relevant pre-clinical and clinical studies capable of advancing the field of personalized nutrition.

1:00-1:15 PM Registration
1:15-2:00 James Allen Olson Memorial Lecture: New Epidemiological Evidence on the Relationship between Carotenoids and Breast Cancer Risk. Heather Eliassen, Department of Epidemiology, Harvard School of Public Health, Boston, MA

2:00–5:00 CARIG Symposium
2:00-2:00 Introduction to Symposium. John W. Erdman, Jr., Division of Nutritional Sciences, University of Illinois, Urbana, IL.
2:10–2:45 Genetic and non-genetic factors impacting carotene absorption, distribution and metabolism. Nancy E. Moran, USDA/ARS Children's Nutrition Research Center at Baylor College of Medicine, Houston, TX.
2:45–3:20 Health aspects of carotenes. John W Erdman, Jr.
3:20–3:40 Break
3:40–4:15 Factors impacting xanthophyll absorption, distribution, metabolism and health aspects of xanthophylls. Elizabeth Johnson; USDA/ARS Human Nutrition Research Center on Aging at Tufts, Boston, MA
4:15–5:00 Panel Discussion: What are the critical variables impacting carotenoid responses and what are the critical questions yet to be answered?(Johnson, Moran, Erdman)
5:00-6:00 Steering Committee meeting
6:00-8:00 CARIG Poster Competition and Reception, McCormick Place Convention Center South Building Foyer

Saturday, April 22. 10:30 AM – 12:30 PM
Minisymposium: Bioavailability and Metabolism of Carotenoids and Vitamin A. Chair: Jessica Cooperstone, Co-Chair: Emily Mohn

Sunday, April 23. 3:00 PM – 5:00 PM
Minisymposium: Carotenoids and Health. Chair: Bryan Gannon, Co-Chair: Sherry Tanumihardjo

Sunday, April 23. **Poster session: CARIG: Bioavailability and Metabolism of Carotenoids and Vitamin A**

FORTHCOMING / RECENT PUBLICATIONS

SIGHT AND LIFE Magazine 30 (2) 2016. PO Box 2116, 4002 Basel, Switzerland, **tel:** 41-61-815-8756, **website:** www.sightandlife.org
See especially:
James Allen Olson Memorial Lecture: Vitamin A, carotenoids and inflammation in infancy. Rubin LP, pp 25-30.
2016 CARIG Conference convenes in San Diego. Solomons NW, pp 138-140.
Malnutrition and psychosis in Don Quixote. Steffen J, pp 118-122.

Carotenoids in Nature. Ed. Stange C. Springer 2016.

A gold standard to accurately assess vitamin A status: are we there yet? Quadro L**.** *J Nutr 146: 1929-30 (2016).*

Lutein and zeaxanthin isomers in eye health and disease. Mares J. *Ann Rev Nutr 36:561-602.*

Recent insight into health benefits of carotenoids. Copperstone J, Schwartz SJ. In *Handbook of Natural Pigments in Food and Beverages*. Eds. Carle R, Schweiggert R, Woodhead Publishing 2016: 473-497.

Natural Bioactive Compounds from Fruits and Vegetables as Health Promoters: Part 1, doi: 10.2174/97816810823941160101, Part 2, doi: 10.2174/97816810824311160101. Eds. da Silva LR, Silva BM, Bantham Books 2016 (printed or e-book).

Carotenoid derivates in achiote (*Bixa orellana*) seeds: synthesis and health promoting properties. [Review]. Rivera-Madrid R, Aguilar-

Espinosa M, Cárdenas-Conejo Y, Garza-Caligari LE. *Front Plant Sci, doi: org/10.3389/fpls.2016.01406, 9/21/2016.*

Indian Food Composition Table, including fat-soluble vitamins and carotenoids. Released 1/17/2017, **website**: www.ifct2017.com

A comprehensive picture of the ultrafast excited-state dynamics of retinal. Flender O, Scholz M, Hölzer J, Oum K, Lenzer T. *Phys. Chem. Chem. Phys 18: 14941-48, 2016.*

Plasma retinol kinetics and β-carotene bioefficacy are quantified by model-based compartmental analysis in healthy young adults with low vitamin A stores. Green MH, Ford JL, Oxley A, Green JB, Park H, Philip Berry P, Boddy AV, Lietz G. *J Nutr 146: 2129-36, 2016.*

