



HUMAN TRIALS WITH FLORAGLO® LUTEIN

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KEY CONCLUSIONS

- *Human studies published in peer-reviewed scientific journals show that consumption of FloraGLO® Lutein may provide significant benefits to the eyes and skin.*
- *Plasma concentration of lutein and macular pigment density increased following ingestion of FloraGLO Lutein.*
- *Visual function improved in patients suffering from age-related macular degeneration following ingestion of FloraGLO Lutein.*
- *Skin hydration, lipid content, and elasticity were increased while lipid peroxidation was decreased in women supplemented with FloraGLO Lutein.*
- *No toxicological side effects were reported in any of these studies.*

A total of 23 studies have been completed in humans using FloraGLO Lutein. These have either been published in peer-reviewed journals, or presented as abstracts at scientific meetings. The majority of the research has been in the eye health area where lutein is known to be deposited in the macula and lens. However, new research also supports lutein's beneficial role in certain skin health parameters, such as improvements in skin hydration, elasticity, lipids, and reduction in lipid peroxidation.

EYE HEALTH

Visual function studies

The following is a listing of clinical studies that show positive relationships between FloraGLO Lutein supplementation and visual function, including those affected by age-related macular degeneration (AMD), a disease that is the leading cause of blindness in the Western world (3):

- Dr. Stuart Richer from the Department of Veteran Affairs Medical Center in North Chicago and colleagues published the results of the Lutein Antioxidant Supplementation Trial (LAST) in the journal *Optometry*. In this double-masked, placebo-controlled trial, age-related macular degeneration (AMD) patients were supplemented for 12 months with either 10 mg FloraGLO Lutein, an antioxidant nutritional supplement which also contained 10 mg FloraGLO Lutein, or placebo (21). Ophthalmic testing, including macular pigment optical density (MPOD), glare recovery, near and distance visual acuity, and contrast sensitivity, was conducted at baseline and at 4, 8, and 12 months. Activities of daily living, night driving, and glare recovery symptoms were evaluated on a VFQ-14 rating scale used by the National Eye Institute. Similarly, subjects were provided with an Amsler grid to monitor changes in vision over time.

Results: In this study, AMD patients experienced a significant increase in MPOD after FloraGLO Lutein supplementation alone or together with other antioxidants (36 and 43% respectively; $p=0.03$ between baseline and final visit). Glare recovery, contrast sensitivity, visual acuity, quality of vision, and visual function over time also improved in both groups receiving FloraGLO Lutein supplements. The authors conclude that the improvements seen in this study may be due to lutein's dual role in the body as a blue light filter and antioxidant. These results also confirm that AMD may be a nutrition responsive disorder.

- Bahrami and colleagues at Johns Hopkins University School of Medicine investigated the effects of lutein supplementation on visual acuity, contrast sensitivity, and central visual field in 34 subjects diagnosed with retinitis pigmentosa. This randomized, double blind, cross-over, placebo controlled study supplemented retinitis pigmentosa subjects with placebo or 10 mg/day FloraGLO Lutein for 12 weeks followed by 30 mg FloraGLO Lutein/day for the following 12 weeks (2).

Results: Although no significant difference in visual acuity or contrast sensitivity was detected, there was a significant reduction in the natural decline of vision due to *retinitis pigmentosa* ($p < 0.05$) in subjects supplemented with FloraGLO Lutein. Significant improvements were also detected ($p = 0.038$) for visual field. No significant adverse events were documented while subjects consumed the FloraGLO Lutein supplements.

- Kvanakul and colleagues used the Lutein Zeaxanthin Eye Accumulation (LUXEA) Study Group to investigate the role of macular pigment in the eye (15). Subjects participating in the LUXEA study ($n = 34$) were supplemented with FloraGLO Lutein (10 mg/day for the first six months, and 20 mg/day for the last six months), zeaxanthin (10 mg/day for the first 6 months, and 20 mg/day for the last six months), or the combination of the two (10 mg each/day) for one year. Contrast acuity thresholds (CATs), macular pigment optical density (MPOD), wavefront aberrations, and scattered light were measured.

Results: A trend of improved CAT was detected in subjects supplemented with FloraGLO Lutein, zeaxanthin, or the two combined; however, this was significant only for the FloraGLO Lutein group. Decreasing trends of light scattering and wavefront aberrations were detected but not significant. This indicates that supplementation with FloraGLO Lutein may improve visual performance at low illumination.

- In a study conducted by researchers at Johns Hopkins University School of Medicine in Baltimore, 16 retinitis pigmentosa patients were supplemented with 40 mg FloraGLO Lutein per day for nine weeks and then placed on a maintenance dose of 20 mg FloraGLO Lutein per day for 15 weeks. Visual acuity tests were self administered on computer screens and wall charts (10).

