Nourishing Better Vision: The ARVO 2021 Mildred Weisenfeld Award Lecture

Paul S. Bernstein
Moran Eye Center, University of Utah, Salt Lake City, Utah, United States

Correspondence: Paul S. Bernstein, Moran Eye Center, University of Utah, 65 Mario Capecchi Drive, Salt Lake City, UT 84132, USA; paul.bernstein@hsc.utah.edu.

Citation: Bernstein PS. Nourishing better vision: The ARVO 2021 Mildred Weisenfeld Award Lecture. Invest Ophthalmol Vis Sci. 2022;63(3):13. https://doi.org/10.1167/iovs.63.3.13

I would like to thank the ARVO Awards Committee and the ARVO Board of Trustees for selecting me to deliver this year’s Mildred Weisenfeld Award Lecture. As a long-time member who attended my ARVO first meeting in the mid-1980s when it was still in Sarasota, I am honored to provide an overview of my career in clinical and basic science vision research with a focus on nutrition and the eye. ARVO is the meeting that I look forward to the most every year, as it is the premier place to present the latest and greatest research work in vision in a casual and unpretentious atmosphere where I am always certain to meet up with mentors, students, and colleagues who have contributed to the work that I will present today. My service on the ARVO Program Planning Committee, as the Biochemistry Section’s representative on the ARVO Board of Trustees, and as an ARVO vice-president, revealed the inner workings of an extraordinarily well-run professional organization that prioritizes nurturing the careers of rising young vision researchers. Unfortunately, coronavirus disease 2019 (COVID-19) forced the cancellation of ARVO 2020, and ARVO 2021 had to be held online only. Thus, I had to deliver this year’s Weisenfeld lecture pre-recorded, with 10 minutes of live questions moderated by Dr. Michael Redmond of the National Eye Institute. Approximately 1000 participants viewed this lecture during its appointed time slot, and presumably many more have watched on-demand since then. I look forward to seeing many of you in person again in Denver next year.

Mildred Weisenfeld was born in Brooklyn, New York in 1921. She lost vision as a teenager due to retinitis pigmentosa, but she continued her education and became an energetic advocate for vision research, ultimately becoming the founder and Executive Director of Fight for Sight. I received my first competitive research grant award from Fight for Sight in 1993, so I am grateful to be able to publicly acknowledge her help in getting my career off to a good start.

As a superstudent who is genetically predisposed to vehemently reject numerous “eye healthy” dark green cruciferous vegetables, such as broccoli, Brussel sprouts, and kale, as bitter and foul tasting, I am periodically asked why I am so passionate about ocular nutrition. First, of course, I do not avoid all vegetables, just the cruciferous one, and I am an avid fruit grower and consumer. Second, and more importantly, I have learned from my mentors and patients that nutrients that are naturally part of our daily diet can be an efficient and rational way to promote eye health and prevent or delay a variety of blinding disorders. From a basic and clinical science standpoint, my laboratory group and others have shown that underlying causes of many eye diseases logically point to nutritional mechanisms, and nutritional treatments can often have fewer side effects than pharmaceutical interventions. Patient compliance can be excellent, and eye healthy nutrients are often relatively low cost. These factors have led to strong and sustained patient and clinician interest in ocular nutrition as an empowering way to combat retinal disease.

Through the years, I have developed a core set of principles to guide my research on ocular nutrition. I have repeatedly found it to be important to take cues from the natural world because if the eye is specifically or uniquely enriched with a nutrient, it is likely to have vital biological functions for vision. There also is a clear and logical progression of ocular nutrition research, starting with clinical observations and epidemiology to generate testable hypotheses. This is followed by laboratory-based biochemistry, physiology, and animal studies. Ultimately, it is desirable to do randomized, controlled human trials to provide reliable evidence and recommendations for clinicians to provide to their patients. This has been done for a handful of nutrients, such as the AREDS2 formulation, but these studies can be quite large, long, expensive, and challenging when dealing with free-living humans eating varied diets. The goal throughout my career has always been to provide the ophthalmology community with the best quality basic and clinical science to guide clinicians and the nutraceutical industry when they advise patients who are eager to know how they can take charge and slow, prevent, or even reverse their retinal diseases using readily available dietary or nutritional supplement interventions. In this lecture, I will focus on three key ocular-enriched nutrients: retinoids, carotenoids, and very-long-chain polyunsaturated fatty acids.

MY FIRST FORAY INTO OCULAR NUTRITION RESEARCH: THE RETINOIDS

As a newly entering MD-PhD student at Harvard Medical School in 1981, I had no preconceived notions about what specialty or research path I would pursue beyond a strong desire that I wanted to work with the best organic chemist that I could find. This led me to the laboratory of Robert R. Rando, PhD, of the Department of Pharmacology.
He outlined a project studying vitamin A metabolism in the vertebrate eye that seemed like a good fit with my organic chemistry background. It would allow me to gain new analytical skills, such as HPLC and live animal work, and it had the potential to have excellent eventual translational relevance.

