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SUPPLEMENTAL MESO-ZEAXANTHIN - NOT WHAT NATURE INTENDED

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KEY CONCLUSIONS

- *Meso-zeaxanthin (MZ) is found in the macula due to the natural conversion by the body of lutein to MZ.*
- *Meso-zeaxanthin, unlike lutein and zeaxanthin, is not found at appreciable levels in the diet.*
- *No studies have been published evaluating the safety or efficacy of meso-zeaxanthin without the presence of lutein and zeaxanthin.*
- *Commercial sources of meso-zeaxanthin are the result of the semi-synthetic chemical conversion of lutein.*
- *Meso-zeaxanthin (sometimes labeled as "zeaxanthin isomers") is not a replacement for dietary zeaxanthin.*

WHAT IS MESO-ZEAXANTHIN?

Meso-zeaxanthin (MZ), sometimes labeled as "zeaxanthin isomers", is a stereoisomer of zeaxanthin that is not found as a natural constituent of food but instead is converted by the body from lutein and deposited in the macula along with lutein and zeaxanthin as part of the macular pigment. (*1, 2*) Monkeys fed a xanthophyll-free diet and subsequently supplemented with lutein alone had both lutein and *meso*-zeaxanthin present in the macula. In contrast, no *meso*-zeaxanthin accumulated in retinas of monkeys fed only zeaxanthin.(*3*) These findings are supported by studies performed in Japanese quail.(*4*)

Meso-zeaxanthin and zeaxanthin differ in their biochemical structure as shown in Figure 1. Zeaxanthin refers to (3R, 3'R)-zeaxanthin, which is the structure found in dietary sources of zeaxanthin, such as peppers, corn and eggs. *Meso*-zeaxanthin is a different stereoisomer from zeaxanthin with a (3 R, 3'S) spatial orientation at position 3. Commercially available *meso*-zeaxanthin (sometime referred to as "zeaxanthin isomers") cannot be extracted from plant materials; it is synthesized by exposing lutein to heat and alkaline conditions to generate different isomeric ratios and chemical profiles from naturally occurring zeaxanthin from plant sources.(*5*) *Meso*-zeaxanthin is not a replacement for the zeaxanthin found naturally in the diet. Moreover, *meso*-zeaxanthin, being a chemical alteration of lutein, is considered semi-synthetic in the published literature.(*6*)

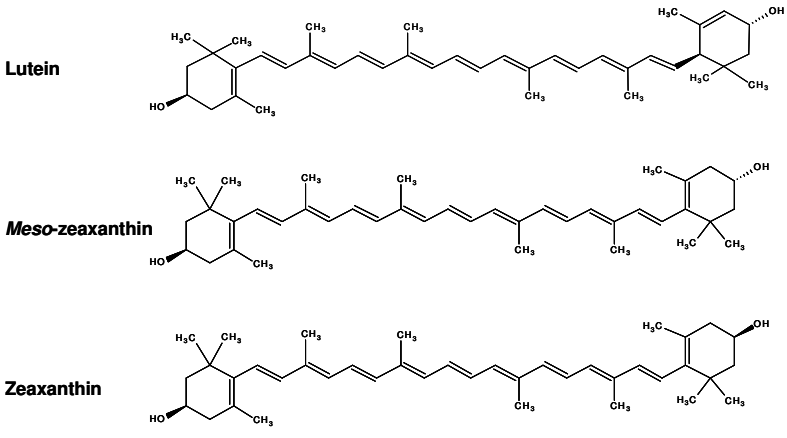

Figure 1: Molecular structures of lutein, *meso*-zeaxanthin, and zeaxanthin.

Several large studies have linked increased consumption of dietary lutein and (3R, 3'R)-zeaxanthin to reduced risk of developing age-related macular degeneration (AMD) (*7-9*) and cataracts.(*10, 11*) Additionally, numerous studies show that

supplementing with dietary lutein and dietary zeaxanthin results in increased macular pigment and improved visual function parameters such as glare tolerance and contrast sensitivity in both healthy adults and patients with eye diseases such as AMD and retinitis pigmentosa (RP).(*12-36*) Notably, dietary lutein (as FloraGLO® Lutein) and dietary zeaxanthin are included in the National Eye Institute's Age-Related Eye Disease Study II (AREDS2) which is investigating whether these nutrients reduce the risk of progression to advanced AMD.(*37*) *Meso*-zeaxanthin is not being studied in AREDS2.

