FloraGLO® by Kemin: The Pioneers of Lutein Science and Discovery

2010 FLORAGLO SUPPORTS BABY'S EYES

2016 BLUE LIGHT PROTECTION PATENT*

2008 FLORAGLO SUPPORTS COGNITION

2014 FLORAGLO PROMOTES QUALITY OF VISION

2007 FLORAGLO IMPROVES SKIN HYDRATION

1996

FloraGLO® LUTEIN

2004 FLORAGLO INCREASES MACULAR PIGMENT

2013 FLORAGLO USED IN AREDS2

KEMIN

* Roberts, Richard. "Methods of Treating Ocular Disorders." Kemin Industries, Inc., assignee. Patent US9226940 B2. This patent covers three conditions for all ages; presbyopia, hyperopia, and astigmatism.

20 FloraGLO™ 20 YEARS OF LUTEIN EXCELLENCE

FloraGLO® Lutein: The First, The Foundation

FloraGLO® Lutein is naturally-sourced, unesterified lutein with an established reputation for quality, safety and efficacy, and the first lutein brand introduced into the global marketplace. Since then, FloraGLO® revolutionized the supplemental lutein market and provided the foundation for the development and advancement of lutein for human health. At the time of its introduction, researchers were just beginning to recognize that lutein and zeaxanthin, two dietary carotenoids found to selectively accumulate in the retina, played an important role in protecting human vision. At about the same time, Kemin also recognized that supplemental lutein provided a significant opportunity to fill the dietary gap that continues to exist due to low fruit and vegetable intakes[1], and more importantly, confirm the hypothesis that supplemental lutein is important for vision and other aspects of human health.

This year, Kemin and DSM celebrate the 20th anniversary of the introduction of FloraGLO® Lutein into the global marketplace. We would like to take this opportunity to review the critical historical milestones behind the understanding of this important nutrient and highlight key lutein research breakthroughs with FloraGLO®.

More recently, there is also growing evidence and recognition of lutein's key role in skin health, cognition, maternal and infant nutrition, highlighting that there are still areas to explore where lutein may prove to be important. This advancement of lutein science is evidenced by the numerous reviews of lutein over the past two decades[2–16] and with many in the scientific community today recommending lutein as an essential part of an optimal diet.

FloraGLO® Lutein has been used in over 70 human clinical trials, including the largest multi-center trial investigating the role of nutrition in supporting human vision, namely the second Age-Related Eye Disease Study (AREDS2).[17] All of this research established the framework that lutein is an essential part of the human diet for not only reducing the risk of disease, but also for maintaining optimal health.

Kemin and DSM are proud to have been at the forefront of lutein research, involved in collaborations that advanced the science and driven the awareness of this important nutrient, which was relatively unknown in 1994. We are extremely grateful to all the many carotenoid researchers over the two decades that have contributed to furthering the science and would like to specifically extend our heartfelt gratitude to the influential researchers who contributed their perspectives on several key discoveries detailed below. And finally, we hope you enjoy this opportunity to take a step back with us for a retrospective view on the story of FloraGLO® and the science behind lutein.

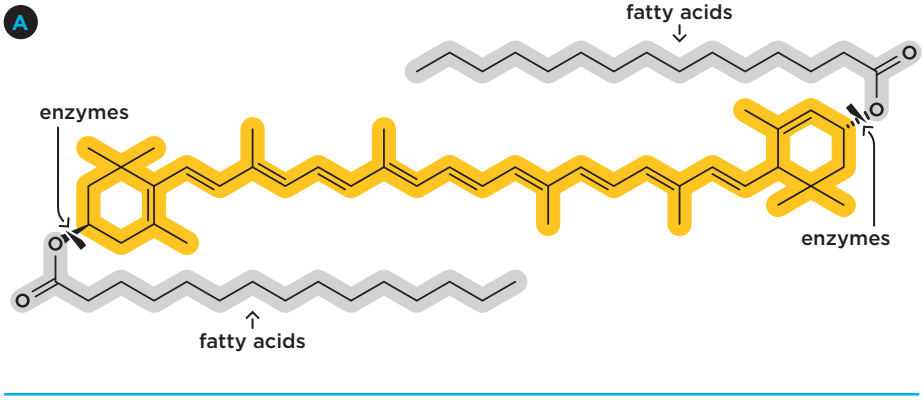


Figure 1: Chemical structure of a lutein ester (A) and lutein (B)

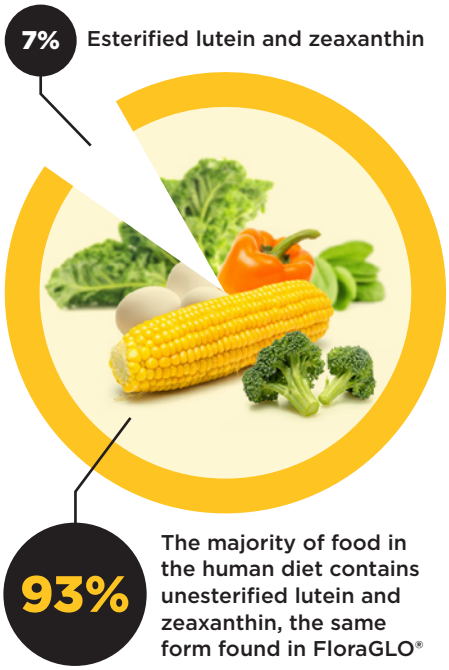


Figure 2: Dietary lutein and zeaxanthin

What are Lutein & Zeaxanthin?

Lutein (CAS 127-40-2) and zeaxanthin (CAS 144-68-3) are carotenoids characterized by the presence of a hydroxyl group on each terminal ring of these molecules. They are found together in nature either esterified to fatty acids (e.g. lutein palmitate) or in an unesterified ("free") form (see Figure 1). They are esterified when found in the flowers or fruits of plants, whereas they are largely present in the free form in certain fruits and vegetables such as spinach and kale. However, lutein and zeaxanthin cannot be synthesized *de novo* in the body and must be acquired through dietary sources.[18]

In their unesterified forms, lutein and zeaxanthin are integral components of plant chloroplasts, and work as accessory pigments in the photosynthetic apparatus to provide antioxidative photoprotection under conditions of high light intensity.[11]

Lutein and zeaxanthin esters are primarily found in the flowers or fruits of plants because the esterified forms are more stable against oxidation and are better suited as pigments.[19,20]

The distinction between esterified and unesterified lutein and zeaxanthin is important since 93% of the foods that humans consume contain lutein and zeaxanthin in the unesterified form whereas only 7% contain lutein and zeaxanthin in the esterified form.[21-23] As described in a later section, unesterified lutein and zeaxanthin are more readily absorbed into the body[24] and are found in the human bloodstream as well as organs of the human body.

Where are Lutein & Zeaxanthin Found in the Human Body?

Lutein and zeaxanthin accumulate in several organs of the human body. Although not pro-vitamin A carotenoids, this accumulation is indicative of lutein and zeaxanthin's important physiological role.[25] Lutein and zeaxanthin accumulate in the retina of the eye. They are the only two dietary carotenoids selectively and exclusively deposited in the *macula lutea*, where they are referred to as macular pigment.[26] They are also the only carotenoids reported to be present in eye lens where they have an important functional role.[27] More recently, lutein has also been found to be the predominant carotenoid in the brain of elderly individuals[28] and infants.[29] Lutein and zeaxanthin have also been found in the skin,[30] breast tissue,[31] ovaries[32] and uterus.[33] They are even found in breast milk and neonatal plasma.[34] The recognition of the presence of lutein and zeaxanthin in these additional tissues indicates a potential functional role beyond that of maintaining the health of the eyes.

A Historical Perspective on the Physiological Importance of Lutein & Zeaxanthin

The physiological importance of lutein and zeaxanthin in the eye can be traced back to 1782.[35] The nature of that research into the macular pigment and human vision has been reviewed by Nussbaum.[2] This history includes the discovery by Wald in 1945 that the macular pigment is comprised of carotenoids and the proposal by Kirschfeld in 1982 that the macular pigment plays an important photoprotective role in the eye.[36,37] However, research into the macular pigment accelerated significantly beginning with the findings of Snodderly and co-authors in the early 1980s. They described the localization and distribution of the macular pigment within the primate retina in great detail with sophisticated tissue fixing and sectioning techniques of ocular tissues, as shown in Figure 3.[38,39] That work demonstrated that the macular pigment is localized in front of the photoreceptors in the primate retina, effectively creating an optical filter to absorb the damaging wavelengths of light, specifically blue wavelengths of light (400 nm – 480 nm).

As we consider the implications of macular pigment for photoprotection in the eye, an important aspect is the focusing of light onto the retina.

1782	BUZZI	First description of yellow macular pigment
1945	WALD	Macular pigment first identified as containing carotenoids
1982	KIRSCHFIELD	Protective function of macular pigment established
1985 1988,1993	BONE, et al.	Lutein and zeaxanthin identified as the carotenoids in macular pigment
1992	NIERENBERG & NANN	Lutein found in the skin and other tissues
1994	GIULIANO, et al.	Lutein identified in breast milk
	SEDDON, et al.	Association of lutein and zeaxanthin intake with reduced risk of age-related macular degeneration (AMD)
	UNITED STATES GOVERNMENT	Passage of Dietary Supplement Health and Education Act (DSHEA)
	KEMIN	FloraGLO® Lutein becomes first lutein brand commercially available to market
1996	KEMIN	FloraGLO® Lutein first mass market product launch

Table 1: Key discoveries in the history of macular pigment leading up to the launch of FloraGLO® Lutein

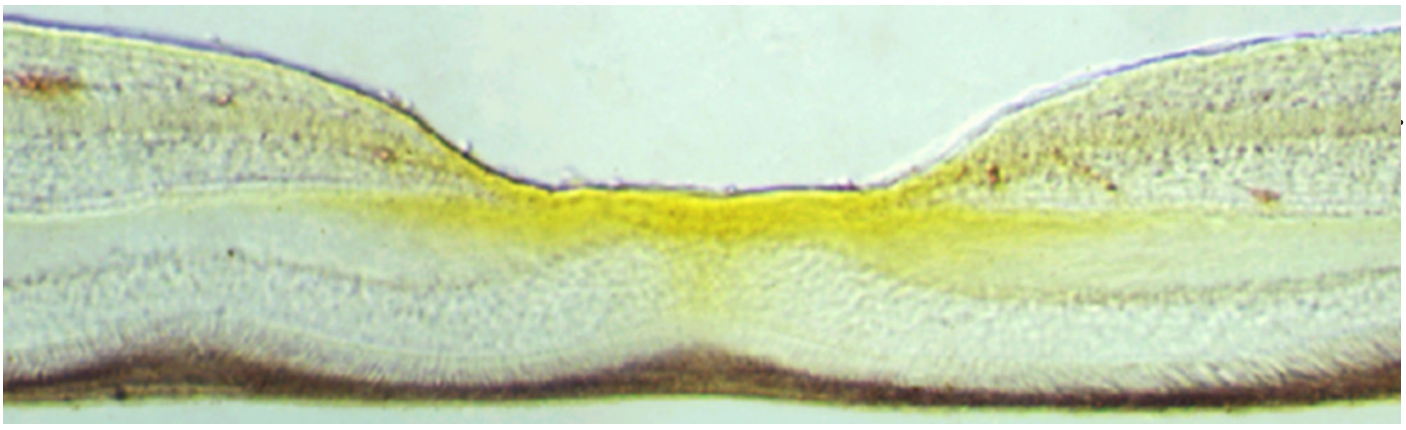


Figure 3: Cross-section of the primate retina showing macular pigment, image courtesy of Max Snodderly

Although the macula is very small, it is the area of the retina with the greatest impact upon visual acuity. This highlights the importance of protecting light sensing photoreceptors in the eye from damaging wavelengths of light.