A retinol isotope dilution equation predicts both group and individual total body vitamin A stores in adults based on data from an early postdosing blood sample. Green MH, Ford JL, Green JB, Berry P, Boddy AV, Oxley A, Lietz G. *J Nutr 146: 2137-42, 2016.*

Retinol isotope dilution is applied during restriction of vitamin A intake to predict individual subject total body vitamin A stores at isotopic equilibrium. Green MH, Ford JL, Green JB. *J Nutr 146: 2407-11, 2016.*

Alphabetical Listing of Recent Publications may be found at www.carotenoidsociety.org/articles-books-and-databases**.** It is prepared by Dr. Harold Furr, Department of Nutritional Sciences, University of Wisconsin, Madison.

TECHNICAL NOTE

Natural pigments from autumn leaves for industry

Autumn leaves contain a range of interesting substances such as pigments, carbohydrates, proteins and compounds that inhibit the growth of harmful bacteria. VTT Technical Research Centre of Finland is developing leaf-processing technologies, which could be used by the cosmetics, textile, and feed and food industries. Very little use has been made of fallen leaves so far. They are either left on the ground, composted or burned resulting in full landfills and a growing carbon dioxide load. Autumn leaves derive their color from orange and yellow carotenoids and red anthocyanins. There is a fast-growing need for natural pigments in various industries around the world. In a process developed by VTT, leaves gathered in gardens and parks are dried and ground, and compounds are extracted. The processing stages were developed by VTT in laboratory experiments; R&D has now entered the piloting stage, using leaf material collected in the Otaniemi area by waste disposal company Lassila & Tikanoja. Special attention has been paid to the environmental friendliness of the overall process and the safety of the compounds produced. Pigments from autumn leaves can be used to color cosmetics and textiles. The chemical composition of leaves varies largely between different tree species. Added value can be obtained by processing the autumn leaves of certain tree types only, thereby producing well-defined compounds. Residual biomass, remaining after extraction, is high in nutrients and suitable for soil improvement in home gardens. Compounds obtained from the leaves may be suitable for use as food coloring and preservatives, and as nutritional supplements. Novel compounds could be obtained for the cosmetic and pharmaceutical industries, by using biotechnological methods to modify pigments.

Phys.org/news (11/2/2016)

NEWS AND VIEWS

Tropical bat as mammalian model for skin carotenoid metabolism

Animals cannot synthesize carotenoid pigments *de novo*, and must consume them in their diet. Most mammals, including humans, are indiscriminate accumulators of carotenoids but inefficiently distribute them to some tissues and organs, such as skin. This limits the potential capacity of these organisms to benefit from the antioxidant and immunostimulatory functions of carotenoids. Indeed, to date, no mammal has been known to have evolved physiological mechanisms to incorporate and deposit large amounts of carotenoids in the skin or hair, and mammals have therefore been assumed to rely entirely on other pigments such as melanins to color their integument. We used HPLC in combination with time-of-flight mass spectrometry

(HPLC-TOF/MS) to show that the frugivorous Honduran white bat *Ectophylla alba* colors its skin bright yellow with the deposition of the xanthophyll lutein. The Honduran white bat is thus a mammalian model that may help developing strategies to improve the assimilation of lutein in humans to avoid macular degeneration. This represents a change of paradigm in animal physiology showing that some mammals actually have the capacity to accumulate dietary carotenoids in the integument. In addition, we have also discovered that the majority of the lutein in the skin of Honduran white bats is present in esterified form with fatty acids, thereby permitting longer-lasting coloration and suggesting bright color traits may have an overlooked role in the visual communication of bats.