It is important to point out than an overwhelming majority of the science linking supplemental lutein and zeaxanthin was conducted with forms of lutein and zeaxanthin found in the diet. *Meso*-zeaxanthin is not found in a typical diet. Maoka *et al. (38)* found *meso*-zeaxanthin present in trace levels in shrimp shells, fish skin, and turtle fat. It was not found in eggs and corn where dietary zeaxanthin is present in high concentrations. Rasmussen *et al.* attempted to repeat this study, but was unable to find any *meso*-zeaxanthin in any of the seafood samples.(*39*) *Meso*-zeaxanthin was found in one egg from southern California and eggs from Mexico where feed is commonly supplemented with pigment containing semi-synthetic *meso*-zeaxanthin. The Rasmussen study also analyzed pure standards of lutein using the methods employed by Maoka and found that this process created *meso*-zeaxanthin, suggesting that the prior findings were the result of an artifact created by the analysis method. The authors clearly reported that "*foods do not appear to be the source of (meso-zeaxanthin) in the macula in the US."* This conclusion can be intuitively extrapolated to other geographical regions such as Europe. Because of this lack of presence in the diet, *meso*-zeaxanthin supplementation is not supported by the body of epidemiological data linking diets rich in lutein and zeaxanthin with eye health benefits and reduced risks of eye disease.

SCIENCE IN ITS INFANCY

No published evidence exists on the effects of supplementation with meso-zeaxanthin alone in the absence of lutein and zeaxanthin. The number of studies supporting supplementation of *meso*-zeaxanthin in addition to dietary lutein and zeaxanthin is quite meager especially when compared to the overwhelming body of evidence supporting supplementation of lutein and zeaxanthin alone. Table 1 provides a summary comparing the body evidence for dietary lutein and zeaxanthin alone to the nominal research that reports on supplementation of *meso*-zeaxanthin in addition to lutein and zeaxanthin.

Table 1. Comparison of the Eye-Related Research on Lutein/Zeaxanthin alone versus in Combination with Meso-zeaxanthin

	Dietary Lutein (as FloraGLO) and Zeaxanthin	*Meso*-zeaxanthin (in combination with lutein and zeaxanthin)
Years of Study	18*	6
Total Number of Published Human Clinicals	63*	7
Serum Absorption	25*	6
Macular Pigment	21*	5
Visual Function	15*	1
Trials with Different Investigators**	✓	⊘
Found Naturally in…		
Diet	✓	⊘
Macula	✓	✓
Skin	✓	⊘
Breast Milk	✓	⊘
Brain	✓	⊘
Included in AREDS2	✓	⊘

*Based on a biannual PubMed search. Counts include studies using FloraGLO® Lutein – the most clinically researched lutein brand worldwide.(*40*) Numbers are considerably higher when other sources of dietary lutein and zeaxanthin are considered.
**Refers to trials where there were not individual investigators in common among all trials

There is currently no published evidence of the safety of pure *meso*-zeaxanthin in the absence of other xanthophylls (lutein and zeaxanthin). Additionally, *meso*-zeaxanthin does not have the long-term evidence of safety from history of use that exists for lutein and zeaxanthin. The safety of several *meso*-zeaxanthin formulations has been evaluated in three studies employing rat and mice models.(*41-43*) The three studies used test articles of varying *meso*-zeaxanthin purity.

