Original Communication

Lutein Complex Supplementation Increases Ocular Blood Flow Biomarkers in Healthy Subjects

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Abstract: *Introduction:* To investigate the effects of a lutein complex supplementation on ocular blood flow in healthy subjects. *Materials and Methods:* Sixteen healthy female patients (mean age 36.8 ± 12.1 years) were enrolled in this randomized, placebo-controlled, double-blinded, two-period crossover study. Subjects received daily an oral dose of the lutein with synergistic phytochemicals complex (lutein (10 mg), ascorbic acid (500 mg), tocopherols (364 mg), carnosic acid (2.5 mg), zeaxanthin (2 mg), copper (2 mg), with synergistic effects in reducing pro-inflammatory mediators and cytokines when administered together in combination) and placebo during administration periods. Measurements were taken before and after three-week supplementation periods, with crossover visits separated by a three-week washout period. Data analysis included blood pressure, heart rate, intraocular pressure, visual acuity, contrast sensitivity detection, ocular perfusion pressure, confocal scanning laser Doppler imaging of retinal capillary blood flow, and Doppler imaging of the retrobulbar blood vessels. *Results:* Lutein complex supplementation produced a statistically significant increase in mean superior retinal capillary blood flow, measured in arbitrary units (60, $p = 0.0466$) and a decrease in the percentage of avascular area in the superior (−0.029, $p = 0.0491$) and inferior (−0.023, $p = 0.0477$) retina, as well as reduced systolic (−4.06, $p = 0.0295$) and diastolic (−3.69, $p = 0.0441$) blood pressure measured in mmHg from baseline. Data comparison between the two supplement groups revealed a significant decrease in systemic diastolic blood pressure (change from pre- to post-treatment with lutein supplement (mean (SE)): −3.69 (1.68); change from pre- to post-treatment with placebo: 0.31 (2.57); $p = 0.0357$) and a significant increase in the peak systolic velocity (measured in cm/sec) in the central retinal artery (change from pre- to post-treatment with lutein supplement: 0.36 (0.19); change from pre- to post-treatment with placebo: −0.33 (0.21); $p = 0.0384$) with lutein complex supplement; data analyses from the placebo group were all non-significant. *Discussion:* In healthy participants, oral administration of a lutein phytochemicals complex for three weeks produced increased ocular blood flow biomarkers within retinal vascular beds and reduced diastolic blood pressure compared to placebo.

Keywords: Ocular blood flow, nutraceuticals, eye disease, health, diet

Introduction

Age-Related Eye Disease (ARED) is a group of conditions that are associated with an increased risk of manifesting later in life and include: age-related macular degeneration (AMD), cataract, diabetic retinopathy, glaucoma, dry eye, and low vision. AMD and cataract are the leading causes of visual impairment in the United States [1]. According to the National Eye Institute (NEI), approximately 1.7 million Americans suffer from some type of AMD, and over 1.5 million cataract surgeries are performed annually [1, 2]. Additionally, the NEI estimates that glaucoma may affect over 6 million Americans by the year 2050 [3, 4].

Among ARED, AMD, a degenerative and progressive disease of the macula, is the leading cause of irreversible central vision loss in developed countries. Specifically, AMD is expected to affect over 288 million people worldwide by 2040 [5]. Recently, vascular abnormalities have been postulated to play a contributory role in AMD pathophysiology [6]. The more common and less severe form is the atrophic subtype, in which extracellular deposits called drusen disrupt the flow of blood and nutrients from the choroid to the retinal pigment epithelium, resulting in retinal cell death and atrophy [7]. In approximately 10-20% of cases, atrophic AMD progresses to exudative AMD via choroidal neovascularization (CNV), where new vessels form around

drusen deposits that result in fluid and blood leakage into the retina causing functional and anatomical disruption of the overlying structures [7]. Exudative AMD is rapidly progressive and is responsible for the majority of the severe vision loss associated with AMD [7]. While anti- vascular endothelial growth factor (VEGF) agents benefit patients with exudative AMD, there is currently no definite preventative or curative treatment for atrophic AMD [7].

The severity and irreversibility of ARED pathology has created an interest in researching methods to either prevent or slow its progression. Nutritional methods have demonstrated improvements in microvascular function in hypertensive patients [8], and consequently, the influence of vitamin supplementation has been studied in both healthy volunteers and those with ARED to determine its role in disease prevention and progression. Although it has not been confirmed to be beneficial in glaucoma patients, the AREDS vitamin formulation study found that high-risk patients with AMD may experience up to a 25% risk reduction when taking certain vitamin combinations [9].