It has been known since antiquity that night blindness can be treated nutritionally by eating foods such as liver or certain fruits and vegetables, but it was not until the 19th and 20th centuries that various chemists identified all-trans-retinol (vitamin A) and its metabolic precursor β-carotene as the active compounds in these foods. In the 1930s and 1940s, Dr. George Wald identified a non-dieta

My first step was to try the various unsuccessful published isomerase assays to confirm that no production of 11-cis-retinoids was detectable using the then relatively new technique of HPLC, and I found that this was indeed the situation. Next, my advisor and I devised a series of experiments to try to determine the likely substrate(s) and product(s) of the isomerase reaction in the living frog and rat eye. I focused on two known inhibitors of the visual cycle including dianiminoxypentane (DAPP, an aromatic amine)⁴ and 4-methyl pyrazole (4-MP; an alcohol dehydrogenase inhibitor)⁵ that cause night blindness in humans. I was able to show that DAPP and a whole series of related hydrophobic aromatic amines induce a short circuit in the visual cycle by forming Schiff bases with free 11-cis-retinal and catalyzing the thermodynamically favored isomerization back to all-trans-retinol and that 4-MP primarily disrupts the visual cycle by inhibiting the oxidation of 11-cis-retinol to 11-cis-retinal.⁶,⁷ I then used these two inhibitors in conjunction with radiotracers studies with intravitreally injected all-trans-retinol double-labeled with ⁳H and ¹⁴C at the C15 position to definitively show that in vivo isomerization of all-trans- to 11-cis retinoids must occur at the alcohol oxidation state.⁸ In other words, all-trans-retinol could not be the direct substrate for a putative retinoid isomerase.

Armed with knowledge that in vivo isomerization must use either all-trans-retinol or all-trans-retinol esterified with long-chain fatty acids as the substrate, I then revisited in vitro isomerase assays. I chose to focus initially on all-trans-retinol labeled with ⁳H at the 11 and 12 positions because it was commercially available in high purity and high specific activity as opposed to the esters, which would require additional chemical modification and repurification. I noted that previously published assays used disturbingly high levels of ethanol ranging between 1 and 10% to solubilize retinoid substrates. These high ethanol concentrations could potentially denature proteins and destroy enzymatic activity, so I reduced the ethanol level to 0.1% and added 0.5% bovine serum albumin (BSA) to solubilize hydrophobic retinoids without danger of denaturation. Using these conditions with crude homogenates of frog RPE membranes, I could readily detect the time-dependent formation of radioactive 11-cis-retinol in the dark.⁹ This activity was absent from retinal homogenates and from the soluble proteins in the RPE and could be destroyed by boiling and 1% ethanol.¹⁰ Subsequent experiments by other members of the Rando laboratory showed that all-trans-retinyl palmitate was the actual substrate for the isomerization reaction and that what I

---

**Figure 1.** Knowledge of the retinoid visual cycle in the 1980s (A) and today (B). 4-MP, 4-methyl pyrazole.
was measuring was a coupled reaction with an endogenous esterification enzyme lecithin retinol acyl transferase (LRAT) that generated all-trans-retinyl palmitate in situ from the originally added tritiated all-trans-retinol.\textsuperscript{11} Thus, the thermodynamically unfavored all-trans- to 11-cis isomerization reaction was actually an energy-coupled isomerohydrolase activity using retinyl ester hydrolysis as the energy source (Fig 1B).

Nearly 2 decades later, the notoriously fastidious retinol isomerohydrolase was identified in other laboratories as RPE65,\textsuperscript{12} the target of the first US Food and Drug Administration (FDA)-approved gene therapy for an inherited retinal disease. Only three of my patients at the Moran Eye Center have had bi-allelic mutations in RPE65, and the first two of them were already at the end stage of the disease and not eligible for treatment. More recently, one of my patients was identified in the much earlier stages of Leber congenital amaurosis and was successfully treated with Luxturna, so it was very gratifying to see my very basic research performed in the 1980s eventually translated to clinical practice more than 3 decades later.

**Establishing My Own Nutritional Research Program: The Macular Carotenoids**

As I transitioned from my post-doctoral and fellowship training to a faculty position, I needed to develop my own independent research program. In keeping with the core principles of ocular nutrition research outlined above, I wanted to focus on another class of nutrients besides the retinoids that was also ocular enriched and likely to perform a vital biological function. While assisting and performing vitrectomies as a resident and fellow, I had become fascinated by the yellow pigment of the human macula that was a reliable marker of the fovea even in a chronically detached retina. I delved through the published literature and found that Wald had concluded in the 1940s that the macular pigment was likely to be a xanthophyll carotenoid, based on its chemical properties and absorption spectrum,\textsuperscript{13,14} and more recently Bone and Landrum had identified the components as dietary lutein and zeaxanthin and a related non-dietary carotenoid, \textit{meso}-zeaxanthin.\textsuperscript{15}

Snodderly had elegantly shown the cross-sectional localization of the macular pigment to the Henle fiber layer of the monkey inner retina,\textsuperscript{16,17} and several research groups had developed psychophysical methods to measure macular pigment levels in clinical research subjects. Intriguingly, one of my retina fellowship mentors, Dr. Johanna Seddon, had just come out with a manuscript linking high levels of dietary lutein and zeaxanthin consumption with significantly lower risk of advanced age-related macular degeneration (AMD).\textsuperscript{18}

Lutein and zeaxanthin are common constituents of the human diet, especially in dark green leafy vegetables and in various orange and yellow fruits and vegetables. Plants and micro-organisms synthesize these colorful isoprenoid compounds for light harvesting, coloration, and photoprotection, but animals must obtain them from their diets. There are over 700 carotenoids in nature, and humans consume approximately 50 in a typical diet, yet only lutein, zeaxanthin, and \textit{meso}-zeaxanthin are present in the primate macula, whereas most other mammals have little, if any, detectable carotenoids in their eyes.\textsuperscript{19} The concentration of xanthophyll carotenoids at the fovea is extraordinarily high, up to 1 mM, while only millimeters away the concentration drops nearly 100-fold. The high degree of specificity of the macular carotenoids on a species, tissue, and spatial level, coupled with a potential protective role against a common eye disease caught my attention as a fruitful new direction for my ocular nutritional biochemistry research.