Galván I et al, Proc. Natl. Acad. Sci. USA 113:10932-37(2016)

Macular carotenoid supplementation improves disability glare performance and dynamics of photostress recovery

The so-called macular carotenoids (MC) lutein (L), zeaxanthin (Z), and meso-zeaxanthin (MZ), comprise the diet-derived macular pigment (MP). The purpose of this study was to determine effects of MC supplementation on the optical density of MP (MPOD), repeated-exposure photostress recovery (PSR), and disability glare (DG) thresholds. This was a double-blind, placebo-controlled trial. Young (mean age = 21.7, n=59), healthy volunteers participated in this study. Subjects supplemented their daily diet with either 10 mg L + 2 mg total Z (1 mg Z + 1 mg MZ; $n = 24$), 20 mg L + 4 mg total Z (2 mg Z + 2 mg MZ; $n = 25$), or placebo ($n = 10$) for 12 months. The primary outcome was a composite measure of visual performance in glare, defined by change in DG and PSR. Secondary outcomes included MPOD and visual fatigue. The primary endpoint for outcomes was 12 months. MPOD was assessed with customized heterochromatic flicker photometry. PSR times for an 8 cycle/degree, 15% contrast Gabor patch target were determined after each of five successive exposures to intense LED lights. DG threshold was defined as the intensity of a ring of lights through which subjects were able to maintain visibility of the aforementioned target. Measures of all parameters were conducted at baseline, 6 months, and 12 months. Repeated-measures ANOVA, and Pearson product-moment correlations were used to determine statistically significant correlations, and changes within and between groups. MPOD for subjects in both supplementation groups increased significantly versus placebo at both 6- and 12-month visits ($p < 0.001$ for all). Additionally, PSR times and DG thresholds improved significantly from baseline compared to placebo at 6- and 12-month visits ($p < 0.001$ for all). At baseline, MPOD was significantly related to both DG thresholds ($r = 0.444$; $p = 0.0021$) and PSR times ($r = -0.56$; $p < 0.001$). As a function of MPOD, the repeated-exposure PSR curves became more asymptotic, as opposed to linear. The change in subjects' DG thresholds were significantly related to changes in PSR times across the study period ($r = -0.534$; $p < 0.001$). Increases in MPOD lead to significant improvements in PSR times and DG thresholds. The asymptotic shape of the repeated-exposure PSR curves suggests that increases in MPOD produce more consistent steady-state visual performance in bright light conditions. The mechanism for this effect may involve both the optical filtering and biochemical (antioxidant) properties of MP.

Stringham JM et al. Eye and Vision (11/11/2016) doi: 10.1186/s40662-016-0060-8

Lutein linked to brain health and intelligence in older adults

Although diet has a substantial influence on the aging brain, the relationship between dietary nutrients and aspects of brain health remains unclear. We hypothesized that higher serum levels of lutein are associated with better performance on a task of crystallized intelligence, and that this relationship is mediated by gray matter structure of regions within the temporal cortex. Crystallized intelligence is the ability to use the skills and knowledge acquired over a lifetime. This investigation aims to contribute to a growing line of evidence, which suggests that particular nutrients may slow or prevent aspects of cognitive decline by targeting specific features of brain aging. We examined 76 cognitively intact adults between the ages of 65 and 75 to investigate the relationship between serum lutein (measured by HPLC), tests of crystallized intelligence (measured by the Wechsler Abbreviated Scale of Intelligence), and gray matter volume of regions within the temporal cortex (measured by MRI). A three-step mediation analysis was implemented using multivariate linear regressions to control for age, sex, education, income, depression status, and body mass index. The mediation analysis revealed that gray matter thickness of one region within the temporal cortex, the right parahippocampal cortex (Brodmann's Area 34), partially mediates the relationship between serum lutein and crystallized intelligence. These results suggest that the parahippocampal cortex acts as a mediator of the relationship between serum lutein and crystallized intelligence in cognitively intact

older adults. Prior findings substantiate the individual relationships reported within the mediation, specifically the links between (i) serum lutein and temporal cortex structure, (ii) serum lutein and crystallized intelligence, and (iii) parahippocampal cortex structure and crystallized intelligence. This report demonstrates a novel structural mediation between lutein status and crystallized intelligence, and therefore provides further evidence that specific nutrients may slow or prevent features of cognitive decline by hindering particular aspects of brain aging. Future work should examine the potential mechanisms underlying this mediation, including the antioxidant, anti-inflammatory, and membrane modulating properties of lutein.