Although the incidence of ARED including AMD continues to rise, often the pathogenesis of ARED are not fully elucidated. Among potential mechanisms, studies have shown that systemic inflammation, which affects choroidal blood flow and results in vascular disturbances, is involved in the pathophysiology of AMD [10, 11]. As a key inducer of inflammatory pathways, tumor necrosis factor-α (TNF-α) causes increased endothelial permeability through activation of VEGF and nuclear factor κβ (NF-κβ) that results in the upregulation of other inflammatory mediators [12, 13]. The role of carotenoids (beta-carotene, lutein, and zeaxanthin) in the prevention of AMD has recently been highlighted with focus on macular pigments (MP) such as lutein and zeaxanthin, which are hypothesized to function as potent antioxidants [14–16]. A study by Hadad et al. demonstrated a significant synergistic effect of lycopene, lutein, beta-carotene, and carnosic acid in reducing pro-inflammatory mediators and cytokines such as TNF-α and interleukin-1 [17]. Multiple studies have also demonstrated that carotenoids have an anti-inflammatory effect on endothelial and vascular smooth muscle cells by suppressing NF-κβ activation, suggesting that carotenoids may decrease vascular perturbations and modify CNV formation via their anti-inflammatory properties [12, 17–19]. Additionally, carotenoids may improve ocular blood flow by modifying the production of vascular activating molecules, such as nitric oxide (NO) and endothelin-1 (ET-1), which are potent vasodilators and vasoconstrictors, respectively. Further, ET-1 has been shown to be a major determinant of choroidal blood flow, and higher blood concentrations have been found in patients with AMD compared to healthy subjects [12, 20].

Carotenoids and phenolic compounds are hypothesized to attenuate AMD through their inhibitory actions on NF-κβ, stimulatory effects on endothelial function, and their preservative influence in ocular perfusion homeostasis [12, 17–20]. Furthermore, studies have shown that lutein supplement of $\geq$ 4 mg/day significantly increases MP levels in AMD patients, and at doses of 10 mg/day, it can improve visual acuity [21, 22]. The purpose of this present study is to quantify the effect of lutein complex supplementation on retinal capillary and retrobulbar blood flow (RBF) in healthy subjects to better understand the potential impact of these frequently taken supplements on ARED vascular-related risk.

Materials and Methods

This investigation was conducted at the Glaucoma Research and Diagnostic Center within the Glick Eye Institute in Indianapolis, Indiana in conjunction with the Division of Biostatistics at the Indiana University School of Medicine (IUSM) from December 2014 to January 2015. This study was conducted following the guidelines outlined in the Declaration of Helsinki. All procedures involving human subjects were approved by the Institutional Review Board at IUSM (Protocol number: 1311824589). Written informed consent was obtained from all subjects.

Subjects

This study was open to all healthy subjects (free of eye disease or uncontrolled blood pressure/diabetes, self reported) with one randomly selected eye from each subject examined. Subjects had to meet the following inclusion criteria: healthy males or females of $\geq$ 18 years of age, no diagnosis of eye disease (other than myopia) or uncontrolled systemic disease, willingness to sign an informed consent statement, and ability to comply with the examination requirements. Participants were excluded for the following self-reported reasons: women who were pregnant, lactating, or planning to become pregnant during the study duration or within one month after study completion; patients receiving medications or dietary supplements with known interaction with the study supplements; smoking during the last ten years; concurrent participation or prior participation in any other clinical trial during the past 30 days involving an investigational drug or device; and concurrent use of any of the components of the study supplement.