As I had learned in the retinoid field, whenever a tissue exhibits a high degree of specificity when accumulating a chemical compound at very high concentration and with long-term stability, it is likely that high-affinity specific binding proteins are involved. Such carotenoid binding proteins were well known in plants, microorganisms, and invertebrates, with the astaxanthin binding protein \textit{β}-crustacyanin of lobster shells a notable example, but in the 1990s nothing was known about carotenoid binding proteins in vertebrates.\textsuperscript{20} Several researchers had speculated that the hydrophobic macular pigment carotenoids were simply dissolved in lipid membranes, but this scenario could not possibly account for the exquisite spatial and chemical specificity of the human macula. While still a post-doctoral fellow in Dr. Rando’s laboratory, I used \textit{14C}-canthaxanthin to photo-affinity label potential carotenoid binding proteins in bovine retina.\textsuperscript{21} We identified tubulin as a carotenoid binding protein, but this protein did not exhibit the degree of chemical and spatial specificity that we were seeking.\textsuperscript{22}

Next, my laboratory used hundreds of human donor maculas and classical protein purification techniques to isolate membrane-associated proteins that co-purified with endogenous macular pigment carotenoids, and we identified GSTP1 as a zeaxanthin and \textit{meso}-zeaxanthin binding protein in 2004;\textsuperscript{23} however, when we tried the same approach to identify the macula’s lutein binding protein, we could confirm that such a protein was present, but we could not purify it sufficiently to identify it. We decided to take a cue from the invertebrate world and searched for homologs to the well-characterized lutein binding protein of silkworms, CBP. We found that an antibody to silkworm CBP labeled the appropriate layer of monkey macula and that a human macular protein, STARD3, shared strong sequence homology to CBP.\textsuperscript{24,25} Ultimately, we were able to show using surface plasmon resonance (SPR) binding assays and immunohistochemistry that GSTP1 and STARD3 fulfilled all of the necessary criteria to be the primary binding proteins for the zeaxanthins and lutein, respectively.\textsuperscript{26}

The origin and role of \textit{meso}-zeaxanthin in the human retina was another major puzzle to be addressed. \textit{Meso}-Zeaxanthin is not a significant constituent of the human diet, yet it accounts for over one-third of the macular pigment and is not found elsewhere in the human body. Johnson and colleagues fed purified lutein or zeaxanthin to carotenoid-deficient monkeys and concluded that lutein was the precursor for \textit{meso}-zeaxanthin,\textsuperscript{27} and we came to the same conclusion when we fed deuterium-labeled lutein or zeaxanthin to Japanese quail.\textsuperscript{28} With this knowledge, we then set out to identify the enzyme responsible for this ocular-specific biochemical reaction. We utilized the developing chicken eye as a potential animal model. It was already known that the yolk of free-range chicken eggs is rich in lutein and zeaxanthin, with no detectable \textit{meso}-zeaxanthin, yet, at hatching, the chicken’s eye has abundant \textit{meso}-zeaxanthin. We performed a developmental study and determined that \textit{meso}-zeaxanthin is never detectable outside of the chicken’s eye, and that it first becomes detectable in the RPE at embryonic day 17 (E17) and then appears in the retina several days later.\textsuperscript{29} We then did an mRNA expression study of the developing chicken RPE to identify likely candidates for
the lutein to \(\textit{meso}\)-zeaxanthin isomerase and concluded that RPE65 was the most promising candidate. Using chicken and human RPE65 expressed in HEK293 cells, we were able to show in 2017 that RPE65 indeed had a second enzymatic activity to make \(\textit{meso}\)-zeaxanthin from lutein in addition to its better known function as the all \(\textit{trans}\)-to \(\textit{11-cis}\)-retinoid isomeroiodhase, and we confirmed this finding when we demonstrated that a specific inhibitor of RPE65’s retinoid isomerohydrolase activity could also shut down \(\textit{meso}\)-zeaxanthin production in the developing chicken eye when injected into the egg at E17 and E19.\(^{30}\)