The advent of high performance liquid chromatography (HPLC) enabled Bone and Landrum to confirm the identification of lutein and zeaxanthin as the dietary carotenoids that accumulate as macular pigment.[26,40] It is now understood that macular pigment also includes a third xanthophyll called *meso*-zeaxanthin, which is a structural isomer resulting from conversion of lutein to *meso*-zeaxanthin in the retina.[41,42] Although there is currently no data that suggests *meso*-zeaxanthin is found in appreciable amounts in the foods human typically consume, this xanthophyll has not been found in the human bloodstream or liver and is not present in the vegetables or fruits known to contain lutein and zeaxanthin.[43–49]

Perhaps the most influential report in the history of the lutein and zeaxanthin science came in 1994 when Johanna Seddon and co-authors investigated the associations of dietary antioxidant and carotenoid intake with age-related macular degeneration (AMD). They observed that intake of lutein and zeaxanthin from dark green leafy vegetables was strongly associated with a decreased risk of AMD.*[50]

*AMD is a progessive, age-related eye condition that attacks macula of the eye resulting in blurry, dim images or black holes in the center of vision robbing people of their independence (https://nei.nih.gov/health/maculardegen/armd_facts). There are nearly 2.07 million cases currently diagnosed in the United States alone and by 2050, the estimated number is expected to more than double to 5.44 million (https://nei.nih.gov/eyedata/amd).

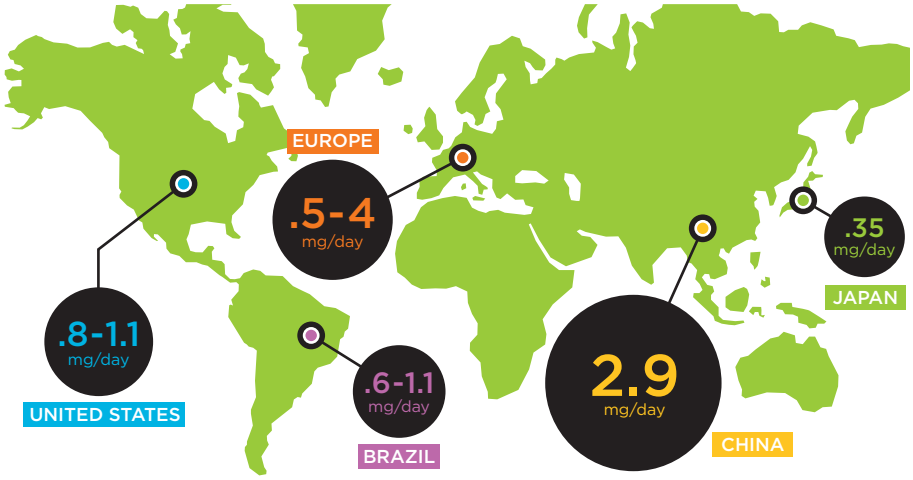


Figure 4: Average daily lutein and zeaxanthin consumption across the world

Where are Lutein & Zeaxanthin Found in the Diet?

Lutein and zeaxanthin are typically found together in nature. They are naturally present in a variety of fruits and vegetables, particularly corn, spinach, broccoli and kale.[51–53] This is not surprising considering the role of lutein and zeaxanthin in photosynthesis. Although poultry do not themselves make carotenoids, lutein and zeaxanthin accumulate in the egg yolk, which depends upon diet.[54]

It is estimated that 80% of total daily dietary intake of these carotenoids is as unesterified lutein and 13% as unesterified zeaxanthin. Only about 7% of the lutein and zeaxanthin in the typical diet is present in the form of lutein esters and zeaxanthin esters.[21] Human pancreatic digestive enzymes are capable of separating the unesterified lutein from a lutein ester, but the efficiency of this process is poor.[55] A head-to-head comparison study on the bioavailability of lutein esters compared to unesterified lutein has shown that a significantly larger amount of lutein ester must be consumed to achieve the same blood levels of lutein as compared with unesterified lutein.[24] Therefore, it is important to provide a supply of lutein already in the unesterified form so it may be readily absorbed by the body.

Based on the results of several randomized trials (discussed in the next section), daily intakes of 10 mg lutein and 2 mg zeaxanthin are typically recommended. However, considering the relatively low intake of fruits and vegetables in many diets around the world, it's not surprising that intakes of lutein and zeaxanthin are far below those levels. In US adults, combined intakes of lutein and zeaxanthin ranged from 0.8 to 1.1 mg/day, with differences observed among age and ethnic groups.[1] Intakes in other countries are also relatively low, with total lutein and zeaxanthin intakes ranging from 0.5-4.0 mg in Europe,[56–58] 0.6 to 1.1 mg/day in Brazil,[59] and 0.35 and 2.9 mg/day in Japan and China, respectively.[60,61] Given that average intakes of dietary lutein are commonly far less than the amounts associated with reductions in risk for AMD, there is a clear need for supplemental sources of dietary lutein. FloraGLO® Lutein addresses this need and presents an opportunity to evaluate the physiological impact of lutein supplementation on human health.

FloraGLO® Lutein: Providing an Opportunity for Lutein Enrichment in the Human Diet

In the early 1990's, Kemin began investigating the potential for lutein as a dietary supplement ingredient. Kemin recognized the gap between the typical intake of lutein in the human diet and a need for lutein in the eye health marketplace. They were able to leverage the knowledge and production technologies from their existing business of providing purified lutein to the animal health market. Kemin's foresight was validated by articles published in 1992 and 1993 indicating that antioxidants, especially carotenoids, may be important in reducing the risk of AMD.[62,63] Subsequently, two events occurred in the fall of 1994 that changed the culture toward: **1.** dietary supplements in general and **2.** lutein supplementation specifically.

The first event was the passage of the Dietary Supplement Health and Education Act of 1994, commonly called DSHEA.[64] This law established standards for the safety of dietary supplements as well as applying existing food standards to labeling requirements for this class of products.

The second event was the publication of the results of an epidemiological study by Dr. Johanna Seddon and other members of the Eye Disease Case Control Study Group (EDCCS) in the November 9, 1994 issue of the prestigious Journal of the American Medical Association (JAMA).[50] This multicenter study was consistent with the earlier findings of the EDCCS indicating that increased intake of carotenoids may reduce the risk of AMD. After adjusting for other risk factors, the study results indicated that people consuming 6 mg of lutein and zeaxanthin on average per day from their diet had a significant 57% reduction in risk of developing AMD compared to those who consumed 0.6 mg of lutein and zeaxanthin on average per day. Such an important finding set the stage for FloraGLO® to breakthrough as the most studied lutein brand for eye health and age-related eye disease prevention.[65]

Quality, Safety and Efficacy of FloraGLO® Lutein

The basic tenets behind FloraGLO® Lutein have always been, and will always be, quality, safety and efficacy as shown in Figure 5. FloraGLO® is the most studied lutein brand worldwide with more than 70 different published human clinical trials supporting its efficacy.[65] Because of this, currently FloraGLO® Lutein is the lutein brand most studied by researchers[65] and recommended by eye doctors.[66] No other lutein brand is backed by this amount of clinical research support.[67]

Figure 5: The basic tenents of FloraGLO® Lutein

Quality

The *quality* of FloraGLO® Lutein begins at the source – the marigold flowers from which FloraGLO® Lutein is derived. Although the highest amounts of lutein in our diet come primarily from dark green, leafy vegetables, these plants are not as abundant in lutein content as the petals of the marigold flower. FloraGLO® Lutein is obtained from marigold flowers grown under Good Agricultural Practices (GAPs) and traceable back to the farms on which the flowers are grown. Once harvested, the flowers are dried, pelleted and extracted with hexane to separate and concentrate the lutein contained in the flower petals from the other materials. The extraction solvent is then removed to yield a semisolid mass called marigold oleoresin. This oleoresin contains lutein and zeaxanthin esters along with other oil soluble plant materials. Marigold oleoresin, which complies with strict specifications, is shipped to Kemin headquarters in Des Moines, Iowa where it is further processed into unesterified FloraGLO® Lutein.

Kemin's process of commercially converting the marigold flower lutein ester to unesterified ("free") lutein, and ultimately the various forms of FloraGLO® Lutein available in the marketplace, is depicted in Figure 6. This proprietary process involves: 1) the removal of the fatty acids from the esterified lutein and zeaxanthin via saponification to yield unesterified forms of both; 2) crystallization and separation of the unesterified lutein and zeaxanthin crystals from the mixture; 3) drying of crystalline lutein; and finally 4) converting crystalline lutein into liquid and dry product forms.

Although the saponification process is not chemically difficult, it is challenging to produce the unesterified forms of lutein and zeaxanthin without generating significant quantities of isomers. The process for manufacturing FloraGLO® Lutein is therefore carefully controlled to assure that the naturally-occurring lutein and zeaxanthin are not degraded

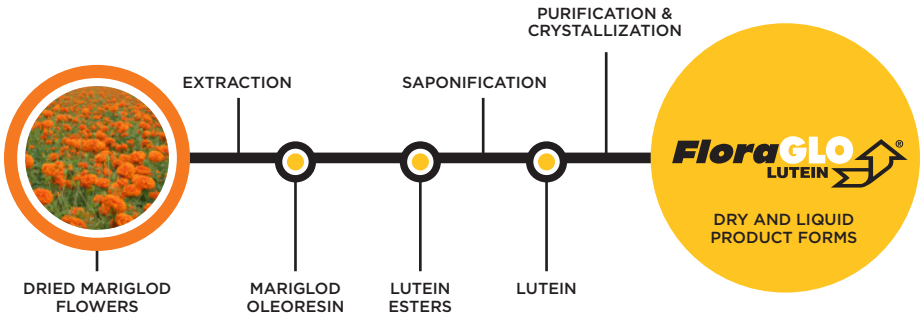


Figure 6: Schematic diagram of FloraGLO® Lutein's proprietary production process

to heat-generated stereoisomers such as *meso*-zeaxanthin. Unlike some other lutein-containing ingredients in the marketplace, FloraGLO® Lutein provides only those forms commonly found in the diet. This is where the proprietary processes that have been developed and refined over the last 20 years are important to maintaining and ensuring the quality of this lutein brand.