Study Design

The study was a randomized, double-blinded, placebo-controlled, two-period crossover study with comparisons

of administration of a lutein complex supplementation (lutein (10 mg), ascorbic acid (500 mg), tocopherols (364 mg), carnosic acid (2.5 mg), zeaxanthin (2 mg), copper (2 mg)) versus placebo, which were identical in appearance to the lutein complex supplement. Treatment and placebo capsules were provided by Lycored Ltd., Beer Sheva, Israel. Testing took place over a period of 6 months. Participants were given detailed medication instructions and were instructed to avoid caffeine intake, smoking, and exercise for 3 hours before each study visit. The first clinic visit obtained pre-supplementation measurements and a questionnaire regarding the participant's medical history and demographic information. Each participant was then randomly assigned to lutein complex or placebo group (Figure 1). Randomization was accomplished with a randomization table and was maintained in a double-blind manner. For each subject (16), one qualified eye (N = 16; 8 right eyes, 8 left eyes) was randomly assigned as the observational study eye. Participants were instructed to take their Period 1 supplement dose once daily and return for a follow-up visit three weeks later to repeat testing. After a washout period of three weeks, participants returned for a second pre-supplementation visit and were given the other study supplement to take daily for three weeks during Period 2 before returning for a final evaluation.

Objective measures

For consistency and bias limitation, all measurements were taken in the same order at the same time of day and by the same examiner for each patient. At each visit, any changes in health or medication history were recorded, and subjects were questioned for any adverse effects of the supplementation. The following measurements were obtained at every visit: best corrected visual acuity (VA) using the Early Treatment Diabetic Retinopathy Study (ETDRS) eye charts, contrast sensitivity with Vector vision analysis, and intraocular pressure (IOP) via Goldmann applanation tonometry. Brachial artery systolic and diastolic blood pressure (SBP and DBP, respectively) and heart rate were also assessed in the seated/supine position after a 5-minute resting period using a calibrated automated sphygmomanometer at the beginning of each study visit. Ocular perfusion pressure (OPP) was calculated as OPP = ((2/3 MAP) – IOP), where MAP is mean arterial pressure. Mean OPP (MPP) was calculated by subtracting IOP from MAP. Systolic ocular perfusion pressure (SOPP) and diastolic ocular perfusion pressure (DOPP) were determined by subtracting IOP from SBP and DBP, respectively.

Velocities and vascular resistance of the retrobulbar vasculature and the blood vessels supplying the optic nerve head and choroid were measured with Doppler ultrasonography in the central retinal artery (CRA), nasal (NPCA) and temporal (TPCA) posterior ciliary arteries and with angle of measurement in the ophthalmic artery (OA). In each vessel peak systolic velocity (PSV) and end diastolic velocity (EDV) were assessed, and Pourcelot's resistivity index (RI) was calculated (RI = (PSV-EDV)/PSV). Perfusion within peripapillary retinal capillary beds was assessed by confocal scanning laser Doppler flowmetry, providing mean retinal capillary blood flow in superficial retinal vascular beds and the degree of vascularity of the retina by differentiating avascular tissue (measured as zero flow pixels) from perfused tissue.

Statistical analysis

The primary statistical endpoint, accomplished using paired t-tests, was to evaluate the efficacy of lutein complex supplement in increasing ocular vascular biomarkers compared to placebo. Secondary effects due to supplementation sequence and study period were analyzed with repeated measures analysis of variance (ANOVA). A two-tailed p-value of 0.05 was set as the threshold of statistical significance. Previous studies of populations and test/retest variability of Doppler measurements of RBF velocities have

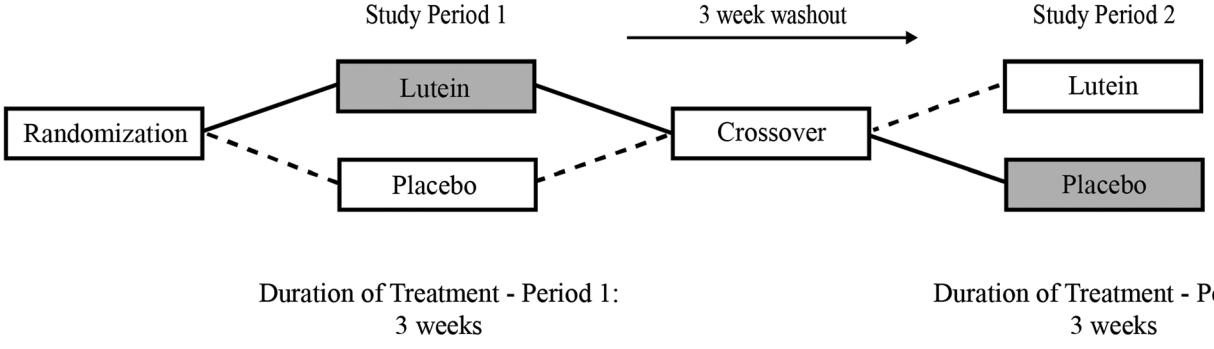

Figure 1. Study design: this experiment involved a randomized, placebo-controlled, double-blinded, two-period crossover study as shown in the figure. Subjects received lutein complex supplement or placebo orally and daily during treatment periods, which were 3 weeks in duration. The two periods were separated by a 3-week washout period.