We and others had noticed that no other mammals accumulate large amounts of carotenoids in their eyes in the same way that humans and fellow nonhuman primates do(3,7) This was an unfortunate situation because it rendered the mouse, a common animal model for environmental and genetic eye research, essentially useless for investigations of carotenoids in retinal health and disease. If we could understand the origin of this problem and bypass it, we might produce a “macular pigment mouse” that would be invaluable for small animal ocular carotenoid research. We initially speculated that lack of a suitable binding protein in their retina precluded uptake into their eye, but when we over-expressed human GSTP1 in the mouse retina under the control of the rhodopsin promoter, we still had no detectable uptake of orally administered zeaxanthin into the retina. We then hypothesized that mice might have unusually active carotenoid cleavage enzymes relative to humans and monkeys that would break down dietary carotenoids before they can be delivered to the retina. We obtained mice deficient in the major xanthophyll cleavage enzyme, Bco2, and found that indeed they could accumulate orally administered lutein and zeaxanthin into the retina at levels comparable to the human peripheral retina, whereas wild-type mice had no detectable levels.\(^{31-32}\) We have subsequently used these \(\textit{Bco2}\)−/− mice to show that supplemental carotenoids enhance visual function as assessed by ERG and optokinetic response and that supplemental \(\textit{zeaxanthin} + \textit{meso}-\text{zeaxanthin}:\text{lutein}\) ratio of 9:1 at the fovea (Fig. 2).\(^{40}\) Directing a blue laser at the palm of the hand or the sole of the foot has proven to be a powerful, validated method to rapidly assess systemic carotenoid status noninvasively in adults, children, and newborns.\(^{41-45}\) This technique was licensed by NuSkin/Pharmanex as the basis for their BioPhotonic Scanner, which they successfully used to encourage nutritional supplement sales, and research grade versions of skin Raman scanners and related skin reflectance scanners have been enthusiastically embraced by nutrition researchers for numerous epidemiological and interventional studies.

I have been pleased to be actively involved in numerous single-center and multicenter clinical studies of carotenoid function and therapeutics through the years. Highlights included being a local principal investigator and writing committee member for the AREDS2 study which showed that 10 mg/day of lutein and 2 mg/day of zeaxanthin were a safe and effective alternative to the \(\beta\)-carotene of the original AREDS study.\(^{46,47}\) My group also conducted a zeaxanthin intervention trial to try to correct the anomalous ring-shaped redistribution of macular pigment in subjects with macular telangiectasia type 2 (MacTel), which showed that we simply enhanced the ring without filling it in, suggesting that macular pigment binding proteins may be absent in the fovea of patients with MacTel.\(^{48}\) More recently, we have begun to focus on the role of the macular pigment carotenoids early in life when they may play a role in promoting foveal development and enhancement of visual and cognitive function.\(^{49}\) We were able to show using blue light reflectance measurements with the RetCam that macular pigment is detectable at birth and steadily rises over the first 7 years of life and that maternal serum and skin carotenoid levels are excellent predictors of their infants’ ocular and systemic carotenoid status at birth, consistent with the well-established trans-placental maternal to infant carotenoid transfer that occurs during the third trimester of pregnancy.\(^{50,51}\) Based on these results, we are currently conducting a National Institutes of Health (NIH)-sponsored randomized, placebo-controlled, single-site clinical trial of 10 mg/day of lutein and 2 mg/day of zeaxanthin added to standard-of-care prenatal vitamins (the L-ZIP study) to determine if the active intervention can combat pregnancy-induced depletion of maternal carotenoids and enhance the infant’s ocular and systemic carotenoid status and foveal
FIGURE 2. Comparison of images of the macular pigment carotenoids in monkey and human foveal sections using blue light absorption or confocal resonance Raman spectroscopy (RRS). (A) A cross section of a monkey’s fovea shows that the yellow macular pigment appears dark when illuminated with blue light. This image was originally prepared by D. Max Snodderly, PhD, in 1984 and is reprinted with permission from Webvision (http://webvision.med.utah.edu/). (B) An RRS image of total macular carotenoids (lutein + zeaxanthin + meso-zeaxanthin) is overlaid on a light microscopic image of the human fovea that provided the RRS image. (C) An RRS intensity map of human foveal zeaxanthin (zeaxanthin + meso-zeaxanthin) generated using Classical Least Squares (CLS) fitting demonstrates that zeaxanthin + meso-zeaxanthin accounts for the vast majority of the foveal macular pigment. (D) An RRS intensity map of lutein generated using CLS fitting shows that lutein is diffusely distributed across the human fovea at much lower concentrations relative to zeaxanthin + meso-zeaxanthin. For details on how the RRS images were prepared, see Reference 40. B–D Reprinted from Li B, George EW, Rognon GT, et al. Imaging lutein and zeaxanthin in the human retina with confocal resonance Raman microscopy. Proc Natl Acad Sci USA. 2020;117(22):12352–12358. (E) Chemical structures of the three major xanthophyll carotenoids of the primate fovea. GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; OS, outer segments; RPE, retinal pigment epithelium.

structure at birth.52 We recently completed enrollment of 47 subjects and expect to report our findings within a year.