Zamroziewicz MK et al, Front Aging Neurosci (12/6/2016) doi.org/10.3389/fnagi.2016.00297

Carotenoid bioaccumulation in tissues of infant rhesus macaques

Lutein is the predominant carotenoid in the developing primate brain and retina, and may have important functional roles. However, its bioaccumulation pattern during early development is not understood. In this pilot study, we investigated whether carotenoid supplementation of infant formula enhanced lutein tissue deposition in infant rhesus macaques. Monkeys were initially breastfed; from 1 to 3 months of age they were fed either a formula supplemented with lutein, zeaxanthin, β-carotene and lycopene, or a control formula with low levels of these carotenoids, for 4 months (*n* = 2/group). All samples were analyzed by HPLC. Final serum lutein in the supplemented group was 5 times higher than in the unsupplemented group. All brain regions examined showed a selective increase in lutein deposition in the supplemented infants. Lutein differentially accumulated across brain regions, with highest amounts in occipital cortex in both groups, β-carotene accumulated, but zeaxanthin and lycopene were undetectable in any brain region. Supplemented infants had higher lutein concentrations in peripheral retina but not in macular retina. Among adipose sites, abdominal subcutaneous adipose tissue exhibited the highest lutein level which was 3-fold higher in the supplemented infants. The supplemented formula enhanced carotenoid deposition in several other tissues. In rhesus infants, increased intake of carotenoids from formula enhanced their deposition in serum and numerous tissues and selectively increased lutein in multiple brain regions.

Jeon S et al. Nutrients 9, 51-65 (2017) doi:10.3390/nu9010051

Maternal serum retinol and β-carotene concentrations and neonatal bone mineralization

Studies in older adults and animals have suggested contrasting relations between bone health and different vitamin A compounds. To our knowledge, the associations between maternal vitamin A status and offspring bone development have not previously been elucidated. We examined the associations between maternal serum retinol and β-carotene concentrations during late pregnancy and offspring bone mineralization assessed at birth with the use of dual-energy X-ray absorptiometry. In the Southampton Women's Survey mother-offspring birth cohort, maternal health, lifestyle, and diet were assessed prepregnancy and at 11 and 34 wk of gestation. In late pregnancy, maternal serum retinol and β-carotene concentrations were measured. Offspring total body bone mineral density (BMD), bone mineral content (BMC), and bone area (BA) were measured within 2 wk after birth. In total, 520 and 446 mother-offspring pairs had measurements of maternal serum retinol and β-carotene, respectively. Higher maternal serum retinol in late pregnancy was associated with lower offspring total body BMC (b = 20.10 SD/SD; 95% CI: 20.19, 20.02; P = 0.020) and BA (b = 20.12 SD/SD; 95% CI: 20.20, 20.03; P = 0.009) but not BMD. Conversely, higher maternal serum β-carotene concentrations in late pregnancy were associated with greater total body BMC (b = 0.12 SD/SD; 95% CI: 0.02, 0.21; P = 0.016) and BA (b = 0.12 SD/SD; 95% CI: 0.03, 0.22; P = 0.010) but not BMD. Maternal serum retinol and β-carotene concentrations had differing associations with offspring bone size and growth at birth: retinol was negatively associated with these measurements, whereas β-carotene was positively associated. These findings highlight the need for further investigation of the effects of maternal retinol and carotenoid status on offspring bone development.

Händel MN et al. Am J Clin Nutr 104:1183-38(2016)

Marginal vitamin A deficiency facilitates Alzheimer's pathogenesis

Deposition of amyloid β protein (Aβ) to form neuritic plaques in the brain is the unique pathological hallmark of Alzheimer's disease (AD). Aβ is derived from amyloid β precursor protein (APP) by β- and γ-secretase cleavages and turned over by glia in the central nervous system (CNS). Vitamin A deficiency (VAD) has been shown to affect cognitive functions. Marginal vitamin A deficiency (MVAD) is a serious and widespread public health problem among pregnant women and children in developing countries. However, the role of MVAD in the

pathogenesis of AD remains elusive. Our study showed that MVAD is approximately twofold more prevalent than VAD in the elderly, and increased cognitive decline is positively correlated with lower VA levels. We found that MVAD, mostly prenatal MVAD, promotes beta-site APP cleaving enzyme 1 (BACE1)-mediated Aβ production and neuritic plaque formation, and significantly exacerbates memory deficits in AD model mice. Supplementing a therapeutic dose of VA rescued the MVAD-induced memory deficits. Taken together, our study demonstrates that MVAD facilitates AD pathogenesis and VA supplementation improves cognitive deficits. These results suggest that VA supplementation might be a potential approach for AD prevention and treatment.