shown that a sample size of 16 individual provides 90% power to detect a > 12% difference in PSV and EDV and a 6% difference in RI between groups. Normality was evaluated using Shapiro-Wilk tests and visual examination of the residual plots. Equality of variance was not assumed for this analysis; variances were allowed to vary by treatment. Because this is a pilot study, no multiple comparisons adjustment was employed because adjustment may be too conservative in this setting, obscuring important exploratory differences worthy of further follow-up in a larger study.

Results

Sixteen healthy female patients (age 36.8 ± 12.1 years; range 20-56 years) were recruited, and there were no patient dropouts throughout the study. The remaining baseline demographic data can be found in Table 1. Patients with hypertension and diabetes were well controlled and complied with the inclusion criteria. No adverse events with either lutein complex supplement or placebo were reported.

Minor differences were detected between groups within the pre-lutein complex supplementation data, such as lower starting values of IOP before treatment with lutein complex ($p = 0.0058$), higher starting values of VA before treatment with lutein complex ($p = 0.0321$), lower starting values of Row D contrast sensitivity before treatment with lutein complex ($p = 0.0465$), lower starting values of CRA EDV before treatment with lutein complex ($p = 0.0101$), and higher starting values of CRA RI before treatment with lutein complex ($p = 0.0011$). Additional pre-treatment differences were found between study periods for contrast sensitivity (lower contrast sensitivity values of Row C and Row D in period 1 compared to period 2, $p = 0.0037$ and $p = 0.0386$, respectively) and CDI-CRA-DYS (higher CRA EDV in period 1 compared to period 2, $p = 0.0405$). Pre-supplementation sequence effects were seen with MAP (lutein complex-placebo lower than placebo-lutein complex) and OPP (lutein complex-placebo lower than placebo-lutein complex). However, as the lutein complex supplement had less of an effect on these values than placebo, it is unlikely to adversely affect conclusions.

Changes in measurements from pre-supplementation to post-supplementation with lutein complex supplement demonstrated significance (Table 2) including increased mean retinal capillary blood flow in the superior retina (Figure 2), decreased percentage of avascular area in the superior and inferior retina (Figure 3), and reduced systolic and diastolic arterial blood pressures (Figure 4). None of these parameters changed significantly in the placebo group. Lutein complex administration also increased mean retinal capillary blood flow in the inferior retina, but did not reach statistical significance (Table 2). There were no significant differences in RBF velocities or RI with either lutein complex or placebo administration; however, there was a trend increase in the PSV of the OA in the lutein complex group. There were also no significant differences in IOP reduction, visual acuity, contrast sensitivity detection, or OPPs with either supplement.

Table 1. Patients' characteristics at baseline. The numbers in parentheses represent percentage of total study group.

	N (% of subjects)
Female	16 (100%)
Race	White: 12 (75%)
	African American: 2 (13%)
	Asian: 1 (6%)
	Other: 1 (6%)
Hypertensive	6 (38%)
Diabetic	5 (31%)
Eye examined	Left: 8 (50%)
	Right: 8 (50%)

N, number. The diabetic or hypertensive statuses of the subjects were defined as self-reported physician-diagnosed conditions.

Data comparison between the two supplement groups revealed a significant decrease in systemic DBP ($p = 0.0357$) and an increase in CRA PSV ($p = 0.0384$) with lutein complex supplement (Table 2). Data analyses from the placebo group were all non-significant. Within the study periods a decrease in MPP during period 1 ($p = 0.0428$), an increase in CRA PSV during period 2 ($p = 0.0282$), and a decrease in CRA EDV in period 1 ($p = 0.0159$) were observed. An increase in IOP ($p = 0.0412$) was demonstrated in the lutein complex-placebo arm while the placebo-lutein complex arm showed a non-significant IOP decrease. This finding was determined to not have adversely affected data interpretation upon further examination of the IOP trends, as we further discussed in the Discussion section.