A NEW FRONTIER OF OCULAR NUTRITION RESEARCH: THE VERY-LONG-CHAIN POLYUNSATURATED FATTY ACIDS

Among the reasons that I chose to join the faculty of the Moran Eye Center of the University of Utah was the opportunity to study inherited retinal diseases in a region of the country with unrivaled access to large, close-knit families and comprehensive genealogical databases. Early on in my career, I came upon a large Utah family with a diverse-appearing maculopathy reminiscent of Stargardt disease and pattern dystrophy that was inherited in an apparent autosomal dominant pattern. In collaboration with geneticists Dr. Mark Leppert of the University of Utah and Dr. Rando Allikmets of Columbia University, we identified two non-contiguous one base-pair deletions in the ELOVL4 gene that corresponded closely with a five base-pair deletion in ELOVL4 that had been recently identified by Dr. Kang Zhang and colleagues as causative for Stargardt-3 disease (STGD3).53,54 Interestingly, ELOVLs in yeast were known to be the enzymes responsible for elongation of fatty acids, and it was soon demonstrated that mammalian ELOVL4 is the enzyme required to synthesize very-long-chain polyunsaturated fatty acids (VLC-PUFAs), rare non-dietary lipids found exclusively in the retina, RPE, and testes.55 Human ELOVL4 is the primary enzyme for production of C28-C38 VLC-PUFAs in the mammalian eye from dietary precursors, such as the LC-PUFAs eicosapentaenoic acid (EPA; 20:5 n-3), docosapentaenoic acid (DPA; 22:5 n-3), and arachidonic acid (AA; 20:4 n-6), and to a lesser extent docosahexaenoic acid (DHA; 22:6 n-3; Fig. 3A).56 Using biomarkers of dietary lipid intake, we were able to show that phenotypic severity in our large Utah family could be correlated with consumption of fish rich in omega-3 LC-PUFAs that could serve as precursors for retinal VLC-PUFAs.57

With the discovery of the genetic defect underlying STGD3, there was enhanced interest in learning about the role of VLC-PUFAs in retinal health and disease. Although the presence of VLC-PUFAs in the retina had been known for decades,58,59 scant attention had been paid to them because they were low abundance lipids that were difficult to analyze and not commercially available. Whereas other laboratories strove to develop mouse models for VLC-PUFA deficiency, these models proved to be problematic because complete knockout of ELOVL4 turned out to be neonatal lethal due to loss of ELOVL4’s other physiological function, the production of very-long-chain saturated fatty acids (VLC-SFAs) in the dermis, lipids essential to prevent catastrophic drying of the skin in an air-exposed environment.60,61 Thus, to study the role of VLC-PUFAs in mouse models, various knock-in and conditional knockout approaches had to be implemented, which proved to be less than ideal due to incomplete deletion of VLC-PUFAs and late-onset subtle ocular pathology.62–64
My laboratory initially chose to focus on improving VLC-PUFA analysis. We developed optimized gas chromatography mass spectrometry (GC-MS) protocols to measure VLC-PUFAs on very small tissue samples, such as pairs of mouse eyes and small punches of human retina. We then applied these techniques to human donor eyes and learned that VLC-PUFA levels in normal eyes peak in middle age and that AMD donor eyes have significantly lower levels of VLC-PUFAs and lower n-3/n-6 ratios relative to age-matched control eyes. We were also able to show that retinal VLC-PUFA and n-3/n-6 ratios were correlated with systemic biomarkers of dietary intake of VLC-PUFA precursors. This suggested that AMD could be due in part to age-related ELOVL4 dysfunction and/or inadequate intake of VLC-PUFA precursors and that interventions to increase retinal VLC-PUFA levels might be protective against AMD.

We were therefore interested in determining whether or not we could raise retinal levels of VLC-PUFA through direct supplementation, but VLC-PUFAs were reputed to be very difficult to synthesize and were not commercially available in more than milligram quantities. Fortunately, I was able to establish a collaboration with a University of Utah synthetic organic chemist, Dr. Jon Rainier, who developed a protocol to synthesize a high-purity VLC-PUFA (32:6 n-3) in quantities approaching one gram (see Fig. 3B). With this lead compound in hand, we were able to show in wild-type and Elolv4 conditional knockout mice that this orally administered VLC-PUFA was rapidly taken up from the gut and was specifically targeted for delivery to the retina and RPE. After 15 days of supplementation, visual function as measured by electroretinography and optokinetic response improved as well. Going forward, we are planning to expand our VLC-PUFA supplementation studies using synthetic n-3 and n-6 VLC-PUFAs longer and shorter than our lead 32:6 n-3 compound in additional animal and cell culture models of VLC-PUFA dysfunction. Ultimately, our goal is to scale-up production of VLC-PUFAs to make enough material to conduct human clinical trials for AMD prevention.

CONCLUSIONS

Looking back on my nearly 4 decades of work in ocular nutrition research, I am pleased with the progress that has been made. Purely academic questions directed at how and why ocular-enriched nutrients and their metabolites, such as retinoids, carotenoids, and VLC-PUFAs, function in retinal health and disease are being answered in a scientifically
rugged manner, and clinical trials designed to alter retinal levels of these compounds to improve ocular health may have been conducted for retinoids and carotenoids and may even
tually be done for VLC-PUFAs as well. In my clinical practice, I am gratified by the interest and motivation of my patients to incorporate better nutrition through diet or supplements into their treatment regimen. I particularly enjoy speaking with the children of my patients with AMD (the “worried well”) of the value of improving nutritional habits now to lessen their risk of visual loss later in life, and I am looking forward to reporting results of our studies expanding ocular nutrition interventions to the prenatal realm. I continually learn from my patients as they inform me about the latest and greatest dietary supplements that they have heard about on the internet or from friends and family, but I caution them that rigorous scientific support for many of these claims may not yet exist. Ultimately, I still emphasize to them the importance of a sound whole-foods diet rich in colorful fruits and vegetables and healthy unsaturated fats as the primary intervention against ocular disease throughout the lifespan.