A small number of *in vitro* and animal studies evaluating the potential health benefits of *meso*-zeaxanthin have been published.(*6, 44-49*) One noteworthy *in vitro* study by Li *et al.* is sometimes incorrectly provided as proof that *meso*-zeaxanthin is the strongest individual macular antioxidant. This study, designed to investigate the proposed protective mechanisms of macular pigment (MP), measured the ability of lutein (L), zeaxanthin (Z) and *meso*-zeaxanthin (MZ) alone and in a 1:1:1 combination to quench singlet oxygen in an *in vitro* assay. The combination regimen had the greatest ability to quench singlet oxygen followed by MZ, Z and L. The authors state that "*MP carotenoids composed in this ratio may optimally quench singlet oxygen and other reactive oxygen species in human retina, although in vivo data are still not available*." *In vitro* antioxidant data is simply not sufficient to support the use of supplemental *meso*-zeaxanthin alone or in combination with dietary lutein and zeaxanthin.

At the date of this document, seven publications report on the human clinical findings for supplementation that included lutein, zeaxanthin and *meso*-zeaxanthin. Six of these studies report on serum response, five studies report on macular pigment response and only one study reports on the effect of *meso*-zeaxanthin, in combination with lutein and zeaxanthin, on visual function. In contrast to lutein and zeaxanthin, there are no published studies reporting on the effect of *meso*-zeaxanthin supplementation on visual function in patients with AMD. Additionally, none of the published studies supplemented with *meso*-zeaxanthin in the absence of lutein or zeaxanthin. Because of this, it is impossible to attribute the positive effects seen in these supplementation studies to *meso*-zeaxanthin alone. A summary of the published studies is provided in Table 2. A brief critical review is subsequently presented.

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Table 2. Summary Table of Human Studies that Included *Meso*-zeaxanthin Along with Lutein and Zeaxanthin

Study	Subjects	Design	N	L (mg)	Z (mg)	MZ (mg)	Dosage Form	Duration (weeks)	Outcome Measures	Conclusions
Bone *et al.* 2007 ***(50)***	Healthy	PC	10	5.5	1.4	14.9	Oil/ Gel cap	17	Serum, MPOD	• Non-significant increase in serum L and Total Z (Z + MZ) for treated group • Significant increase in the rate of change of MPOD in 60% of eyes tested for treated group
			9	0	0	0				
Thurnham *et al.* 2008 ***(51)***	Healthy	Open	19	10.8	1.2	8	Oil/ Capsule	3	Serum	• No MZ was found in the serum at baseline • Serum MZ levels increased from compared to baseline • Plasma response to L and Z appeared to be depressed by the presence of MZ
Connolly *et al.* 2010 ***(52)***	Healthy/ Early AMD	Open	10	3.7	0.8	7.3	Encapsulated/ Capsule	8	Serum, MPOD	• Significant increase in serum L and MZ from baseline • Significant increase in average MPOD
Connolly *et al.* 2011 ***(53)***	Healthy	RDBPC	22 (18)	5.9	1.2	10.6	Encapsulated/ Capsule	24	Serum, MPOD	• Significant increase in serum L and Total Z (Z + MZ) • Significant increase in central MPOD in treated group
			22 (17)	0	0	0				
Nolan *et al.* *2012* ***(54)***	Healthy	RDB	10[a]	20	2	0	Oil/ Softgel	8	MPOD	• Significant increase in MPOD at 0.25° and 0.5° for Group 2
			10[b]	10	2	10				
			10[c]	3	2	17				
Meagher *et al.* *2012* ***(55)***	Healthy/AMD	RDB	21[a]	20	2	0	Oil/ Softgel	8	Serum	• Significant increase in serum L for Groups 1 and 2 compared to baseline • Significant increase in serum Z for Groups 1 and 2 compared to baseline • Small but significant increase in serum MZ in all three groups compared to baseline*
			20[b]	10	2	10				
			13[c]	3	2	17				
Loughman *et al.* *2012* ***(56)***	Healthy	RSBPC	11[a]	20	2	0	Oil/ Softgel	26	Serum, MPOD, Visual Performance	• Non-significant increase in serum L for Group 1 compared to baseline • Significant increase in serum L for Group 2 compared to baseline • Significant increase in serum Z for Groups 1 and 2 compared to baseline • Small but significant increase in serum MZ for Group 2 compared to baseline* • Significant increase in MPOD for Group 2 compared to baseline • Significant improvement in mesopic contrast sensitivity for Group 1 compared to baseline • Significant improvement in mesopic and photopic contrast sensitivity for Group 2 compared to baseline
			11[b]	10	2	10				
			10[c]	0	0	0				