Discussion

ARED patients are susceptible to ocular tissue and vascular damage, and previously the beneficial effects of carotenoids on various aspects of ocular health have been reported by numerous studies [12, 14-19, 22, 31-32]. In patients who develop cataracts and glaucoma, evidence that supports nutritional supplementation to counteract disease pathways have not been confirmed. However, although the pathogenesis of AMD is not completely understood, carotenoids and phenolic compounds have been proposed to interfere with AMD disease pathways through reductions in TNF-α and interleukin-1, inhibition of

Table 2. Comparison of change from pre- to post-treatment in lutein complex supplement and placebo groups. The numbers in parentheses represent mean or standard error.

	Change from pre- to post-treatment with lutein supplement: mean (SE)	P-value	Change from pre- to post-treatment with placebo: mean (SE)	P-value	Comparison of change of lutein to placebo group (p-value)
HR	−3.31 (1.59)	0.0552	0.00 (3.39)	1.000	0.4334
Systolic BP	−4.06 (1.69)	**0.0295**	0.06 (2.29)	0.9786	0.0506
Diastolic BP	−3.69 (1.68)	**0.0441**	0.31 (2.57)	0.9048	**0.0357**
MAP	−1.52 (1.50)	0.3265	−1.02 (2.55)	0.6947	0.6447
IOP	0.50 (0.41)	0.2396	−0.38 (0.34)	0.2875	0.0546
OPP	−1.01 (1.00)	0.3265	−0.68 (1.70)	0.6947	0.6447
Systolic OPP	−0.19 (2.73)	0.9461	0.44 (2.23)	0.8469	0.7419
Diastolic OPP	−2.94 (1.67)	0.0989	−1.19 (3.13)	0.7094	0.4331
Mean OPP	−2.02 (1.49)	0.1955	−0.65 (2.61)	0.8078	0.3984
VA	−0.029 (0.020)	0.1777	−0.006 (0.018)	0.7326	0.3022
Row A	−0.018 (0.034)	0.6128	−0.009 (0.038)	0.8084	0.7162
Row B	0.064 (0.039)	0.1252	0.010 (0.042)	0.8165	0.3074
Row C	−0.006 (0.058)	0.9158	−0.019 (0.061)	0.7635	0.9598
Row D	−0.003 (0.061)	0.9597	−0.107 (0.052)	0.0559	0.0908
OA PSV	1.46 (0.70)	0.0545	0.89 (0.57)	0.1360	0.2813
OA EDV	−0.02 (0.18)	0.9183	0.36 (0.21)	0.1002	0.2931
OA RI	0.012 (0.008)	0.1459	−0.009 (0.011)	0.4233	0.1870
CRA PSV	0.36 (0.19)	0.0714	−0.33 (0.21)	0.1444	**0.0384**
CRA EDV	−0.11 (0.16)	0.5254	−0.24 (0.15)	0.1145	0.6906
CRA RI	0.022 (0.014)	0.1494	0.016 (0.014)	0.2859	0.5786
NPCA PSV	−0.11 (0.15)	0.4977	0.29 (0.21)	0.1729	0.0990
NPCA EDV	−0.01 (0.13)	0.9637	0.16 (0.12)	0.2177	0.3771
NPCA RI	−0.010 (0.016)	0.5501	−0.008 (0.015)	0.5982	0.8144
TPCA PSV	0.06 (0.12)	0.6587	−0.09 (0.27)	0.7375	0.7280
TPCA EDV	−0.03 (0.17)	0.8860	−0.07 (0.22)	0.7465	0.9241
TPCA RI	−0.001 (0.020)	0.9622	−0.004 (0.023)	0.8635	0.8518
SUP zero flow	−0.029 (0.014)	**0.0491**	−0.007 (0.008)	0.3710	0.1994
SUP mean FV	60 (28)	**0.0466**	−56 (72)	0.4498	0.1639
INF zero flow	−0.023 (0.011)	**0.0477**	−0.003 (0.016)	0.8658	0.3894
INF mean FV	47 (24)	0.0747	−52 (57)	0.3792	0.1672

P-values of statistical significance ($p < 0.05$) are in bold.