Acknowledgments

Supported by NIH grants EY11600, EY14800, and EY029857; Foundation Fighting Blindness; Research to Prevent Blindness.

Disclosure: P.S. Bernstein, Tesseract (C), NuSkin/Pharmanex (R)

References

1. Wald G. Molecular basis of visual excitation. *Science*. 1968;162:230–239.
2. Dowling JE. Chemistry of visual adaptation in the rat. *Nature*. 1960;188:114–118.
3. Rando RR, Chang A. Studies on the catalyzed interconversions of vitamin A derivatives. *J Am Chem Soc*. 1983;105:2879–2882.
4. Bernstein PS, Rando RR. The specific inhibition of 11-cisretinyl palmitate formation in the frog eye by diaminopimelate, an inhibitor of rhodopsin regeneration. *Vis Res*. 1985;25:741–748.
5. Jurgensemeier C, Bhosale P, Bernstein PS. Evaluation of 4-methylpyrazole as a potential therapeutic dark adaptation inhibitor. *Curr Eye Res*. 2007;32:911–915.
6. Bernstein PS, Fulton BS, Rando RR. Mechanism of action of aromatic amines that short-circuit the visual cycle. *Biochemistry*. 1986;25:3370–3377.
7. Bernstein PS, Lichtman JR, Rando RR. Short-circuiting the visual cycle with retinotopic aromatic amines. *Proc Natl Acad Sci USA*. 1986;83:1632–1635.
8. Bernstein PS, Rando RR. In vivo isomerization of all-trans-11-cis-retinoids in the eye occurs at the alcohol oxidation state. *Biochemistry*. 1986;25:6473–6478.
9. Bernstein PS, Law WC, Rando RR. Isomerization of all-trans-retinoids to 11-cis-retinoids in vitro. *Proc Natl Acad Sci USA*. 1987;84:1849–1853.
10. Bernstein PS, Law WC, Rando RR. Biochemical characterization of the retinoid isomerase system of the eye. *J Biol Chem*. 1987;262:16848–16857.
11. Deigner PS, Law WC, Canada FJ, Rando RR. Membranes as the energy source in the endogenous conversion of vitamin A to 11-cis-retinol. *Science*. 1989;244:968–971.
12. Redmond TM, Yu S, Lee E, et al. Rpe65 is necessary for production of 11-cis-vitamin A in the retinal visual cycle. *Nat Genet*. 1998;20:344–351.
13. Wald G. Human vision and the spectrum. *Science*. 1945;101:653–658.
14. Wald G. The photochemistry of vision. *Doc Ophthal mol*. 1949;3:94–137.
15. Bone RA, Landrum JT, Hime GW, Cains A, Zamor J. Stereochemistry of the human macular carotenoids. *Invest Ophthalmol Vis Sci*. 1993;34:2033–2040.
16. Snodderly DM, Auran JD, Delori FC. The macular pigment. II. Spatial distribution in primate retinas. *Invest Ophthalmol Vis Sci*. 1984;25:674–685.
17. Snodderly DM, Brown PK, Delori FC, Auran JD. The macular pigment. I. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. *Invest Ophthalmol Vis Sci*. 1984;25:660–673.
18. Seddon JM, Ajani UA, Sperduto RD, et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. *Eye Disease Case-Control Study Group. JAMA*. 1994;272:1413–1420.
19. Bernstein PS, Li B, Vachali PP, et al. Lutein, zeaxanthin, and meso-zeaxanthin: The basic and clinical science underlying carotenoid-based nutritional interventions against ocular disease. *Prog Retinal Eye Res*. 2016;50:34–66.
20. Bhosale P, Bernstein PS. Vertebrate and invertebrate carotenoid-binding proteins. *Arch Biochem Biophysics*. 2007;458:121–127.
21. Bernstein PS, Choi SY, Ho YC, Rando RR. Photoaffinity labeling of retinoid acid-binding proteins. *Proc Natl Acad Sci USA*. 1995;92:654–658.
22. Bernstein PS, Balashov NA, Tsong ED, Rando RR. Retinal tubulin binds macular carotenoids. *Invest Ophthalmol Vis Sci*. 1997;38:167–175.
23. Bhosale P, Larson AJ, Frederick JM, Southwick K, Thulin CD, Bernstein PS. Identification and characterization of a Pi isoform of glutathione S-transferase (GSTP1) as a zeaxanthin-binding protein in the macula of the human eye. *J Biol Chem*. 2004;279:49447–49454.
24. Bhosale P, Li B, Sharifzadeh M, et al. Purification and partial characterization of a lutein-binding protein from human retina. *Biochemistry*. 2009;48:4798–4807.
25. Li B, Vachali P, Frederick JM, Bernstein PS. Identification of StARD3 as a lutein-binding protein in the macula of the primate retina. *Biochemistry*. 2011;50:2541–2549.
26. Vachali P, Li B, Nelson K, Bernstein PS. Surface plasmon resonance (SPR) studies on the interactions of carotenoids and their binding proteins. *Arch Biochem Biophys*. 2012;519:32–37.
27. Johnson EJ, Neuringer M, Russell RM, Schalch W, Snodderly DM. Nutritional manipulation of primate retinas; III. Effects of lutein or zeaxanthin supplementation on adipose tissue and retina of xanthophyll-free monkeys. *Invest Ophthalmol Vis Sci*. 2005;46:692–702.
28. Bhosale P, Serban B, Zhao DY, Bernstein PS. Identification and metabolic transformations of carotenoids in ocular tissues of the Japanese quail Coturnix japonica. *Biochemistry*. 2007;46:9050–9057.
29. Gorusupudi A, Shyam R, Li B, et al. Developmentally Regulated Production of meso-Zeaxanthin in Chicken Retinal Pigment Epithelium/Choroid and Retina. *Invest Ophthalmol Vis Sci*. 2016;57:1853–1861.
30. Shyam R, Gorusupudi A, Nelson K, Horvath MP, Bernstein PS. RPE65 has an additional function as the lutein to meso-zeaxanthin isomerase in the vertebrate eye. *Proc Natl Acad Sci USA*. 2017;114:10882–10887.
31. Li B, Vachali PP, Gorusupudi A, et al. Inactivity of human β,β-carotene-9’,10’-dioxygenase (BCO2) underlies retinal accumulation of the human macular carotenoid pigment. *Proc Natl Acad Sci USA*. 2014;111:10173–10178.
32. Li B, Vachali PP, Shen Z, et al. Retinal accumulation of zeaxanthin, lutein, and β-carotene in mice deficient in carotenoid cleavage enzymes. *Exp Eye Res.* 2017;159:123–131.