Zeng J et al. Acta Neuropathol (1/27/2017) doi: 10.1007/s00401-017-1669-y

Common SNP rs6564851 in the BCO1 gene affects the circulating levels of β-carotene and the intake of carotenoids

The circulating levels of β-carotene are modulated not only by sex, but also by autosomal gene variations and fruit intake. The aim of this study was to investigate the interactions between β-carotene metabolism-related gene single nucleotide polymorphisms (SNPs; genetic factors) and nutrient intake (environmental factors) relating to their effects on circulating β-carotene. The serum concentrations of β-carotene and the habitual food intake of 92 healthy Japanese women were examined. All subjects were genotyped for three common SNPs: rs6564851 in the β-carotene 15,150-oxygenase 1 (BCO1) gene, rs2278986 in the scavenger receptor class B member 1 (SCARB1) gene and rs362090 in the intestine-specific homeobox (ISX) gene. Univariate analysis revealed that the circulating β-carotene levels were significantly higher in rs6564851 GG homozygotes (p=0.003). Additionally, the daily intake of β-cryptoxanthin was positively associated with the circulating β-carotene levels in female GG homozygotes of rs6564851 (p=0.023), and the daily intake of α- and β-carotenes, and β-cryptoxanthin was significantly lower in female rs6564851 T allele carries than in female GG homozygotes (p = 0.009, 0.008, 0.009, respectively). The present study apparently indicates that higher circulating β-carotene levels in female rs6564851 GG homozygotes depend on carotenoid intake.

Yabuta S et al, PLOS ONE doi:10.1371/journal.pone.0168857 (12/22/2016)

Bioaccessibility and intestinal cell uptake of astaxanthin from salmon and supplements

Although the keto-carotenoid astaxanthin (Ast) is not typically present in human plasma due to its relative scarcity in the typical diet, global consumption of salmon, the primary source of Ast in food, and Ast supplements continues to increase. The first objective of the present study was to investigate the bioaccessibility of Ast from uncooked and cooked fillets of wild and aquacultured salmon, Ast-supplements and krill oil, during simulated gastric and small intestinal digestion. Uptake of *E*-Ast from micelles generated during digestion of wild salmon by monolayers of Caco-2 was also monitored. Both wild and aquacultured salmon flesh contained *E*-Ast and *Z*-isomers of unesterified Ast, whereas Ast esters were the predominant form of the carotenoid in commercial supplements and krill oil. Flesh from wild salmon contained approximately 10 times more Ast than aquacultured salmon. Common styles of cooking flesh from wild and aquacultured salmon decreased Ast content by 48–57% and 35–47%, respectively. Ast in salmon flesh, supplements and krill oil was relatively stable (>80% recovery) during *in vitro* digestion. The efficiency of transfer of Ast into mixed micelles during digestion of uncooked wild salmon was 43%, but only 12% for uncooked acquacultured salmon. Cooking wild salmon significantly decreased Ast bioaccessibility. The relative bioaccessibility of Ast (41–67%) after digestion of oil vehicle in commercial supplements was inversely proportional to carotenoid content (3 – 10 mg/capsule), while bioaccessibility of endogenous Ast in phospholipid-rich krill oil supplement was 68%. More than 95% of Ast in mixed micelles generated during digestion of supplements and krill oil was unesterified. Caco-2 intestinal cells accumulated 11–14% of *E*-Ast delivered in mixed micelles generated from digested wild salmon. Apical uptake and basolateral secretion of *E*-Ast by Caco-2 cells grown on inserts were greater after digestion of Ast-enriched krill oil compared to uncooked wild salmon. These data suggest that the bioacessibility of Ast in wild salmon and soft-gel capsules is greater than that in aquacultured salmon, and that uptake and basolateral secretion of the carotenoid by enterocyte-like cells is enhanced by the digestion products of phospholipid-rich krill oil.