BP, blood pressure (mmHg); CRA, central retinal artery; EDV, end diastolic velocity (cm/sec); FV, flow volume (arbitrary units); HR, heart rate (beats per minute); INF zero flow, inferior zero flow (arbitrary units); IOP, intraocular pressure (mmHg); MAP, mean arterial pressure (mmHg); NPCA, nasal posterior ciliary artery; OA, ophthalmic artery; OPP, ocular perfusion pressure (mmHg); PSV, peak systolic velocity (cm/sec); RI, resistivity index (dimensionless quantity); SUP zero flow, superior zero flow (arbitrary units); TPCA, temporal posterior ciliary artery; Row A/B/C/D refers to contrast sensitivity (Log Units); VA, visual acuity (Early Treatment Diabetic Retinopathy Study, ETDRS Log score).

NF-κβ, stimulation of endothelial function, and preservation of ocular blood flow [12–23].

The relationship of ARED and ocular blood flow have been investigated in several studies. Previously, a longitudinal study by Metelitsina et al. found that AMD patients who progressed to CNV had a 24% lower choroidal blood volume and a 20% lower choroidal blood flow at baseline compared to AMD patients who did not progress [24]. Furthermore, AMD eyes with reduced baseline choroidal blood flow and volume were found to have a 2.3 times the risk of significant visual loss compared to AMD eyes with above-average baseline choroidal blood flow [24]. In glaucoma patients, Tobe et al. demonstrated that increased avascularity of the retina correlated with an increased cup/disk ratio over an 18-month period [25]. Our study analyzed retinal blood flow over 3 weeks in healthy patients taking oral lutein complex supplements and found that mean superior retinal capillary blood flow ($p = 0.0466$) increased by 5.6%, and the percentage of avascular area in the inferior ($p = 0.0477$) and superior ($p = 0.0491$) temporal retinal decreased by 8.5% and 10% following lutein complex administration (Table 2). This supports the hypothesis that lutein and/or other ingredients of the complex (i.e. zeaxanthin, beta carotene, carnosic acid), over several weeks may

Figure 2. Mean change (standard error of mean) in superior retinal capillary blood flow assessed by Heidelberg retinal flowmeter from pre-supplementation to post supplementation measured in arbitrary units. Changes in data from pre-treatment to post-treatment with lutein complex supplement revealed significantly increased mean retinal capillary blood flow in the superior retina ($p = 0.0466$) compared to non-significant changes with the placebo. The number of subjects was 16.

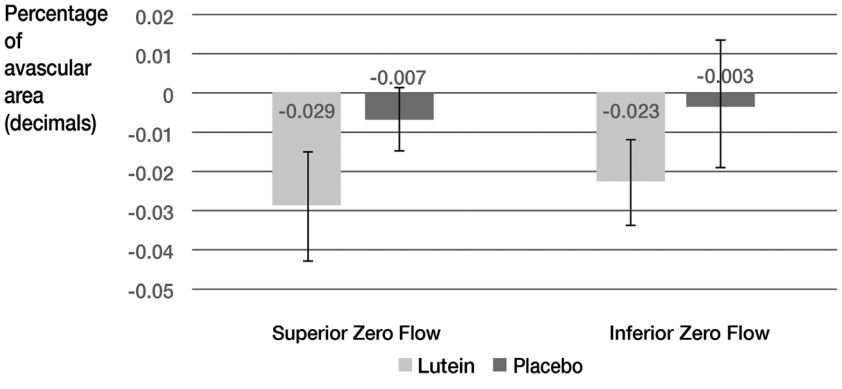

Figure 3. Mean change (standard error of mean) in the percentage of avascular area in the superior and inferior retina assessed by Heidelberg retinal flowmeter with lutein complex and placebo. Changes in data from pre-treatment to post-treatment with lutein complex supplement revealed significantly decreased percentage of avascular area shown as decimals both in the superior and inferior retina ($p = 0.0491$, $p = 0.0477$, respectively) compared to non-significant changes with the placebo. The number of subjects was 16.