33. Arunkumar R, Gorusupudi A, Li B, et al. Lutein and zeaxanthin reduce A2E and iso-A2E levels and improve visual performance in Abca4(-/-)/Bco2(-/-) double knockout mice. *Exp Eye Res.* 2021;209:108680.

34. Li B, Rognon GT, Mattinson T, et al. Supplementation with macular carotenoids improves visual performance of transgenic mice. *Arch Biochem Biophys.* 2018;649:22–28.

35. Bernstein PS, Delori FC, Richer S, van Kuijk FJ, Wenzel AJ. The value of measurement of macular carotenoid pigment optical densities and distributions in age-related macular degeneration and other retinal disorders. *Vis Res.* 2010;50:716–728.

36. Bernstein PS, Yoshiha MD, Katz NB, McClane RW, Gellermann W. Raman detection of macular carotenoid pigments in intact human retina. *Invest Ophthalmol Vis Sci.* 1998;39:2003–2011.

37. Bernstein PS, Zhao DY, Wintch SW, Ermakov IV, McClane RW, Gellermann W. Resonance Raman measurement of macular carotenoids in normal subjects and in age-related macular degeneration patients. *Ophthalmology.* 2002;109:1780–1787.

38. Sharifzadeh M, Zhao DY, Bernstein PS, Gellermann W. Resonance imaging of macular pigment distributions in the human retina. *J Opt Soc Am A, Optics, Image Sci, and Vis.* 2008;25:947–957.

39. Green-Gomez M, Bernstein PS, Curcio CA, Moran R, Roche W, Nolan JM. Standardizing the Assessment of Macular Pigment Using a Dual-Wavelength Autofluorescence Technique. *Trans Vis Sci Technol.* 2019;8:41.

40. Li B, George EW, Rognon GT, et al. Imaging lutein and zeaxanthin in the human retina with confocal resonance Raman microscopy. *Proc Natl Acad Sci USA.* 2020;117:12352–12358.

41. Conrady CD, Bell JP, Besch BM, et al. Correlations Between Macular, Skin, and Serum Carotenoids. *Invest Ophthalmol Vis Sci.* 2017;58:3616–3627.

42. Ermakov IV, Ermakova MR, Bernstein PS, Chan GM, Gellermann W. Resonance Raman-based skin carotenoid measurements in newborns and infants. *J Biophotonics.* 2013;6:793–802.

43. Mayne ST, Cartmel B, Scarmo S, et al. Noninvasive assessment of dermal carotenoids as a biomarker of fruit and vegetable intake. *Am J Clin Nut.* 2010;92:794–800.

44. Scarmo S, Cartmel B, Lin H, et al. Significant correlations of dermal total carotenoids and dermal lycopene with their respective plasma levels in healthy adults. *Arch Biochem and Biophysics.* 2010;504:34–39.

45. Scarmo S, Heneberry K, Peracchio H, et al. Skin carotenoid status measured by resonance Raman spectroscopy as a biomarker of fruit and vegetable intake in preschool children. *Eur J Clin Nut.* 2012;66:555–560.

46. Bernstein PS, Ahmed F, Liu A, et al. Macular pigment imaging in AREDS2 participants: an ancillary study of AREDS2 subjects enrolled at the Moran Eye Center. *Invest Opthalmol Vis Sci.* 2012;53:6178–6186.

47. Age-Related Eye Disease Study 2 (AREDS2) Research Group, Chew EY, Clemons TE, et al. Secondary analyses of the effects of lutein/zeaxanthin on age-related macular degeneration progression: AREDS2 report No. 3. *JAMA Ophthalmol.* 2014;132:142–149.

48. Choi RY, Gorusupudi A, Wegner K, Sharifzadeh M, Gellermann W, Bernstein PS. Macular pigment distribution responses to high-dose Zeaxanthin supplementation in patients with Macular Telangiectasia Type 2. *Retina (Philadelphia, Pa).* 2017;37:2238–2247.