Results: Results showed significant improvements in the visual acuity of these patients. This study was the first to show an improvement in the vision of retinitis pigmentosa patients following lutein supplementation, and suggests that FloraGLO supplementation may be beneficial for eye conditions other than AMD.

- Richer and colleagues used a clinical protocol to investigate the effect of consuming a diet with foods rich in lutein and zeaxanthin or taking a FloraGLO Lutein supplement on patients with atrophic AMD (20).

Results: Using his AMD work-up protocol, Dr. Richer was able to measure moderate vision improvements or complete resolution of scotomas metamorphosis in seven of eight patients that consumed five ounces of spinach four to seven times per week and in three patients consuming FloraGLO Lutein. This was the first study to show that a lutein-containing supplement may improve objective visual parameters in AMD patients.

MACULAR PIGMENT STUDIES

The macular pigment optical density (MPOD) refers to the amount of lutein and zeaxanthin that is present in the macula, the central portion of the retina upon which light is focused. Studies show that there appears to be an inverse association between the levels of MPOD and the risk of developing AMD (6). The following is a listing of clinical studies that show FloraGLO Lutein supplementation can increase MPOD:

- Ten subjects diagnosed with AMD were supplemented with 6 mg FloraGLO Lutein (in a supplement produced by Twinlab® Specialty Corp.) for 40 days by a group of German researchers. The objective of the study was to determine if short-term lutein supplementation increases MPOD. Imaging spectrometry and laser scanner images (488 nm) were used for MPOD measurements. Unlike psychophysical methods (e.g. reflectometry), these techniques are independent of the patient's ability for foveal fixation suggesting they may be more objective and accurate (23).

Results: Increases in MPOD were observed in some subjects after 40 days of FloraGLO Lutein supplementation. The optical density reached a plateau after 30 days of supplementation. Imaging spectrometry and laser scanning techniques both allowed for successful determination of macular pigment. This study suggests that short-term FloraGLO Lutein supplementation may influence MPOD in AMD patients.

- A group from the University of Utah headed by ophthalmologist Dr. Paul Bernstein tested a revolutionary new technology for measuring MPOD. The technology, developed by Dr. Bernstein and colleagues, utilizes Raman spectroscopy to measure carotenoids in certain human tissues. Sixty-three AMD patients were divided into two groups: those reporting consumption of a lutein supplement (≥ 4 mg/day), and those not consuming a lutein supplement. The patients' macular pigment density was measured using the Raman technology and was compared to that for 138 age-matched controls (5).

Results: Of the patients consuming a lutein supplement, the majority reported usage of products containing FloraGLO Lutein, including *Ocuvite with lutein* (Bausch & Lomb®), *OcuGuard Plus* (Twin Labs®), *ICaps with lutein* (Alcon®). These patients also showed a MPOD 43% higher than patients not consuming a lutein supplement and comparable to that of age-matched controls as measured by Raman spectroscopy. This study suggests that consumption of a supplement containing FloraGLO Lutein may help maintain MPOD, which is a factor in eye health. Moreover, this represents the first peer-reviewed publication demonstrating the use of Raman technology to measure MPOD in AMD patients using a lutein supplement.

- Researchers from the University of Pennsylvania supplemented a group of patients suffering from *choroideremia*, a genetically-linked retinal disease that has no known cure, with 20 mg FloraGLO Lutein per day for 6 months (11).

Results: This dose of lutein significantly increased both serum lutein levels and MPOD in these patients. This suggests that FloraGLO Lutein supplementation may benefit those with eye conditions other than AMD.

- Dr.'s Aleman and Duncan of the University of Pennsylvania, supplemented a group of retinitis pigmentosa and usher syndrome patients with 20 mg FloraGLO Lutein per day for six months (1).

Results: This dose of FloraGLO Lutein increased serum levels of lutein in all patients. Macular pigment optical density was significantly increased in half of the patients. This study was one of the first to show that macular pigment density may be improved in retinitis pigmentosa patients with FloraGLO Lutein supplementation.

- At the 2005 Association for Research in Vision and Ophthalmology (ARVO) annual conference two abstracts were presented discussing results from the Lutein Xanthophyll Eye Accumulation (LUXEA) study (13, 22). LUXEA is a randomized, placebo-controlled, prospective pilot supplementation trial investigating the affects of repeated dosing of FloraGLO Lutein (10 mg/day), zeaxanthin (10 mg/day), or the combination of the two (10 mg each/day) for one year.

Results: Supplementation with FloraGLO Lutein and zeaxanthin alone or combined resulted in increased plasma concentrations and MPOD.