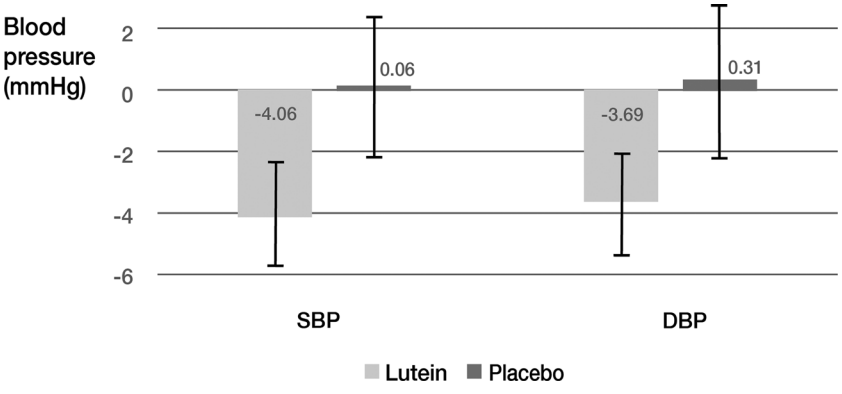

Figure 4. Mean change (standard error of mean) in systolic and diastolic blood pressures (SBP, DBP) measured in mmHg from pre-supplementation to post supplementation. Changes in data from pre-treatment to post-treatment with lutein complex supplement revealed significantly reduced systolic and diastolic blood pressures ($p = 0.0295$, $p = 0.0441$, respectively) compared to non-significant readings with the placebo. The number of subjects was 16.

influence the retinal vasculature with mild increases seen in several biomarkers in healthy subjects free from eye disease.

Several studies have emphasized the importance of retrobulbar blood flow in the development and progression of ARED. Galassi et al. found that glaucoma patients were 6 times more likely to develop visual field deterioration when their baseline OA RI was above 0.78 [26]. Martinez et al. revealed that glaucoma patients whose visual fields had worsened over 3 years had a higher baseline RI of the SPCA and OA [27]. More recently, Moore et al. discovered that glaucoma patients with decreased baseline OA PSV and EDV showed both structural and functional disease progression after 4 years [28]. Our study shows that after daily lutein complex supplementation for three weeks, healthy subjects had a significant increase in CRA PSV

($p = 0.0384$) compared to placebo, suggesting that daily supplementation with lutein complex may improve ocular perfusion in healthy subjects (Table 2).

A slightly unanticipated finding of our study was a significant decrease in SBP ($p = 0.0295$) and DBP ($p = 0.0441$) after lutein supplementation as well as a decrease in DBP ($p = 0.0357$) when comparing the changes within the groups (Table 2). Systemic blood pressures directly influence OPP, and nighttime systemic hypotension has been linked to normal-tension glaucoma progression [29]. Further, hypertensive patients have been found to have lower choroidal blood flow compared to non-hypertensive subjects, and areas of poor perfusion in the choroid have been correlated to locations of neovascular change in patients with AMD [30]. However, this study did not include patients with normal tension glaucoma or AMD, nor was it designed to address questions of blood pressure changes and disease progression. Therefore, the clinical relevance of these findings is currently unclear. In fact, it is important to notice that the blood pressures' reduction detected in our study, even if statistically significant, was still into normal physiological range.

It is important to recognize that our study is not without limitations. One limitation was that our study was a relatively small sample and we might have been unable to determine all effects of lutein complex supplementation. Although it was determined to not have negatively impacted the analysis, the administration sequence provides indication that the washout period might not have been sufficiently long enough to allow subjects to fully return to baseline. To overcome this limitation, we calculated and compared the results of study period 1 and 2 and found no statistically significant differences. Additionally, our study only included healthy female subjects, thereby eliminating gender comparison. Additionally, as our subjects were all free of ocular disease, it can only be speculated how complex administration would compare in the target AMD population. Another limitation is that our study included patients with hypertension and diabetes; however, to be included in the study they had to be well-controlled, therefore the effects were likely due to the complex supplements and not to the disease state. Finally, the hematic concentrations of the ingredients of the lutein complex supplement have not been measured. In fact, we monitored the compliance to the treatment via a self-reported capsules count that was recorded in a logbook. Also, we did not evaluate the macular pigment optical density. The collection of blood samples and the evaluation of the blood concentration of the ingredients of the lutein complex supplement, and the measurement of the macular pigment optical density were beyond the scope of this study, and future studies with these specific aims are therefore needed.

Our pilot study shows that daily supplementation with a lutein complex may have the ability to increase biomarkers of ocular circulation in healthy subjects. In theory, the development and progression of AREDs, such as AMD and glaucoma that have previously identified vascular mechanistic pathways, may be affected by lutein complex supplement ingestion. It is important to highlight that the supplementation period analyzed in our study is relatively very short and in a relatively small sample group. Future studies should focus on larger samples and in more ARED disease specific populations to evaluate its clinical significance.