FloraGLO® Lutein benefits from a legacy of knowledge on carotenoid bioavailability from Hoffman La-Roche's Vitamins and Fine Chemicals division, which was acquired by DSM in 2003. In 2008, Kemin and DSM entered a strategic alliance to more thoroughly develop the lutein market, providing an array of product forms that simultaneously deliver process stability and bioavailability for dietary supplements and the enrichment of food products with lutein. Perhaps the most intriguing of the available product forms is that of the lutein powders employing the Actilease® technology (a trademark of DSM). This microencapsulation technology produces small particles of lutein within a readily dissolvable matrix that is capable of being compressed into product forms such as tablets without significant degradation. Because of the nature of the dissolvable matrix, this powder yields a highly bioavailable lutein as confirmed in two separate clinical studies.[68,69]

Safety

FloraGLO® Lutein established the benchmark for safety of supplemental lutein. The *safety* of FloraGLO® was first demonstrated in an extensive array of tests as described by Kruger and co-authors.[70] These tests included a complete battery of acute and chronic feeding and mutagenicity studies where FloraGLO® Lutein was administered in significant quantities in order to demonstrate its safety. The data generated was reviewed by the Joint Food and Agricultural Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) to assess the safety of this lutein brand for use in foods in June of 2004. Based upon their review, JECFA established an Acceptable Daily Intake (ADI) for lutein and zeaxanthin from marigolds at 2 mg/kg body weight/day (i.e., 140 mg/day for a person weighing 70 kg or about 154 pounds). This amount is considerably higher than the 10 mg lutein and 2 mg zeaxanthin commonly recommended

FloraGLO® is the #1 doctor recommended lutein brand.[66]

for daily intake in vitamin/dietary supplement products sold in the market.

The safety of FloraGLO® Lutein was also reviewed by experts and determined to be Generally Recognized as Safe (GRAS) for a variety of food product categories. A subsequent GRAS Notification was successfully reviewed and acknowledged by the U.S. Food and Drug Administration (FDA) (GRAS Notice Inventory, GRN 140). Additionally, the FDA reviewed subsequent GRAS dossiers on FloraGLO® in both term (GRN No. 221) and preterm (GRN No. 390) infant formula for which letters of non-objection were received. To date, these represent the only approvals for the use of lutein in infant formula, a product category where safety is considered to be paramount. Importantly, since the commercialization of FloraGLO® Lutein over twenty years ago, there have been no reports of serious adverse events related to FloraGLO® noted in any clinical trial where it was administered. More recently, the European Food Safety Authority (EFSA) confirmed the safety of lutein using a more conservative Acceptable Daily Intake (ADI) of 1 mg/kg body weight/day while simultaneously confirming the safety of lutein for nutritional uses by infants and young children in 2006.[71]

Then again in 2008, EFSA after evaluating the data presented on FloraGLO® for infant nutrition concluded that the data did not "raise concerns about the safety of lutein in infant formulae at the levels achieved through the natural content of ingredients nor at the level of use (concentration of added lutein 250 µg/l) proposed by the applicant for infant formulae with a low natural lutein content (about 20 µg/l or lower)".[72] All these safety assessments have endorsed the safety of FloraGLO®, hence making FloraGLO® Lutein the lutein brand you can trust that is proven safe for use across the lifespan.

FloraGLO® is the lutein brand clinically proven safe for babies and selected for infant nutrition.

Efficacy

The *efficacy* of FloraGLO® Lutein, as compared with other brands, and as demonstrated in human clinical trials, time and time again, is unparalleled. As of the beginning of 2016, FloraGLO® Lutein was used in more than 70 separate published human clinical studies, including the prestigious National Institutes of Health (NIH), National Eye Institute (NEI) AREDS2 study.[17] In total, these studies, as described in Table 2, cover a broad range of clinical parameters from the amount of lutein in the bloodstream to specific measurable changes in cognitive function as a result of the administration of FloraGLO® Lutein. This number of clinical studies is significantly more than that of any other lutein brand available in the marketplace today. A direct result of being the product used in so many human clinical studies is that FloraGLO® Lutein is the lutein brand most studied by researchers[65] and recommended by doctors.[66]

PARAMETER EVALUATED	NUMBER OF STUDIES
Serum Lutein	30
Macular Pigment Optical Density	28
Visual Function	22
Infant/Maternal Health	14
Skin Health	4
Cognition	1

Table 2: Published studies conducted on FloraGLO® Lutein by the parameter evaluated

FloraGLO® is the most studied lutein brand worldwide with proven efficacy in more than 70 human clinical trials.[65]

A History of Lutein and FloraGLO®

1945 — MACULAR PIGMENT FIRST IDENTIFIED AS CONTAINING CAROTENOIDS—*WALD*

1985 — LUTEIN AND ZEAXANTHIN IDENTIFIED AS THE CAROTENOIDS IN MACULAR PIGMENT—*BONE AND LANDRUM*

1992

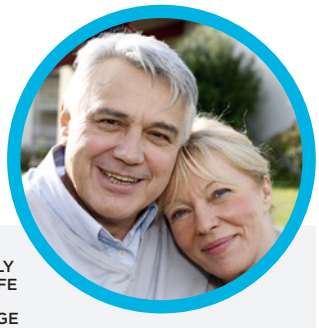
- LUTEIN FOUND IN THE SKIN AND OTHER TISSUES —*NIERENBERG AND NANN*
- LUTEIN IDENTIFIED IN HUMAN BREASTMILK —*GIULIANO AND CO-AUTHORS*

1994

- THE ASSOCIATION BETWEEN DIETARY LUTEIN AND AMD ESTABLISHED —*SEDDON AND CO-AUTHORS*
- PASSAGE OF THE DIETARY SUPPLEMENT HEALTH AND EDUCATION ACT (DSHEA)
- FLORAGLO® BECOMES THE FIRST LUTEIN BRAND COMMERCIALLY AVAILABLE TO THE MARKET

1996

- FLORAGLO® LUTEIN FIRST MASS MARKET PRODUCT LAUNCH
- FLORAGLO® IS FDA NOTIFIED GENERALLY RECOGNIZED AS SAFE (GRAS) IN MULTIPLE FOOD AND BEVERAGE CATEGORIES (GRN NO. 140)
- FLORAGLO® SAFETY ESTABLISHED BY GLOBALLY-RECOGNIZED WORLD HEALTH ORGANIZATION JOINT EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA)
- FLORAGLO® (10 MG) SHOWED IMPROVEMENTS IN MPOD, GLARE RECOVERY, VISUAL ACUITY, AND CONTRAST SENSITIVITY IN AMD PATIENTS—*RICHER AND CO-AUTHORS*
- LUTEIN FOUND IN THE ADULT BRAIN—*CRAFT AND CO-AUTHORS*



1997

- LUTEIN SUPPLEMENTATION SHOWN TO IMPROVE MACULAR PIGMENT DENSITY —*LANDRUM*
- NATIONAL EYE INSTITUTE INITIATES RECRUITMENT FOR AREDS2 STUDY, THE LARGEST LUTEIN SUPPLEMENTATION STUDY EVER CONDUCTED

2004

- FLORAGLO® IS FDA NOTIFIED GRAS FOR USE IN TERM INFANT FORMULA (GRN NO. 221)
- FLORAGLO® (10 MG) FOUND TO INCREASE SKIN HYDRATION AND ELASTICITY —*PALOMBO AND CO-AUTHORS*

2006

- STRATEGIC ALLIANCE WITH DSM ESTABLISHED
- FLORAGLO® (10 MG) SHOWED IMPROVEMENTS IN MPOD, GLARE RECOVERY, VISUAL ACUITY AND CONTRAST SENSITIVITY IN HEALTHY YOUNG ADULTS —*STRINGHAM AND HAMMOND*
- FLORAGLO® SHOWN TO SUPPORT COGNITIVE FUNCTION IN ADULTS —*JOHNSON AND CO-AUTHORS*

2007

- FLORAGLO® IS THE ONLY LUTEIN BRAND USED IN LUTEIN MATERNAL AND INFANT FEEDING STUDIES

2008

- EXPANDED GRAS STATUS OF FLORAGLO® INCLUDING THE USE OF PRE/POSTNATAL SUPPLEMENTS AT 6-12 MG LUTEIN PER DAY IN A PREGNANT/LACTATING POPULATION
- FLORAGLO® IS FDA NOTIFIED GRAS FOR USE IN PRETERM INFANT FORMULA (GRN NO. 390)
- FLORAGLO® BECOMES THE MOST RECOMMENDED LUTEIN BRAND AMONG EYE DOCTORS

2010–2016

- AREDS2 STUDY IS PUBLISHED AND RECOMMENDS 10 MG OF LUTEIN AND 2 MG OF ZEAXANTHIN FOR AREDS2 TYPE FORMULATIONS —*CHEW AND CO-AUTHORS*

10+2

2012

2013

2014

- FLORAGLO® LUTEIN (10 MG) RESULTED IN IMPROVEMENTS IN MPOD, GLARE RECOVERY, CHROMATIC CONTRAST AND PHOTOSTRESS RECOVERY IN HEALTHY YOUNG ADULTS —*HAMMOND AND CO-AUTHORS*

2016

- FLORAGLO® IS MOST STUDIED LUTEIN BRAND WORLDWIDE, WITH OVER 70 PUBLISHED HUMAN CLINICAL TRIALS
- KEMIN GRANTED PATENT FOR BLUE LIGHT PROTECTION WITH LUTEIN*

*Roberts, Richard. "Methods of Treating Ocular Disorders." Kemin Industries, Inc., assignee. Patent US9226940 B2. This patent covers three conditions for all ages; presbyopia, hyperopia, and astigmatism.

FloraGLO: The Pioneers of Lutein Science and Discovery

The commercialization of FloraGLO® Lutein was important for the advancement of research on the dietary supplementation with lutein.