49. Bernstein PS, Arunkumar R. The emerging roles of the macular pigment carotenoids throughout the lifespan and in prenatal supplementation. *J Lipid Res.* 2021;62:100038.

50. Bernstein PS, Sharifzadeh M, Liu A, et al. Blue-light reflectance imaging of macular pigment in infants and children. *Invest Ophthalmol Vis Sci.* 2013;54:4034–4040.

51. Henriksen BS, Chan G, Hoffman RO, et al. Interrelationships between maternal carotenoid status and newborn infant macular pigment optical density and carotenoid status. *Invest Ophthalmol Vis Sci.* 2013;54:5568–5578.

52. Addo EK, Gorusupudi A, Allman S, Bernstein PS. The Lutein and Zeaxanthin in Pregnancy (L-ZIP) study-carotenoid supplementation during pregnancy: ocular and systemic effects-study protocol for a randomized controlled trial. *Trials.* 2021;22:300.

53. Bernstein PS, Tammur J, Singh N, et al. Diverse macular dystrophy phenotype caused by a novel complex mutation in the ELOVL4 gene. *Invest Ophthalmol Vis Sci.* 2001;42:3331–3336.

54. Zhang K, Kniazeva M, Han M, et al. A 5-bp deletion in ELOVL4 is associated with two related forms of autosomal dominant macular dystrophy. *Nat Genet.* 2001;27:89–93.

55. Agbagha MP, Brush RS, Mandal MN, Henry K, Elliott MH, Anderson RE. Role of Stargardt-3 macular dystrophy protein (ELOVL4) in the biosynthesis of very long chain fatty acids. *Proc Natl Acad Sci USA.* 2008;105:12843–12848.

56. Yu M, Benham A, Logan S, et al. ELOVL4 protein preferentially elongates 20:5n3 to very long chain PUFA's over 20:4n6 and 22:6n3. *J Lipid Res.* 2012;53:494–504.

57. Hubbard AF, Askew EW, Singh N, Leppert M, Bernstein PS. Association of adipose and red blood cell lipids with severity of dominant Stargardt macular dystrophy (STGD3) secondary to an ELOVL4 mutation. *Arch Ophthalmol (Chicago, Ill : 1960).* 2006;124:257–263.

58. Aveldaño MI, Sprecher H. Very long chain (C24 to C36) polyenic fatty acids of the n-3 and n-6 series in dipolyunsaturated phosphatidylcholines from bovine retina. *J Biol Chem.* 1987;262:1180–1186.

59. Rotstein NP, Aveldaño MI. Labeling of lipids of retina subcellular fractions by [1-14C]eicosatetraenoate (20:4(n-6)) docosapentaenoate (22:5(n-3)) and docosahexaenoate (22:6(n-3)). *Biobichimica et Biophysica Acta.* 1987;921:221–234.

60. Vastreddy V, Uchida Y, Salem N, Jr., et al. Loss of functional ELOVL4 depletes very long-chain fatty acids (>or =C28) and the unique omega-O-acycleramides in skin leading to neonatal death. *Hum Mol Genet.* 2007;16:471–482.

61. Cameron DJ, Tong Z, Yang Z, et al. Essential role of ELOVL4 in very long chain fatty acid synthesis, skin permeability barrier function, and neonatal survival. *Int J Biol Sci.* 2007;3:111–119.

62. Barabas P, Liu A, Xing W, et al. Role of ELOVL4 and very long-chain polyunsaturated fatty acids in mouse models of Stargardt type 3 retinal degeneration. *Proc Natl Acad Sci USA.* 2013;110:5181–5186.

63. Barabas P, Gorusupudi A, Bernstein PS, Kriizaj D. Mouse Models of Stargardt 3 Dominant Macular Degeneration. *Adv Exp Med Biol.* 2016;854:137–143.

64. Bennett LD, Brush RS, Chan M, et al. Effect of reduced retinal VLC-PUFA on rod and cone photoreceptors. *Invest Ophthalmol Vis Sci.* 2014;55:3150–3157.

65. Liu A, Terry R, Lin Y, Nelson K, Bernstein PS. Comprehensive and sensitive quantification of long-chain and very long-chain polyunsaturated fatty acids in small samples of human and mouse retina. *J Chromatogr A.* 2013;1307:191–200.
66. Liu A, Chang J, Lin Y, Shen Z, Bernstein PS. Long-chain and very long-chain polyunsaturated fatty acids in ocular aging and age-related macular degeneration. *J Lipid Res.* 2010;51:3217–3229.

67. Gorusupudi A, Liu A, Hageman GS, Bernstein PS. Associations of human retinal very long-chain polyunsaturated fatty acids with dietary lipid biomarkers. *J Lipid Res.* 2016;57:499–508.

68. Wade A, Rallabandi R, Lucas S, et al. The synthesis of the very long chain polyunsaturated fatty acid (VLC-PUFA) 32:6 n-3. *Org Biomol Chem.* 2021;19:5563–5566.

69. Gorusupudi A, Rallabandi R, Li B, et al. Retinal bioavailability and functional effects of a synthetic very-long-chain polyunsaturated fatty acid in mice. *Proc Natl Acad Sci USA.* 2021;118:e2017739118