Chitchumroonchokchai C, Failla ML. Food Res Int (in press) doi.org/10.1016/j.foodres.2016.10.010

Contribution of the first two enzymes of the MEP pathway to carotenoid and chlorophyll biosynthesis in carrot

Carotenoids and chlorophylls are photosynthetic pigments synthesized in plastids from metabolic precursors provided by the methylerythritol 4-phosphate (MEP) pathway. The first two steps in the MEP pathway are catalyzed by the deoxyxylulose 5-phosphate synthase (DXS) and reductoisomerase (DXR) enzymes. While DXS has been recently shown to be the main flux-controlling step of the MEP pathway, both DXS and DXR enzymes have been proven to be able to promote an increase in MEP-derived products when overproduced in diverse plant systems. Carrot (*Daucus carota*) produces photosynthetic pigments (carotenoids and chlorophylls) in leaves and in light-exposed roots, whereas only carotenoids (mainly α- and β-carotene) accumulate in the storage root in darkness. To evaluate whether DXS and DXR activities influence the production of carotenoids and chlorophylls in carrot leaves and roots, the corresponding *Arabidopsis thaliana* genes were constitutively expressed in transgenic carrot plants. Our results suggest that DXS is limiting for the production of both carotenoids and chlorophylls in roots and leaves, whereas the regulatory role of DXR appeared to be minor. Interestingly, increased levels of DXS (but not of DXR) resulted in higher transcript abundance of endogenous carrot genes encoding phytoene synthase, the main rate-determining enzyme of the carotenoid pathway. These results support a central role for DXS on modulating the production of MEP-derived precursors to synthesize carotenoids and chlorophylls in carrot, confirming the pivotal relevance of this enzyme to engineer healthier, carotenoid-enriched products.

Simpson K et al. Front. Plant Sci (8/31/2016) doi.org/10.3389/fpls.2016.01344

Identification of plastoglobules as a site of carotenoid cleavage in plants

Carotenoids play an essential role in light harvesting and protection from excess light. During chloroplast senescence carotenoids are released from their binding proteins and are eventually metabolized. Carotenoid cleavage dioxygenase 4 (CCD4) is involved in carotenoid breakdown in senescing leaf and desiccating seed, and is part of the proteome of plastoglobules (PG), which are thylakoid-associated lipid droplets in chloroplasts. Here, we demonstrate that CCD4 is functionally active in PG. Leaves of *Arabidopsis thaliana ccd4* mutants constitutively expressing CCD4 fused to yellow fluorescent protein showed strong fluorescence in PG and reduced carotenoid levels upon dark-induced senescence. Lipidome-wide analysis indicated that β-carotene, lutein, and violaxanthin were the principle substrates of CCD4 *in vivo* and were cleaved in senescing chloroplasts. Moreover, carotenoids were shown to accumulate in PG of *ccd4* mutant plants during senescence, indicating translocation of carotenoids to PG prior to degradation.

Rottet S et al. Front Plant Sci, (12/8/2016) doi.org/10.3389/fpls.2016.01855

Nutritionally important pigments in purslane (*Portulaca oleracea*)

Purslane (*Portulaca oleracea*) is a succulent weedy annual in much of the United States. In other parts of the world purslane is grown as a specialty crop, valued for its nutritional quality and contributes carotenoid phytochemicals in the typical Mediterranean diet. Nitrogen (N) influences plant growth and alters pigment composition and accumulation. The objective of this study was to evaluate the influence of N fertility levels on biomass and concentrations of nutritionally important carotenoid and chlorophyll pigments in purslane. Green Leaf and Golden Leaf purslane cultivars were grown in nutrient solution culture at N concentrations from 13 to 105 mg/L. Plants were harvested at 45 days after planting, and measured for concentrations of shoot pigments using HPLC. Concentrations of lutein, neoxanthin, violaxanthin, chlorophyll *b*, total xanthophyll cycle pigments, and the chlorophyll *a* to *b* ratio differed between the purslane cultivars. There was no effect of N treatment concentration on purslane shoot tissue fresh weight (FW) accumulation. Nitrogen treatment significantly influenced shoot tissue β-carotene, lutein, neoxanthin, total carotenoids, chlorophyll *a*, chlorophyll *b*, total chlorophyll, and the chlorophyll *a* to *b* ratio in only Green Leaf purslane shoot tissues. Raising nitrogen concentrations from 13 mg to 105 mg/L increased chlorophyll *a* by 110%, chlorophyll *b* by 49%, total carotenoids by 45% (to 30 mg/100g FW) in this cultivar. Therefore, N fertility management and cultivar selection should be considered when producing purslane as a nutritious specialty vegetable crop.