Macular Pigment Density

Aside from the levels of lutein in the bloodstream as a result of supplementation, the second most evaluated parameter in human clinical studies related to lutein supplementation has been the Macular Pigment Optical Density or MPOD. MPOD is a direct measure of the amounts of lutein and zeaxanthin present in the macular area of the eye. The deposition of lutein and zeaxanthin into the macular area of the eye is visible as a yellow coloration as shown in Figures 3 and 7. A careful inspection of Figure 3 reveals that the amount of lutein and zeaxanthin is greatest in the center of the macula and decreases with increasing distance from the center. Although pictures like this one are very helpful in depicting the amounts of lutein and zeaxanthin present in the macular area, they require a complete sacrifice of the eye. Therefore, a non-invasive parameter was needed to quantify the amounts of lutein and zeaxanthin in the eye. That parameter is MPOD. In that regard, virtually every supplementation study with FloraGLO® Lutein has shown that MPOD is increased as a result of the supplementation. One example of such an increase in MPOD following supplementation is shown in Table 3.[72]

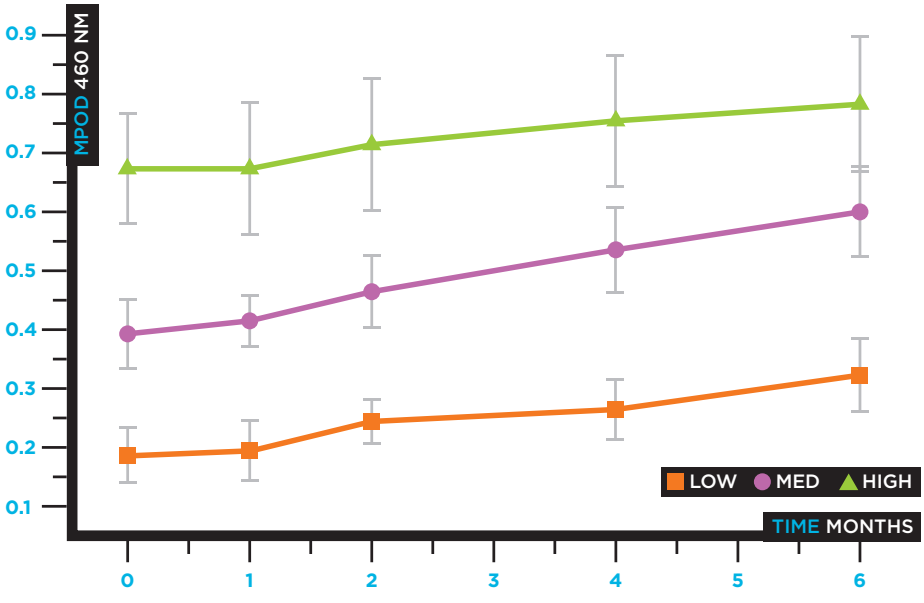


Table 3: Changes in MPOD in healthy subjects adapted from Stringham, et al. (2008)

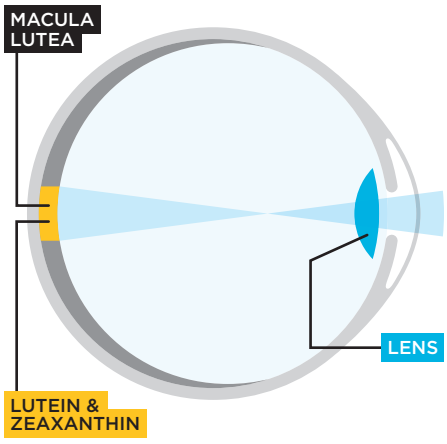


Figure 7: Diagram of the deposition of lutein and zeaxanthin in the macular area of the eye

Not surprisingly, people with the highest initial MPOD are typically found to exhibit the smallest increases whereas those with the lowest initial MPOD typically exhibited the greatest increases.[+] However, using data from several studies encompassing a population of 846 subjects, Wooten and Hammond showed that 43% of this sample of people had a MPOD of less than 0.2 Optical Density Units (ODU) and that 16% had a MPOD of less than 0.1 ODU.[75] Furthermore, although somewhat controversial, studies have shown that MPOD declines with age. This age-related decline in MPOD has been reported in a large case-control study of more than 800 healthy Irish subjects.[76] Studies in smaller groups of American (n = 217) and Japanese (N = 197) subjects showed a similar decline of MPOD with age.[77,78] Studies have also shown that subjects with AMD have lower MPOD than subjects with healthy eyes.[79,80] These points are important because low MPOD and age are two important risk factors associated with the development of AMD.[76]

[+]A panel of experts came together in 2009 to review the background behind MPOD measurements and changes in this value as it pertains to lutein/zeaxanthin supplementation. Amongst their findings, the panelists agreed that central MPOD below 0.2 ODU should be considered to be low. MPOD values between 0.2 and 0.5 are intermediate, and values above 0.5 are considered to be high.[74]

Although MPOD is relatively stable,[81] increased consumption of lutein and zeaxanthin has been shown to increase MPOD, regardless of whether it is a result of changes in diet or via supplementation.[82–85] However, changes in MPOD are not immediate. It can take between 2 to 4 months of daily supplementation with 10 mg of FloraGLO® Lutein for an individual's MPOD to increase significantly. Researchers are now using MPOD as a biomarker for the amounts of lutein and zeaxanthin in the body as well as a long-term indicator of the adequacy of the diet.[86–88] MPOD has also been proposed as a biomarker for the amounts of lutein and zeaxanthin in the brain.[89–91] While there is debate on the predictive value of MPOD and functional outcomes related to vision or AMD risk[74,92–94], it seems to be acknowleged even by EFSA that lutein intake does indeed increase MPOD in most healthy individuals.[95]

Perhaps most importantly, the ability to measure MPOD has moved out of the research laboratory and is making its way into more and more optometric and ophthalmological offices. If optometrists and ophthalmologists begin measuring MPOD in their patients routinely, the public will become more aware of their need for lutein and zeaxanthin in their diet. This would provide a means to regularly assess the steps that the public needs to take to increase the consumption of these nutrients. Since most people do not consume the kinds of foods needed to obtain sufficient amounts of lutein and zeaxanthin (e.g. dark green vegetables), regular accessibility to MPOD measurements may also increase the demand for eye health supplements containing these nutrients. The powerful economic impact of lutein and zeaxanthin supplementation was demonstrated in a Frost and Sullivan report "Smart Prevention—Health Care Cost Savings Resulting from the Targeted Use of Dietary Supplements".[96] The report highlighted that "An average of $3.87 billion per year and a cumulative savings of $30.95 billion from 2013 to 2020 in avoidable healthcare utilization costs is potentially realizable if all U.S. adults over the age of 55 diagnosed with AMD or cataract were to use lutein and zeaxanthin dietary supplements at protective levels." The serious healthcare savings possible with the daily supplementation of 10 mg lutein and 2 mg zeaxanthin to American adults 55 years and over reveals a significant opportunity for improving public health since only about 4% of American adults over 55 years regularly supplement with lutein.[97]

RESEARCHER PERSPECTIVE

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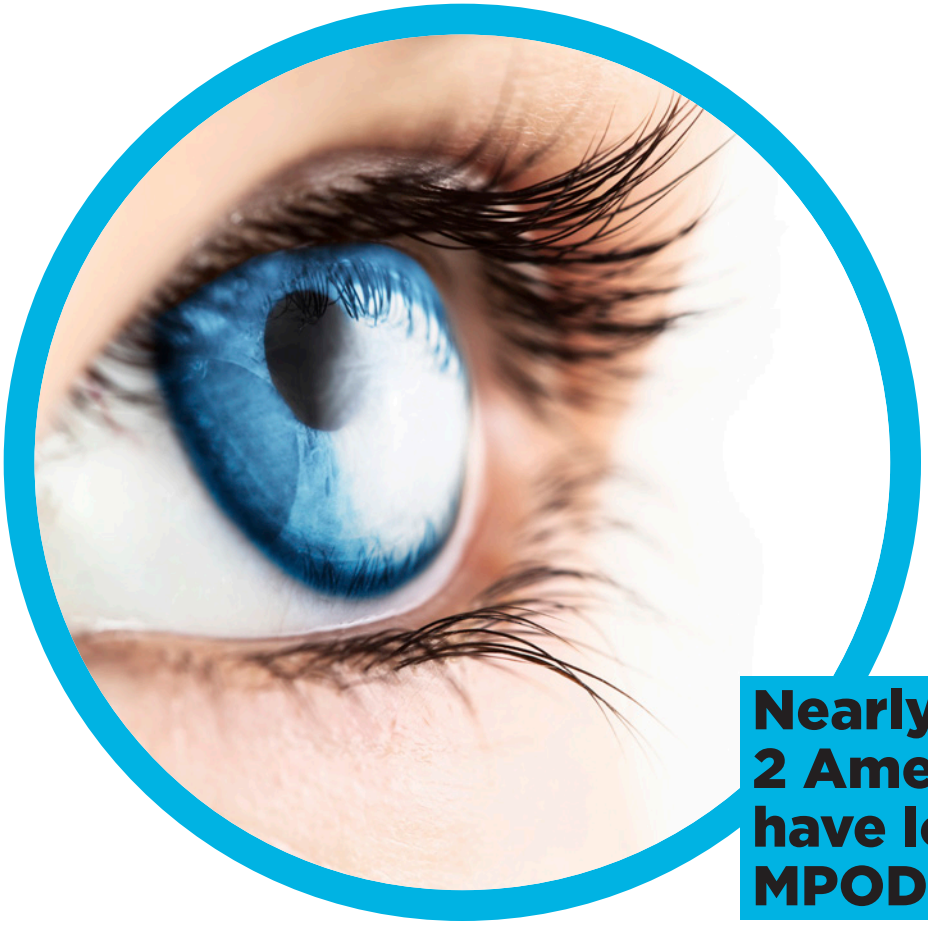
When the intake of lutein is insufficient, MPOD would be lower, that could be the risk of progression of age-related macular degeneration.

Macular pigment optical density (MPOD) is positively correlated with dietary and serum lutein, and negatively correlated with serum oxidized LDL, a biomarker for systemic oxidative stress and artherosclerosis. Those who have artherosclerosis, one of the estimated risks of AMD, might have lower level of MPOD, which could further increase the risk of AMD.



Nearly 1 in 2 Americans have low MPOD[75]

Eye Protection

The amount of macular pigment in the eye as measured by MPOD is important because of the protective activity that lutein and zeaxanthin provide to the cells comprising the macula. Lutein and zeaxanthin act by filtering the damaging blue wavelengths of light striking the retinal tissue thereby preventing oxidative stress and free radical damage of the cells of the macula through their antioxidant activity. Although there is little doubt that MPOD has played a role in helping to define the relationship between lutein supplementation and AMD, the earliest studies in establishing that relationship did not measure MPOD. As noted above, the publication of the epidemiological evidence linking the risk for AMD with dietary lutein intake was first described by Seddon and co-authors.[50] These results were supported by the publication of two small intervention studies in which subjects with AMD consumed increased amounts of lutein.[98,99] Although these studies only included small numbers of AMD subjects, they both showed that increasing the intake of lutein improved visual function parameters. At about the same time that these two studies were being conducted, the NEI was conducting the original Age-Related Eye Disease Study (AREDS). AREDS was a 5-year study designed to learn more about AMD and cataracts while simultaneously testing whether supplementation with certain vitamins and minerals could help prevent or at least slow progression of these diseases.[100] Unfortunately, no commercially available source of lutein and zeaxanthin existed at the time that this study was designed and subject recruitment began and hence, lutein was not included as part of the intervention.