L - Lutein, Z – Zeaxanthin, MZ – *meso*-zeaxanthin, R – Randomized, DB – Double Blind, SB – Single Blind, PC – Placebo Controlled [a] Group 1; [b] Group 2, [c] Group 3 * No MZ was present in the serum at baseline

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ADDITIONAL OBSERVATIONS FROM THE TRIALS TESTING *MESO*-ZEAXANTHIN IN COMBINATION WITH LUTEIN AND ZEAXANTHIN

Interference with Absorption of Lutein and Zeaxanthin. It was noted in five of the six studies that measured serum absorption that the presence of MZ may alter or impair the absorption of lutein and zeaxanthin. In Bone *et al.* the authors commented that the serum responses for L and Z+MZ were modest and perhaps this was due to poor bioavailability of supplement and/or possible competition among the carotenoids for serum uptake. Thurnham *et al.* also found slightly lower response for L and Z in the presence of MZ compare to studies supplementing with pure L and Z.

In Connolly *et al.* the authors commented that the 1.3 fold increase seen in lutein was consistent with other studies by Bone and Berendschot (*57, 58*); however, when the data are compared as µmol/L per mg increase, the response seen (0.019 µmol/L per mg) is quite low when compared to those same studies (Bone – 0.063 µmol/L per mg and Berendschot – 0.072 µmol/L per mg). The lack of a significant response for dietary zeaxanthin is also not consistent with other studies. The authors comment that this is possibly due to the low dose of Z in the formulation (0.8 mg per capsule), however, other studies with similar low doses of zeaxanthin given along with lutein have reported significant serum response(*23*). Also, while the MZ serum response was significant, it is noteworthy to point out that it was much more modest compared to lutein with final serum concentrations for L being six times higher than those for MZ despite being present in the supplement at roughly ½ the dose of MZ (3.7 mg L vs 7.3 mg MZ). While the second study by Connolly *et al.* only evaluated the response of total Z (Z+MZ), the authors still observed a lower serum response for L and Z when compared with previous studies.

The study by Meagher *et al.* highlights the effect of supplementing with a high dose of MZ (17 mg) compared to L (3 mg) and Z (2 mg). Despite the low lutein dose in Group 3, the lack of significant increase in serum L is unexpected based on results of previous studies.(*59, 60*) Additionally, the zeaxanthin dosage was constant across all three groups, however, the serum response was significantly lower (in fact, negative) in Group 3. These observations suggest that there is competition for absorption between MZ and Z and that high MZ can impair L and Z absorption. Also, while the total carotenoid dose remained constant for all three groups, the serum response for total carotenoids was dramatically lower for Group 3 which contained high levels of MZ.

Macular Pigment Response. Significant increases in macular pigment optical density (MPOD) were seen in the five studies that assesses this endpoint. It should be noted; however, that in Bone *et al.* the authors' method of reporting rate of change in MPOD in individual subjects is atypical compared to the body of evidence on regarding the ability of supplementation to improve MPOD and makes it very difficult to compare with other studies. The researcher attempt to attribute the rapid rise seen in central MPOD observed to the presence of MZ in the formulation but method for measuring MPOD is not xanthophyll specific and only measures increases in total macular pigment and not MZ. Additionally, since none of these studies supplemented with MZ alone, one cannot conclude that the rise in MPOD is due to MZ. In fact, in Nolan *et al.* one arm was supplemented with a combination with a much higher MZ content compared to the L and Z and this significant rise in central MPOD was not observed.

Visual Function. Loughman *et al.* was the only study to report on the effect of supplementing with MZ in combination with L and Z on visual function. Statistically significant improvements in visual performance measures including visual acuity and contrast sensitivity with and without glare were observed for Group 2 (10 mg L, 10 mg MZ and 2 mg Z) only. Significant improvement in mesopic contrast sensitivity at one spatial frequency was observed at 6 months for Group 1 (20 mg L and 2 mg Z) ($p < 0.05$). No improvements in any parameters of visual performance were observed for subjects

supplemented with placebo. This general lack of improvement in visual performance parameters after 6 months of supplementation with 20 mg lutein is questionable. The results of Stringham *et al.* showed a significant improvement in glare disability and foveal photostress recovery times at both 4 and 6 months of supplementation with 10 mg L and 2 mg L compared to baseline.(*24*) Additionally, Group 2 was the only arm with significant MPOD response.