- Dr.'s Landrum and Bone from Florida International University presented a supplementation study at the 2000 Association for Research in Vision and Ophthalmology meeting in which 24 normal subjects were given 2.4 mg FloraGLO Lutein per day for six months (16).

Results: This relatively low dose of lutein significantly increased both serum lutein levels and MPOD. Increases in MPOD levels have been linked with reduction in risk of AMD (6). This suggests that even at low doses, FloraGLO Lutein may benefit eye health.

Lutein levels in the serum or plasma

The following is a listing of clinical studies that show relationships between FloraGLO Lutein supplementation and the levels of lutein in the serum or plasma:

- Thurman and colleagues characterized the plasma kinetics of lutein in healthy subjects who consumed 0, 4.1, or 20.5 mg FloraGLO Lutein orally for 42 days. Subjects were asked to avoid lutein and zeaxanthin rich fruits and vegetables while participating in the study (24).

Results: All subjects completed the study and no adverse events related to FloraGLO Lutein consumption occurred. Lutein levels remained steady in control subjects not consuming lutein supplements. Plasma levels of lutein increased after consumption of 4.1 and 20.5 mg of FloraGLO Lutein daily for 42 days (3.5 and 10 fold respectively). No significant changes in lycopene, α -carotene, β -carotene, β -cryptoxanthin, or retinol levels were detected after supplementation of FloraGLO Lutein at low (4.1 mg) or high (20.5 mg) doses.

- Molldrem and colleagues at the University of Wisconsin compared serum lutein concentrations in subjects consuming genetically selected white carrots, genetically selected lutein-containing yellow carrots, or FloraGLO Lutein supplement in a randomized, blinded, crossover trial. White carrots contained no lutein while both lutein-containing carrots and FloraGLO Lutein supplement provided 1.7 mg lutein/day. Subjects consumed the treatments for seven days followed by a seven day washout period between treatments (17).
Results: While consuming white carrots, subjects had a small but significant ($p < 0.0001$) decrease in serum lutein concentration. Both lutein-containing yellow carrots and FloraGLO Lutein supplement significantly increased serum lutein concentrations; however, the relative lutein bioavailability from lutein-containing yellow carrots was 65% of that of the FloraGLO lutein supplement.
- Researchers from three French medical laboratories supplemented 29 non-smoking males with 9 mg FloraGLO Lutein for five weeks. Subjects from two age groups (20-35 and 60-75) were recruited for the study. Lutein was measured by HPLC in fasting serum, adipose tissue, and buccal mucosa cells (BMC) before and after supplementation. MPOD was also measured by reflectometry before and after supplementation (7).
Results: Plasma lutein increased in all subjects in response to FloraGLO Lutein supplementation. BMC also increased significantly in both groups in response to FloraGLO Lutein supplementation. Significant differences between age groups did not exist in the plasma lutein and BMC responses to FloraGLO Lutein supplementation. These results suggest lutein status in the body is not significantly modified in healthy elderly men and that the dietary recommendations for lutein in young adults can be applied to healthy older adults.
- Researchers at the Wageningen Agricultural University, Netherlands, conducted a study to compare the effect of processing spinach (disruption of the plant matrix) on the bioavailability of carotenoids relative to a supplement (9) and the impact of consumption of spinach or supplement on antioxidant enzyme activities (8). Seventy-two subjects were supplemented with various spinach products (10-12 mg lutein per day) or 6.6 mg FloraGLO Lutein per day (in salad dressings) for three weeks.
Results: Disruption of the matrix by various processing means had no effect on the lutein bioavailability from spinach. In addition, lutein absorption from FloraGLO Lutein was double that of any of the spinach products. This suggests lutein from FloraGLO may be more bioavailable than that from spinach. The consumption of spinach or carotenoid supplement resulted in greater responses of erythrocyte glutathione reductase activity and lowered the serum α -tocopherol response. Spinach consumption alone lowered erythrocyte catalase. This effect is therefore not likely associated with the carotenoid content of spinach.
- A group from Unilever Research Vlaardingen, Netherlands, examined the bioavailability of lutein from vegetables. Subjects ingested a low-vegetable diet (containing approximately 3 mg lutein/day), a high-vegetable diet (including spinach, broccoli, and other greens; equivalent to approximately 11 mg lutein/day), or a carotenoid supplement (containing approximately 11 to 12 mg FloraGLO Lutein /day), for four weeks. Bioavailability of a variety of antioxidants was determined by measuring the change in serum levels (25).
Results: Serum lutein in the low-vegetable group increased the least amount, followed by a dramatic increase in serum lutein from the high-vegetable group. However, serum lutein derived from the carotenoid supplement increased the most of all three groups, more than 50% higher than the high-vegetable group. This study supports the idea that FloraGLO Lutein may be more bioavailable than that from vegetable sources.
- Researchers at the USDA Human Nutrition Research Center in Beltsville, MD, supplemented three subjects with 10 mg per day of FloraGLO Lutein (in olive oil) for 18 days (12).
Results: No toxicological side effects were reported. The serum lutein level increased significantly after one week of supplementation with FloraGLO Lutein. Similarly, ingestion of lutein resulted in increased concentration of lutein oxidation products. The data generated from this study revealed the existence of oxidized metabolites of lutein, suggesting lutein acts as an antioxidant *in vivo*.
- Investigators from Iowa State University provided eight subjects with 0.3 mg/kg body weight of FloraGLO Lutein and/or β -carotene in single doses (14).
Results: Results showed that peak lutein concentration in the serum was achieved after 16 hours and returned to baseline at 440 hours. This was one of the first studies to examine the absorption profile for lutein following ingestion of a FloraGLO Lutein supplement in humans.