At about the same time that the AREDS study was being conducted, a clinical study in AMD subjects involving supplementation with lutein was being conducted at the Department of Veterans Affairs Medical Center, North Chicago, Illinois. The study, commonly referred to as the LAST study, evaluated the efficacy of FloraGLO® Lutein or FloraGLO® Lutein plus antioxidants in the improvement of visual function in 90 subjects with AMD. The results showed MPOD increased significantly and that visual function improved in subjects receiving lutein supplementation relative to the placebo.[101] Therefore, the LAST study was the first to demonstrate that FloraGLO® Lutein supplementation increased MPOD and simultaneously improved visual function in AMD subjects. Since the lutein supplements administered in the LAST study were made with FloraGLO® Lutein, this study was the first to link supplementation of FloraGLO® Lutein with visual performance improvements. The results, along with AREDS

LAST demonstrated that FloraGLO® Lutein increased MPOD and improved visual function in AMD subjects.[101]

RESEARCHER PERSPECTIVE

STUART RICHER

OD, PHD, FAAO

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The 2004 LAST (Lutein Antioxidant Supplementation Trial), was the first randomized, double masked, placebo controlled, multiple time point clinical study among patients with early age related macular degeneration consuming dietary lutein, and FloraGLO® Lutein in particular.[101] Subjects consuming 10 mg FloraGLO® Lutein had significant improvements in visual function across a wide range of visual parameters. These results included superior visual acuity, contrast sensitivity, glare recovery, Amsler Grid defect resolution and general visual function. This work has been subsequently validated globally in AMD and non-AMD patients alike, and remains the most cited scientific publication in the history of the Journal of the American Optometric Association. The "LAST Team" was announced as Service to America Medal finalists in Science and Technology, June 2006.

I was later assisted by two scientists from Alcon Laboratories, (Ft Worth, TX), using the LAST data set, but employing more data inclusive 'general estimation equations', rather than the repeated factors ANOVA (analysis of variance) statistics technique used in the LAST study.[102] We found AMD patients having the lowest macular pigment optical density (MPOD) also had the quickest rise in re-pigmentation. Noteworthy is the observation that those individuals with lowest MPOD were most likely to benefit from lutein or lutein plus antioxidant supplementation.

Some five scientists, ophthalmologists and optometrists met in a round table discussion led by Dr. Paul Bernstein. We formulated clinically meaningful guidelines concerning what constitutes low, average and superior macular pigmentation optical density or MPOD, a new ocular parameter that can be non-invasively measured by clinicians.[74] MPOD can be raised thru diet, or supplementation with lutein or zeaxanthin. This is the first clinical metric (guideline) concerning what constitutes superior protective and visually-restoring macular pigmentation, important to eye doctors, who have the ability to re-pigment their patients' macula(s), thru prescriptive xanthophyll carotenoid supplementation.

RESEARCHER PERSPECTIVE

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Retinal specialists have participated in a spectacular expansion of treatment options for age-related macular degeneration (AMD) in the past two decades. A once untreatable condition has now become manageable for many patients, but the treatment burden is enormous, often requiring monthly injections of expensive medications into the eye, and even then, some patients continue to progress to legal blindness. Thus, there has always been considerable interest on the part of eye doctors and their patients in preventative strategies such as nutritional interventions.

In the early 1990s, it was recognized that lutein was an excellent candidate for a nutritional supplement to prevent or slow AMD. Along with its isomer zeaxanthin, these two plant-derived carotenoids are specifically concentrated in the macula of the human eye where they are thought to combat light-induced oxidative damage that can lead to AMD, and various epidemiological studies confirmed that individuals who consume large amounts of lutein and zeaxanthin through diet or supplements have lower risk of AMD.

Twenty years ago, Kemin introduced FloraGLO® Lutein into the market, and it was rapidly embraced by consumers concerned about vision loss from AMD, but retina specialists typically require compelling clinical study evidence before consistently recommending supplement interventions against eye disease. These sorts of studies can be enormous undertakings, often requiring thousands of subjects followed for many years. We are fortunate that the National Eye Institute decided that there was sufficient evidence and interest to evaluate lutein and zeaxanthin in the randomized, placebo-controlled clinical trial known as AREDS2. In 2014, the AREDS2 researchers announced that substitution of lutein and zeaxanthin for the ß-carotene of the original AREDS formula was safe and effective in AMD patients, especially if they were current or former smokers. Since that time, AREDS2 formulations containing FloraGLO® Lutein have become standard-of-care nutritional supplements to combat AMD worldwide.

trials, confirmed that AMD is a nutritionally responsive disorder.

As part of the comprehensive evaluation that the NEI conducted on the results from the AREDS study, the AREDS researchers evaluated the relationship of lutein and zeaxanthin from the diet upon the incidence of AMD in this study population using data from food frequency questionnaires administered at the outset of the AREDS study.[103] This evaluation revealed that people reporting the highest levels of lutein and zeaxanthin intake at the beginning of the AREDS study had a significantly reduced likelihood of having AMD, geographic atrophy, or large or extensive drusen in comparison to those people reporting the lowest lutein and zeaxanthin intake from the diet. In addition to confirming that the lutein and zeaxanthin may be highly important in reducing the risk of AMD, the findings from this analysis became one of the driving factors responsible for the development and implementation of the AREDS2 study.

In 2006, the NEI completed the design and began recruiting subjects for the Age-Related Eye Disease Study 2 (AREDS2), a 5-year study to evaluate a set of variants of the original AREDS formulation in a population of people with existing AMD.[104] One of the primary variants investigated was the replacement of the 15 mg of ß-carotene in the AREDS formulation with 10 mg of lutein (FloraGLO® Lutein) plus 2 mg of zeaxanthin (OPTISHARP® brand, a registered trademark of DSM). The initial evaluation of the results of this study indicated that administration of 10 mg of lutein along with 2 mg of zeaxanthin yielded a 10% risk reduction in the progression rate of AMD when these xanthophylls were ingested along with the existing AREDS formulation that contained 15 mg of ß-carotene.[17] However, when the lutein and zeaxanthin replaced the ß-carotene, a significant 18% reduction in the risk of progression to advanced AMD was found. Furthermore, when the results from the AREDS2 study were evaluated in relation to the dietary intake of the test subjects as reported at the beginning of this study, a significant 26% reduc-

AREDS2 By the Numbers

4,203 Patients Age 50-85
At high risk of progression to advanced AMD

5 Year Study
2006-2013

82 Clinical Sites

Figure 8: Details from the design of the AREDS2 study, the largest lutein supplementation study ever conducted

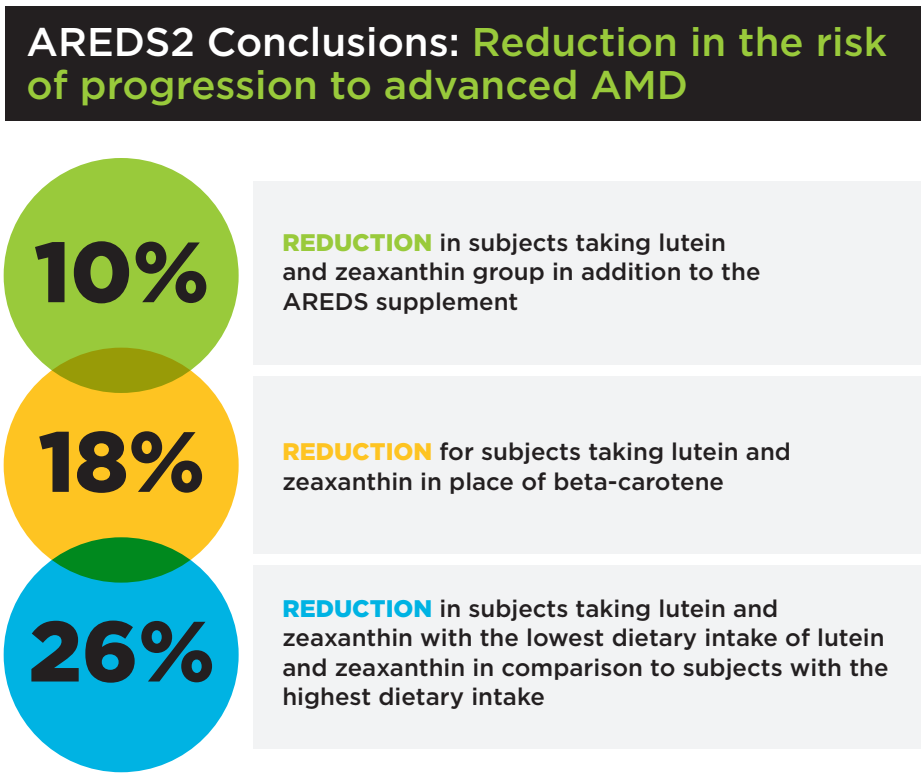

Figure 9: Conclusions of AREDS2

tion in risk of progression to advanced AMD was found in subjects ingesting the lowest levels of dietary lutein and zeaxanthin. This is very important since the low dietary intakes of lutein and zeaxanthin among the AREDS2 subjects closely match the typical levels of lutein and zeaxanthin consumed by people around the world.

AMD is not the only ocular condition where lutein and zeaxanthin have been shown to be important. Cataracts are responsible for the clouding of the lens of the eye thereby reducing visual quality. Lutein and zeaxanthin have been shown to be important antioxidants in the lens of the eye where they help reduce the risk of cataracts. Epidemiological studies have shown that serum levels of lutein and zeaxanthin are inversely related to the incidence of cataracts in men and women.[105–108] This has been confirmed through a meta-analysis of the epidemiological data.[109] Furthermore, as part of the AREDS2 study, an analysis of the results for subjects supplemented with lutein and zeaxanthin with the highest dietary intake of lutein and zeaxanthin in comparison to those that did not receive these carotenoids with the lowest intakes showed a significant reduction in the risk of progression to cataract surgery (32% risk reduction), of any cataract (30% risk reduction) or of any severe cataract (36% risk reduction).[110] Similar to the results previously described for AMD, the available data indicate that cataracts may be a nutritionally responsive ocular disorder.