These unexpected Group 1 results can be easily explained by the lack of serum response which is possibly associated with poor compliance. A statistically significant change in serum L, Z, MZ and total carotenoids concentration across visits was observed in Group 2 only ($p < 0.01$ for L and MZ and $p < 0.05$ for Z). No statistically significant change across visits was observed for serum L or MZ in Group 1 or Placebo. The serum lutein data for this study, showing a lack of typical serum response following lutein supplementation, are suspect and provide doubt about the degree of compliance by the subjects and could likely explain the lack of performance seen by Group 1. While the authors did include methods to ensure compliance, actual data on compliance was not reported. Group 1 serum data had very large deviations at 3 and 6 months such that the increase seen for lutein did not reach significance. Additionally, the serum Z increase was also quite modest. The other unexpected element was that the mean serum concentrations for lutein decreased by 40% from month 3 to month 6 rather than achieve and maintain the expected plateau seen in the body of evidence for lutein serum response to recurrent supplementation. Additionally, the Meagher et al study conducted by the same authors in the same research center with a similar number of subjects and the same commercial L and Z supplement used in Group 1 showed a statistical significant increase in serum L and Z level after 4 and 8 weeks supplementation. With the questionable Group 1 serum data seen in this study, it is impossible to draw any accurate conclusions about the efficacy of lutein and zeaxanthin supplementation (with or without MZ) on MPOD and visual performance.

Investigators. An additional limitation of the seven human studies is the absence of different independent researchers across all studies. The use of unaffiliated, independent investigators across studies is considered by regulatory authorities helpful in insuring reproducibility and reliability of study results(*61*). Alan Howard, Chair, Howard Foundation, who is noted in Bone *et al.* as having a proprietary interest in the use of MZ to raise MPOD and treat macular disorders, is an author on all seven studies. A majority of these studies also cite funding provided by the Howard Foundation.

CONCLUSION

In summary, *meso*-zeaxanthin is not found naturally in the diet. Supplemental *meso*-zeaxanthin is synthesized from lutein in the presence of heat and alkaline conditions and cannot be extracted from plant materials. Often labeled in a confusing manner as “zeaxanthin isomers”, *meso*-zeaxanthin is not a replacement for dietary zeaxanthin. Because *meso*-zeaxanthin is not found in the diet and particularly not in foods like green leafy vegetables, eggs or corn, which are good sources of lutein and zeaxanthin, supplementation of *meso*-zeaxanthin is not supported by epidemiological study data and other research linking the consumption of diets rich in lutein and zeaxanthin with eye health benefits and reduced risk for eye disease Additionally, while three animal studies address the safety of carotenoid mixtures including *meso*-zeaxanthin, this substance cannot rely on the clear long-term evidence of safety from history of use that exists for lutein and zeaxanthin .

Meso-zeaxanthin science is still in its infancy. Only seven human trials supplementing with *meso*-zeaxanthin (in combination with lutein and zeaxanthin) have been published as of the date of publication of this summary and only one assessed its impact upon visual function. However, since none of the studies supplemented with *meso*-zeaxanthin in the absence of lutein or zeaxanthin, it is difficult to determine to what extent, any study effect is directly attributable to *meso*-zeaxanthin.

There is a much larger body of evidence supporting the benefits of lutein and zeaxanthin supplementation on MPOD and visual function in both healthy adults and patients with ocular disease.(*62*) A thorough comparison of the science provides a clear picture of the benefits of supplementing in dietary lutein and zeaxanthin alone. Notably, dietary lutein and

zeaxanthin, and not *meso*-zeaxanthin, are included in the NEI's AREDS2 which is investigating the ability of supplement interventions to reduce the risk of progression to advanced AMD.

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