- At the 2002 American Association of Ophthalmology Annual Meeting Dr. Paul Bernstein, from the University of Utah, presented a study in which 46 elderly subjects received an antioxidant supplement containing FloraGLO Lutein or a diet rich in carotenoids for 12 weeks (4).

Results: Consumption of an antioxidant supplement containing lutein or a high carotenoid diet increased the serum level of lutein significantly in these subjects. These findings suggest that a supplement containing FloraGLO Lutein may be as effective in increasing serum lutein levels as a diet rich in carotenoids.

SKIN HEALTH

The protective functions of lutein seem to be centered on safeguarding tissue from light damage. Light can affect both the eyes and the skin. Emerging science has looked at the relationship between lutein supplementation and skin health. The following is a listing of clinical studies that show relationships between FloraGLO Lutein supplementation and improving skin health parameters:

A clinical study presented at the “Beyond Beauty Paris” 2006 conference in France provided new evidence that FloraGLO Lutein alone may provide specific skin health benefits including increases in skin hydration, skin lipids, and skin elasticity, while decreasing skin lipid peroxidation (19). Dr. Morganti, professor of applied cosmetic dermatology at the University of Naples, supplemented female subjects, age 25-50, over a 12-week period with placebo, 5 mg FloraGLO Lutein taken twice a day orally, a topical suspension containing 50 ppm FloraGLO Lutein applied twice daily to the skin, or the combination of both 5 mg FloraGLO Lutein taken twice a day orally and a topical suspension containing 50 ppm FloraGLO Lutein applied twice daily. Skin hydration, elasticity, lipid peroxidation, and lipid content were followed throughout the course of the study.

Results: Both oral and topical administration of FloraGLO Lutein had a significant effect on all parameters analyzed (**Table 1**). Additive improvements were observed in skin hydration and skin lipid content in subjects supplemented with the combined oral and topical treatments. This study is the first to show improvements in skin health through the supplementation of FloraGLO Lutein alone either orally or topically.

Table 1. Average maximal changes in parameters of skin health following supplementation of FloraGLO Lutein orally, topically, or combined.

	Maximum Percentage Change ^a in			
Route of Administration	Skin Hydration	Skin Elasticity	Skin Lipid Peroxidation	Superficial Skin Lipids
Oral	38	8	55	33
Topical	40	21	62	11
Combined Oral and Topical	60	20	64	50

^a After correcting for the contribution of the placebo treatment

Morganti and colleagues investigated the effect of an oral supplement containing 6 mg of FloraGLO Lutein per day plus other antioxidants, combined with a topically applied antioxidant formulation, upon several crucial human skin health parameters. This study looked at changes in 1) skin hydration, 2) skin lipids, and 3) skin lipid peroxidation. The female subjects in this study were randomly assigned to cells that involved the use of 1) two placebo products (oral and topical), 2) the oral antioxidant product (providing 6 mg FloraGLO Lutein plus other antioxidants) with a placebo topical formulation, or 3) the oral antioxidant product (providing 6 mg FloraGLO Lutein plus other antioxidants) and the topical antioxidant formulation. Following a two-week washout period, the subjects were given their designated products to use. Changes in the study parameters were measured at two-week intervals throughout the eight-week study period. The average values for subjects using the two placebo products were compared to those for subjects using the oral product plus the placebo topical formulation.

Results: The test results showed that the FloraGLO Lutein-containing oral antioxidant product resulted in statistically significant increases in skin hydration and skin lipids as well as decreases in skin lipid peroxidation at the two-week evaluation. These statistically significant differences continued throughout the entire eight-week test period (18).



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