Finally, research has indicated a possible beneficial role of lutein as a retinal protective ingredient in people with diabetes. Scientific evidence consistently indicates that MPOD is significantly lower in diabetic subjects when compared to healthy controls.[111–113] Experimental studies utilizing animal models of diabetes have shown that lutein intake reduces diabetic-induced oxidative stress, preventing the diabetes-induced biochemical and histological changes in the retina and improving retinal functionality.[114–116] Supplementation of patients with diabetic retinopathy using lutein, zeaxanthin and other antioxidants have also been found to be beneficial.[117,118]

All of the studies conducted on the effects of lutein in ocular diseases have been supported by the findings in five classical lutein mechanistic publications, which were based on research funded by DSM and conducted in an animal model, specifically the monkey.[42,119–122] The monkey was used because it is the only animal with an ocular physiology similar to humans that also accumulates macular pigment in the same manner. These studies have shown that monkeys deprived of lutein and zeaxanthin from birth had no lutein or zeaxanthin in their bloodstream and a total absence of macular pigment as compared to monkeys who consumed lutein and zeaxanthin from typical dietary sources. In the absence of macular pigment, these animals exhibited defects in the retinal pigmented epithelial (RPE) cells. RPE cells are important because their role is to recycle the retinal in rhodopsin that is used in visual processes (all-trans retinal 11-cis retinal). These RPE defects were found to be reversible by supplementation with lutein and zeaxanthin. Furthermore, supplementation resulted in the accumulation of macular pigment in the eyes and adipose tissue of the lutein/zeaxanthin deficient monkeys in a manner that was consistent with monkeys fed a normal diet from birth. Perhaps most importantly, monkeys raised from birth on a xanthophyll-free diet and then supplemented with lutein or zeaxanthin showed less photodamage when the eye was irradiated with short wavelength blue light compared to their unsupplemented counterparts. These results have helped clearly demonstrate the importance lutein and zeaxanthin in the physiology and development of the eye.

Visual Performance

In addition to helping reduce damage in the eye as a result of their antioxidant and blue light absorbing capacity, lutein and zeaxanthin have also been shown to have the capability to help improve visual quality in people already diagnosed with AMD as well as in younger people with healthy vision. Several studies have evaluated the ability of lutein and zeaxanthin to improve visual acuity, contrast sensitivity and glare tolerance in individuals with AMD.[85,89,123–128] A recent meta-analysis conducted on the data from seven separate clinical studies found that supplementation with lutein increased MPOD values while simultaneously improving visual acuity and contrast sensitivity in AMD subjects.[129]

Although this finding is important because people with AMD lose central visual acuity, it has also been found that supplementation with lutein and zeaxanthin improves visual performance in young adult subjects with healthy eyes. In a study which was published in 2008, it was found that visual performance could be improved in healthy eyes of young adult subjects by supplementation with FloraGLO® Lutein and zeaxanthin.[73] These results were confirmed in a follow-up randomized, double-blind, placebo-controlled study published in 2014.[130] Together, these studies showed that supplementation with lutein and zeaxanthin resulted in significantly decreased photostress recovery time (i.e., the time it takes to recover from a blinding flash of light) and increased chromatic contrast which was related to the increase in MPOD values following supplementation. These benefits were accompanied by an increased tolerance to glaring light although the latter results were not significant. These visual performance improvements are very important because they demonstrate that lutein and zeaxanthin supplementation provides benefits in the human eye regardless of a person's age or the presence/absence of AMD.

RESEARCHER PERSPECTIVE

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My work in the area of lutein started in 1989 when we were developing methods for non-invasively measuring the amount of lutein and zeaxanthin in the human retina (macular pigment). We found that the very best method was based on a psychophysical technique: a subject's behavioral response to a visual stimulus. We found that macular pigment was influencing visual function in a variety of ways and as a linear function of amount (i.e., the more you had, the bigger its impact on vision).

For decades, work had focused on the role of macular pigment in preventing macular degeneration, but it seems unlikely that we evolved mechanisms for accumulating lutein within the retina only to protect vision past child-bearing age. Lutein and zeaxanthin are fairly ubiquitous in human biology and they have been linked to cardiovascular health, immune function, and the biology of skin and brain. A functional effect on vision, however, would likely have far reaching, immediate and ecologically meaningful influences.

Since macular pigment is a yellow filter anterior to the cone photoreceptors, we hypothesized that it serves very much like internal sunglasses. This hypothesis was confirmed in a series of randomized trials showing that increasing macular pigment directly reduces visual disability due to glare and speeds recovery from blinding flashes of intense light (photostress). We also found improvements in visual range and the ability to detect chromatic borders.

These studies, from our laboratory and others, showed that macular pigment has a basic function in the normal operation of the retina. Hence, deficiency in lutein/zeaxanthin intake would have implications for everyone, not just the elderly or individuals with early stages of eye disease. We are currently extending this work to look at additional roles of lutein within the central nervous system such as visual-motor function, processing speed and brain function.

Maternal and Infant Health

Lutein and zeaxanthin have been shown to support healthy vision in adults. Emerging research has shown that these important nutrients are also beneficial for developing infant eyes. Lutein and zeaxanthin have been found to accumulate in the eye of fetuses as early as 17 to 22 weeks of gestation.[131] This crucial finding initiated the examination of lutein during pregnancy by several carotenoid research groups. Between 1998 and 2007 a number of papers were published on this association, especially the amount of lutein in the bloodstream of the mother during pregnancy.[132–142] The findings revealed that lutein is not only present in the mother's bloodstream during pregnancy, but also that it typically increases during pregnancy and then decreases postpartum. This research also showed that lutein is present in cord blood which provides nutrients to the fetus. The amount of lutein and zeaxanthin in cord blood was found to be approximately 25% of the amount found in the mother's bloodstream.[132] Additionally, lutein has been found to be the most predominant carotenoid in human colostrum, contributing to its characteristic yellow color. Colostrum is the first milk expressed from breast tissue following birth of the baby and the first consumed by newborn infants. Lutein has also been found in breast milk throughout the entire nursing period.[143,144] In total, these findings provide a compelling argument for providing lutein in infant formula, especially given that infant formula is given to many babies in place of breast milk.

To date, FloraGLO® Lutein is the only brand of lutein allowed for use in infant formula.

In 2007 and 2012, GRAS notifications were submitted to the U.S. Food and Drug Administration on the use of FloraGLO® Lutein in term and preterm infant formula and letters of non-objection were received in return. The allowable amount of lutein for use in such products was established at 250 µg/L for term infants (GRN No. 221, 2007) and 210 µg/L for pre-term infants (GRN No. 390, 2012). To date, FloraGLO® Lutein this is the only brand of lutein allowed for use in infant formula and hence, is included in some infant formulas currently on the market. Also noteworthy is that all the published infant lutein supplementation studies (formula or supplement) have used FloraGLO® and they have all shown that the lutein supplementation provided is safe for infants.[145–153]

On the other hand, for breastfed babies to also receive these important carotenoids in their diet, it is important for the mother to be optimally nourished. This is particularly important considering that most adults consume only a fraction of the amounts of lutein and zeaxanthin that they need via their typical diet. Breastfeeding mothers can often be at-risk of nutrient depletion for a host of reasons and subsequent deficiency in the mother and the baby can only be mitigated with improving maternal nutritional status. Therefore, a study was conducted evaluating the effect of lutein and zeaxanthin supplementation upon nursing mothers and their offspring.[154] This study showed that supplementation with 6 to 12 mg of FloraGLO® Lutein per day for 4 months resulted in a significant increase in serum levels of lutein of the mothers as well as significant increases in levels of lutein and zeaxanthin in their breast milk without affecting the amounts of other carotenoids. Additionally, the bloodstream of the infants of mothers receiving lutein and zeaxanthin supplementation contained levels of these xanthophylls that were proportional to that found in the breast milk. Finally, the ratio of lutein and zeaxanthin found in the babies' bloodstream was directly related to the amounts found in the milk that they consumed. In total, these results suggest that nursing mothers should supplement their diet with additional lutein and zeaxanthin intake in order to preclude the potential of depleting their own bodies of these important carotenoids during the nursing period. This is particularly important in light of recent publications supporting the role of lutein in brain development. Lieblein-Boff and co-authors reported the results of an exploratory metabolomic analysis showing that lutein is concentrated in the neural tissues important for learning and memory (frontal cortex, hippocampus and occipital cortex) and is correlated with fatty acids, phospholipids and amino acid neurotransmitters in the brain.[155] The authors suggest that lutein may play a role in regulating brain volume or structural growth in the development or remodeling of neurons. Furthermore, Cheatham and co-authors also explored the relationship between lutein, choline and DHA levels in human milk and recognition memory in 5-month old infants. Their results showed that high levels of choline and lutein in breast milk are associated with better recognition memory.[156]

RESEARCHER PERSPECTIVE

PIERFRANCESCO MORGANTI
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Lutein has shown superior protective effects on human cells because of the presence of -OH groups in the molecule. The photo protective effectiveness of these natural molecules depends on the type of carotenoid used, its crystallinity and purity, the carrier used, and the consumption of a balanced and diet, when taken orally. To obtain the best effectiveness, the carrier used is fundamental. For both topical application and oral intake, the active ingredient must be loaded at the best concentration, delivered to the right sites and released at the right concentration at the right time. For topical applications, our group has shown that chitin nanofibril (CN), because of its nanodimension (140x7x5 nm) and the positive charges covering its exterior surface, can entrap oxygenated molecules such as lutein into its polymeric structure. When lutein has been entrapped into CN, a regular and constant dissolution rate of this molecule during 48 hours has been evidenced. The crystallinity and purity of the lutein is important to obtain high loading and entrapping efficiencies, and our best results have been obtained by the use of FloraGLO® Lutein. Moreover, CN-encapsulated lutein has shown to be easily distributed and delivered to the skin.

Interesting moisturizing, photoprotective and antiaging activities have been shown by the contemporary oral intake and topical applications of FloraGLO® Lutein by a doubled blind in vivo study. For this purpose it is important to remember that, while lutein is a natural antioxidant ingredient obtained from marigold flowers, CN is a natural polymer which is obtained from the crustacean waste. It is characterized for its moisturizing properties and has the same backbone of hyaluronic acid. Both the compounds, in accordance with the so-called green economy, are environmentally-friendly and skin-friendly, respecting the biodiversity of our planet.

Skin Health

Lutein has also been found to play additional functional roles in tissues beyond the eye. In a multi-country cross-sectional analysis of elderly subjects, vegetable consumption was associated with decreased skin wrinkling, prompting speculation that the antioxidant carotenoids found in vegetables may play a role.[157] As mentioned previously, lutein has been identified in various organs of the body, including the skin.[30] Therefore, it is not surprising that lutein supports skin health.

Dietary supplementation of FloraGLO® Lutein (6 mg/day) in combination with other antioxidant micronutrients was found to increase skin hydration, increase skin lipids and decrease skin lipid peroxidation in older women with moderately dry skin.[158] The same researchers later tested the effects of orally administered FloraGLO® Lutein at a higher amount (10 mg/day), either alone or in combination with topical lutein application, in a double-blind, randomized, placebo-controlled trial including 40 women with signs of premature aging.[159] Time-dependent increases in skin hydration, skin elasticity and skin lipids were observed, with concomitant decreases in skin lipid peroxidation and skin redness following UV light exposure. Significant differences were observed in both the orally supplemented and topical application groups as compared to the placebo, with even more pronounced effects in the combined group.