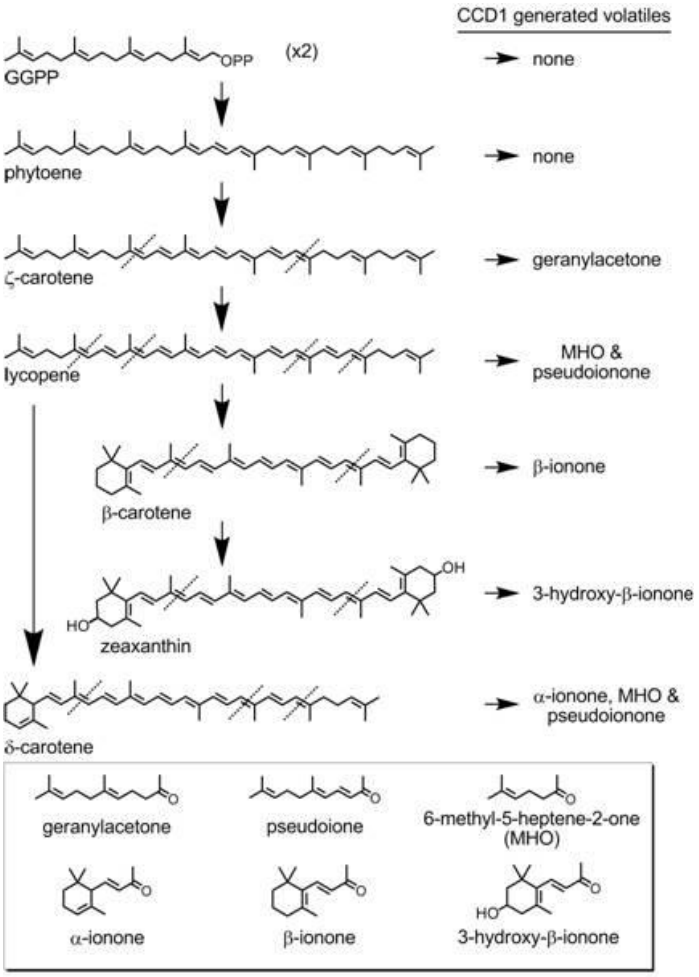
Kopsell DA et al. Hort Science 51: 784-87 (2016)

Tomato flavor

Prof. Harry Klee (Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL) is looking for lost flavor in tomatoes. To address the question of why modern tomato cultivars lack the flavor of older varieties (heirlooms), his team examined the flavor-associated volatiles of 48 modern cultivars vs 236 older varieties, combining tasting panels with chemical and genomic analyses. While several hundred volatiles have been identified in tomato, only about 15-20 actually impact our perception of the fruit. This is because most of these compounds fall below the odor threshold. This threshold is determined by both the concentration of the substance and our ability to detect it. Thus, a poorly detected compound, that is present in quite high levels, will not register. Conversely, a substance, to which we are quite sensitive, will be perceived in very low amounts. Odor thresholds vary markedly between individuals and can be greatly influenced by the way in which the volatile is presented. Several of these volatiles, ß-ionone, ß-damascenone and 6-methyl-5-heptene-2-one (MHO), are produced by oxidative cleavage of carotenoids. Additional carotenoid-derived volatiles just below this threshold include geranyl acetone and pseudoionone. Florida researchers have isolated a pair of closely related tomato carotenoid cleavage dioxygenases (CCDs), that cleave a wide range of carotenoid substrates at the 9,10 and 9',10' positions to release a variety of volatile compounds that are essential components of tomato flavor.

A total of 13 flavor-associated volatiles were significantly reduced in modern varieties relative to heirloom varieties Thus, poor flavor of modern varieties can largely be attributed to the dilution of important volatiles that should be correctable by reintroducing superior alleles of genes controlling their synthesis.

Tieman D et al. Science 355 (6323): 391-94(2017)



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