FloraGLO® increases skin hydration and elasticity.[159]

Cognition

Based upon the fact that visual processes are of neural origin, studies have looked at the connection between lutein and zeaxanthin in the eye and their content in the brain. Such studies have demonstrated that lutein and zeaxanthin are indeed found in brain tissue, not just in primates but also in older adults as well as in infants.[91,160,161] Several studies have investigated the effect of lutein and zeaxanthin supplementation upon cognitive function where MPOD was used as an indicator of brain levels of these carotenoids.[89,90,162–168] These studies have shown that people with higher MPOD values have better reaction speed, better recall, and faster visual processing than people with lower MPOD values. Six interventional studies have also been conducted to evaluate the effects of lutein and zeaxanthin supplementation upon cognition.[168–172]

Although not all of these supplementation studies demonstrated significant effects, all of the studies that included healthy populations reported improved cognitive capabilities, while those that did not included subjects with known cognitive diseases such as Alzheimer's. For example, in one study, supplementation of elderly women with 12 mg of FloraGLO® Lutein per day or this same dose of lutein plus docosahexaenoic acid (DHA) resulted in an improvement in verbal fluency.[171] Most recently, the lutein binding protein previously identified in the retina has also been found in the human brain, at levels associated with lutein concentrations in younger age groups.[173] These findings further support the selective accumulation of lutein, and indicate a specific functional role in the human brain. Further research is needed in this area and is anticipated in the next few years.

Future Directions for Research

In addition to future research into the role of lutein and zeaxanthin on brain health and cognitive function, there are three areas of research associated with lutein and zeaxanthin that have become important in the last few years, namely the effects of these carotenoids in screening blue light, upon eye fatigue, and upon inflammation. Blue light and eye fatigue have become important because of the increased exposure through blue light sources such as computers, video display screens, tablets, e-readers, and cell phones. Additionally, compact fluorescent and LED based lighting emit significant amounts of blue light.[174-178] Given that lutein and zeaxanthin absorb the harmful wavelengths of blue light and reduce damage in the eye, these nutrients may contribute to reducing the progression of ocular diseases which may be a result from harmful blue light exposure.[179,180] Studies have already shown that ingestion of lutein and zeaxanthin reduce eye fatigue associated with prolonged exposure to light from a computer.[181,182]

Lutein and zeaxanthin have also been reported to reduce inflammation for quite some time now. However, the evidence demonstrating that these nutrients exhibit efficacy in reducing inflammation is limited to *in-vitro* and animal studies.[183-188] It is currently suspected that lutein and zeaxanthin reduce oxidative stress within cells, which reduces inflammation by blocking the action and/or release of nuclear factor kappa B (Nf-κB) together with the cytokines and chemokines that the release of Nf-κB stimulates. If a reduction in Nf-κB can be demonstrated in human subjects as a result of supplementation with lutein and zeaxanthin, this could indicate a new mode of action for these nutrients in the human body. Since Nf-κB has been implicated in a host of inflammatory reactions in the human body, such a result may mean that lutein and zeaxanthin could play a more important physiological role in the body than previously suspected. However, additional research is needed to confirm these effects.

Is Lutein an Essential Nutrient?

As the body of scientific knowledge on lutein has grown, the concept of establishing a dietary reference intake (DRI) has been discussed.[12,189-191] In the development of DRIs, "a nutrient requirement is defined as the lowest continuing intake level that will maintain a defined level of nutriture in an individual"[182] using a criterion of biological adequacy (functional indicator). As outlined in the 2000 DRIs for Vitamin C, Vitamin E, Selenium and Carotenoids, Recommended Daily Allowances (RDAs) and Adequate Intakes (AIs) should be based on levels of intake to maintain desirable body stores or reserves, rather than levels of

Studies have shown that lutein and zeaxanthin reduce eye fatigue associated with prolonged exposure to blue light.[181,182]

intake needed to prevent pathologically relevant and clinically detectable signs of dietary inadequacy proposed by 1996 WHO/FAO/IAE expert consultations.[193]

First and foremost it is important to recognize that lutein and zeaxanthin are not synthesized by the human body[18] and must be obtained from the diet or through supplementation. Lutein is not only the predominant carotenoid, but it is also selectively and preferentially accumulated in the human eye, brain and breast-milk.[26–29,34] Lutein is important for the normal functioning of the eye across the lifespan[14], with growing evidence that it also plays a crucial role in the functioning of other vital organs such as the brain.[194] Current science is now even able to support the benefits of lutein supplementation on functional outcomes with plausible mechanisms of action. For instance, the specific xanthophyll-binding proteins that facilitate the uptake of lutein have been identified.[195,196] Additionally, in the classical DSM-funded primate studies discussed above, monkeys consuming xanthophyll-free diets experienced a greater number of retinal lesions upon exposure to damaging blue light, while monkeys that received supplemental lutein and zeaxanthin following such diet had normal number of lesions.[122] And finally, the role of lutein and zeaxanthin in decreasing the risk for eye diseases such as macular degeneration, cataract and diabetic retinopathy are gaining more public attention as plausible mechanisms for efficacy are being substantiated.[17]

Additionally, lutein meets almost all the criteria proposed by Lupton and co-authors as a bioactive to be considered for evaluation through a DRI-like process including: a definition of that is commonly accepted; an established validated method of analysis; database including the amount in foods; prospective cohort studies; clinical trials investigating absorption, digestion, metabolism, transport and excretion; clinical trials proving efficacy and a plausible mechanism for efficacy; and safety data supporting the level of intake expected.[191] The one area that lutein may not entirely fulfill is in the area of systematic reviews or meta-analyses which support efficacy. Nonetheless, lutein is still considered a strong candidate which is evidenced by the recent reviews in support of a DRI for lutein, especially if such a proposed framework for evaluation is accepted.[190,197]

Hence, with everything taken together, there is a strong case that adequate intake of lutein is important for people across the lifespan starting as early as fetal development and is required for not just optimal eye development and function, but also the development and function of several other vital organs such as the brain and skin. Using plasma and tissue concentrations as the criteria of biological adequacy (see IOM DRIs C, E, Selenium & Carotenoids pp 351), we strongly support the establishment of DRI for lutein and zeaxanthin. In the sidebar above, John Erdman provides an expert perspective on the concept of establishing a DRI for lutein.

RESEARCHER PERSPECTIVE

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A Case for Essentiality of Lutein

The Food and Nutrition Board, National Academy of Sciences, has long promulgated RDAs, and since the mid-1990's, Dietary Reference Intakes (DRIs) which still include RDAs designations. The RDAs set dietary levels for essential nutrients to alleviate the risk for developing symptoms of chronic deficiency disease. Essentiality implies that removing a food component from the diet will result in reversible adverse symptoms. Among the 42 DRI nutrients, do all of them meet the classical essentiality criteria? Are there other dietary components that might be considered essential?

A case for considering lutein for DRI status can certainly be made. The Food and Nutrition Board never defined the term essential but they expanded consideration to food components that would alleviate chronic disease. Lutein and zeaxanthin intake has been shown to increase macular pigment optical density (MPOD), which is associated with reduced risk of age-related macular degeneration (AMD) and improved visual performance, visual acuity, and glare sensitivity.

Lutein deposits in the macular fovea pit (at concentrations >500-fold higher than those of other body tissues or the blood). Specific binding proteins for both lutein and zeaxanthin facilitate their substantial deposition in the macula. Nature generally does not construct such a biological process without purpose. As in chloroplasts in green plants, lutein appears to protect the retina from excess light damage and risk of oxidative damage to surrounding tissue.

Based on the available evidence, enhancement of MPOD by increasing lutein intake is associated with a decreased risk of the disease, AMD, providing a foundational argument for the essentiality of lutein. There are additional, on-going clinical trials with lutein supplementation that are probing specific lutein eye function interrelationships. Outcomes from these studies and other work with non-human primates should shed more light on the essentiality of lutein.

The Legacy of FloraGLO® Lutein

Kemin and DSM are proud of the 20 years of initiative, enterprise and determination in establishing the unparalleled reputation and legacy of FloraGLO® and are committed to upholding the FloraGLO® brand standard through continued collaboration, innovation and scientific discovery. FloraGLO® Lutein represents the gold standard for the lutein supplement market because it is the lutein brand most studied by researchers[65] and included in the eye health supplement products that are most recommended by eye doctors.[66] The careful production of FloraGLO® ensures a high-quality product containing primarily lutein, a small amount of zeaxanthin and *de minimus* levels of heat-generated isomers. The strategic alliance of Kemin and DSM also enabled the creation of a diverse array of product forms with guaranteed stability and bioavailability.

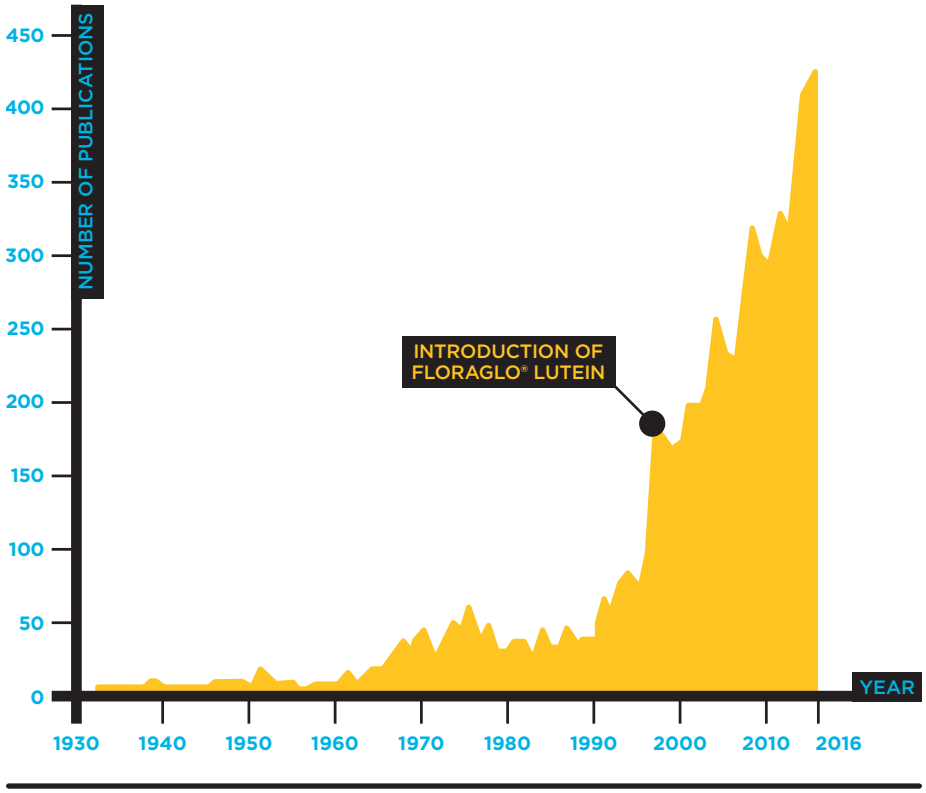


Figure 10: Number of publications making references to lutein by year. Scopus title, abstract, key word search for "lutein," showing results through 2014.

As such, FloraGLO® Lutein is the benchmark that established the safety of supplemental lutein, not only in adults, but across the lifespan, including term and preterm infants. Furthermore, FloraGLO® Lutein as the first lutein brand commercially available was the foundation for several key scientific research discoveries on the physiological benefits of lutein supplementation over the past two decades. Its introduction and commercialization came at a pivotal time, when scientific interest was emerging on the physiological importance of the macular pigment for eye protection and on the beneficial effects of dietary antioxidants in general (Figure 10). Since that time, as described above, many more interesting discoveries have been made with FloraGLO® Lutein, the lutein of choice over the past 20 years, with use in over 70 published human clinical trials and numerous other animal, *in-vitro* and mechanistic investigations.

As we have discussed above, the science of lutein has advanced beyond the known benefit of eye protection. Figure 11 shows a relational view of the emergence of new areas of research based upon some of the most highly cited or otherwise influential research publications. Some of the most exciting findings related to optimal visual performance in healthy adults and the developmental and cognitive aspects of maternal and infant nutrition have only recently been identified.

And last, but not least, the success and reputation of the FloraGLO® Lutein brand is a reflection of the hard work and commitment of the many people who have worked behind the scenes over the past two decades. This includes but is not limited to the combined expertise of Kemin and DSM of scientific, technical, regulatory, operational, quality, innovation, customer service and marketing support. Kemin and DSM are grateful to have had many opportunities to collaborate with the most well-respected researchers in fields of optometry, ophthalmology, infant and maternal nutrition, skin care, aging and cognition. In concert with this network of experts, we continue to look for opportunities to conduct additional research on the health benefits of enriching the human diet with lutein. We believe that our commitment to advancing scientific understanding will continue to create new opportunities to advance public health. Put another way, our vision for the future is bright.

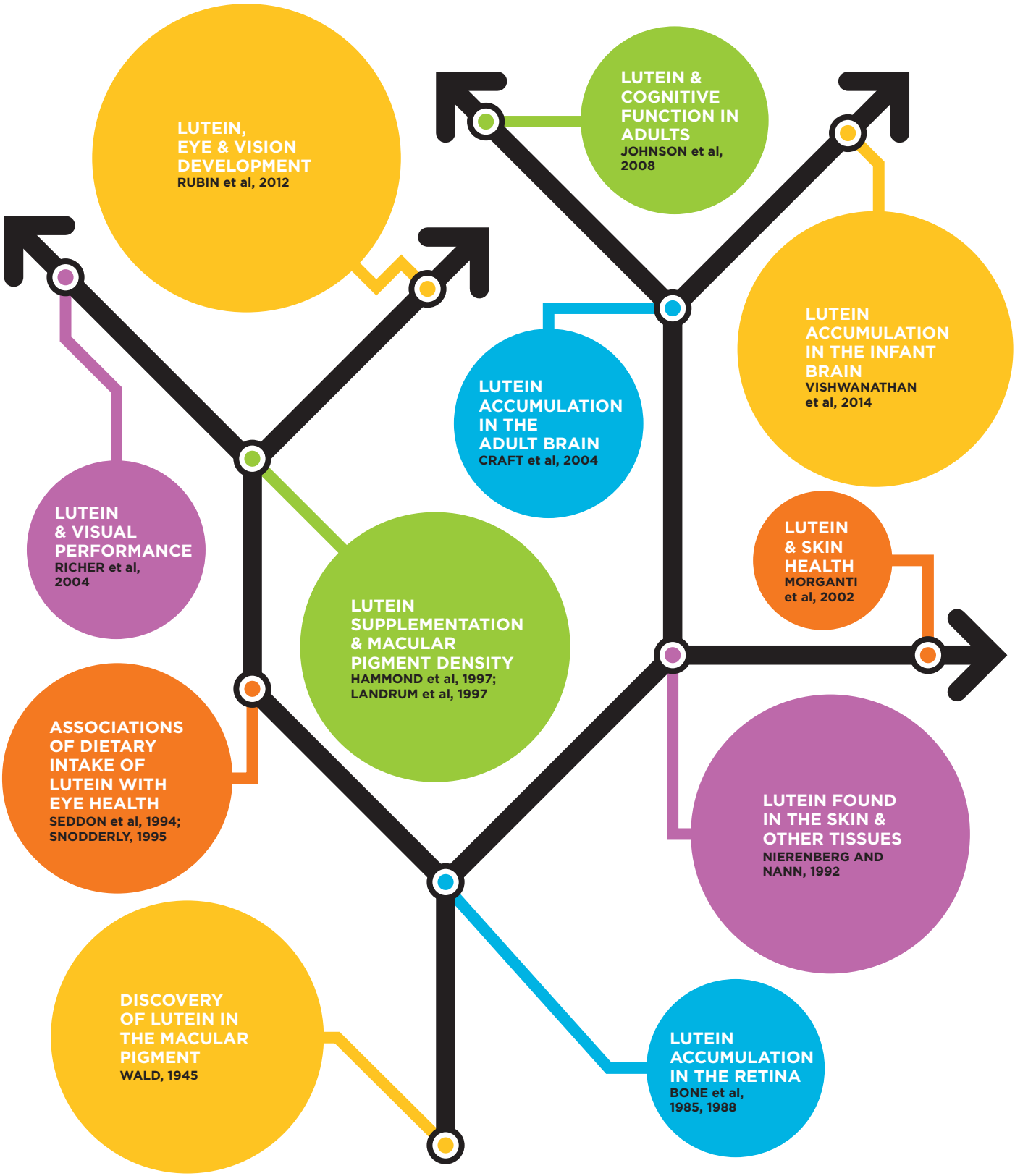
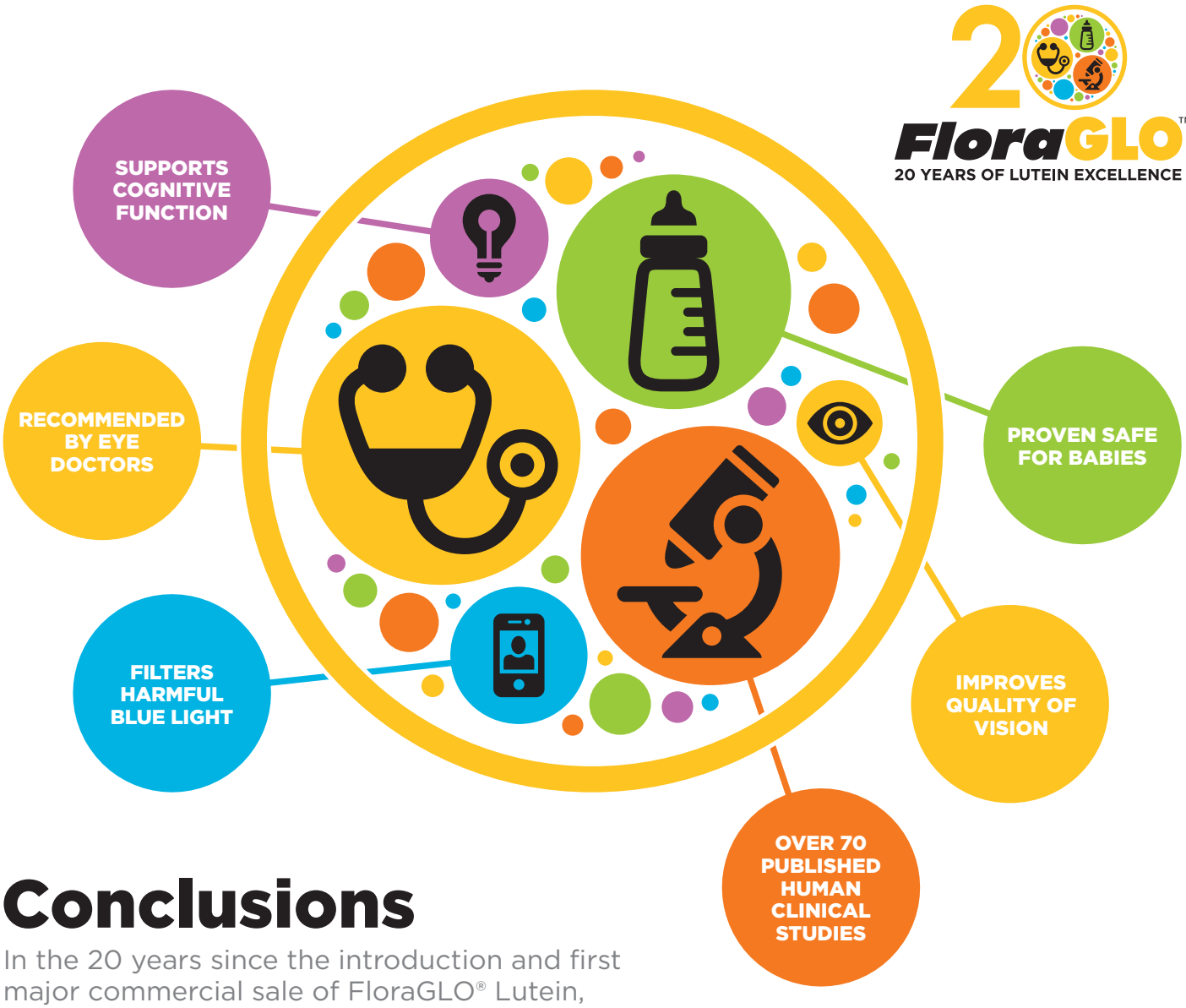


Figure 11: A relational view of some highly influential research publications in the field of lutein and human nutrition



Conclusions

In the 20 years since the introduction and first major commercial sale of FloraGLO® Lutein, great strides have been made in the research of this important nutrient.

These leaps were enabled by the availability of a quality lutein source in FloraGLO®, and the commitment, investment and countless hours of time and effort of Kemin and DSM into driving the science and public awareness of lutein. This commitment is evidenced by the overwhelming amount of published data in support of the FloraGLO® brand. Therefore, it should not be surprising that FloraGLO® is the lutein brand most studied by researchers[65] and recommended by eye doctors.[66] The established reputation of the FloraGLO® brand for safety, quality, and efficacy of FloraGLO® Lutein is evidenced by the over 70 separate publications describing the results of clinical studies that have used FloraGLO® Lutein as the lutein source. No other brand of lutein comes close to that number.

"Science-backed, not borrowed" is more than a slogan for FloraGLO® Lutein. It is a fact. You can trust FloraGLO® Lutein for all your lutein needs, today, tomorrow and forever.

References available upon request.