References

1. National Eye Institute, National Institutes of Health. (2013, May) Age-Related Eye Disease Study-Results. nei.nih.gov/amd. Accessed July 25, 2016
2. Thompson, J., & Lakhani, N. (2015) Cataracts. Prim Care. 42(3), 409–423.
3. National Eye Institute, National Institutes of Health. Glaucoma, Open-angle. nei.nih.gov/eyedata/glaucoma. Accessed July 25, 2016
4. Costa, V.P., Harris, A., Anderson, D., Stodtmeister, R., Cremasco, F., Kergoat, H., Lovasik, J., Stalmans, I., Zeitz, O., Lanzl, I., Gugleta, K., & Schmetterer, L. (2014) Ocular perfusion pressure in glaucoma. Acta Ophthalmol. 92(4), e252–266.
5. Wong, W.L., Su, X., Li, X., Cheung, C.M., Klein, R., Cheng, C.Y., & Wong, T.Y. (2014) Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta analysis. Lancet Glob Health. 2(2), e106–116.
6. Mehta, S. (2015) Age-Related Macular Degeneration. Prim Care Clin Office Pract. 42, 377–391.
7. Ciulla, T.A., Harris, A., & Martin, B.J. (2001) Ocular perfusion and age-related macular degeneration. Acta Ophthalmol Scand. 79(2), 108–115.
8. McCall, D.O., McGartland, C.P., McKinley, C.P., Patterson, C.C., Sharpe, P., McCance, D.R., Young, I.S., & Woodside, J.V. (2009) Dietary intake of fruits and vegetables improves microvascular function in hypertensive subjects in a dose-dependent manner. Circulation. 119(16), 2153–2160.
9. Age-Related Eye Disease Study Research Group. (2001) A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Arch Ophthalmol. 119(10), 1417–1436.
10. Scholl, H.P.N., Issa, P.C., Walier, M., Janzer, S., Pollok-Kopp, B., Börncke, F., Fritsche, L.G., Chong, N.V., Fimmers, R., Wienker, T., Holz, F.G., Weber, B.H.F., & Oppermann, M. (2008) Systemic complement activation in age-related macular degeneration. Plos One. 3(7), e2593.
11. Smailhodzic, D., Klaver, C.C., Klevering, B.J., Boon, C.J., Groenewoud, J.M., Kirchhof, B., Daha, M.R., den Hollander, A.I., & Hoyng, C.B. (2012) Risk alleles in CFH and ARMS2 are independently associated with systemic complement activation in age-related macular degeneration. Ophthalmology. 119, 339–346.
12. Armoza, A., Haim, Y., Basiri, A., Wolak, T., & Paran, E. (2013) Tomato extract and the carotenoids lycopene and lutein improve endothelial function and attenuate inflammatory NF-κβ signaling in endothelial cells. J Hypertens. 31, 1277.
13. Clauss, M., Sunderkotter, C., Sveinbjornsson, B., Hippenstiel, S., Willuweit, A., Marino, M., Haas, E., Seljelid, R., Scheurich,

P., Suttorp, N., Grell, M., & Risau, W. (2001) A permissive role for tumor necrosis factor in vascular endothelial growth factor-induced vascular permeability. Blood. 97, 1321–1329.
14. Kim, Y., Seo, J., & Kim, H. (2010) Beta-carotene and lutein inhibit hydrogen peroxide-induced activation of NF-κβ and IL-8 expression in gastric epithelial AGS cells. J Nutr Sci Vitaminol. 57, 216–223.
15. Seddon, J., & Hennekens, C. (1994) Vitamins, minerals, and macular degeneration: Promising but unproven hypotheses. Arch Ophthalmol. 112, 176–178.
16. Krinsky, N.I. (2002) Possible biologic mechanisms for a protective role of xanthophylls. J Nutr. 132, 540S–542S.
17. Hadad, N., & Levy, R. (2012) The synergistic anti-inflammatory effects of lycopene, lutein, β-carotene, and carnosic acid combinations via redox-based inhibition of NF-κβ signaling. Free Radic Biol Med. 53, 1381–1391.
18. Feng, D., Ling, W., & Duan, R. (2009) Lycopene suppresses LPS-induced NO and IL-6 production by inhibiting the activation of ERK, p28MAPK, and NFκβ in macrophages. Inflamm Res. 59, 115–121.
19. Izumi-Nagai, K., Nagai, N., Ohgami, K., Satofuka, S., Ozawa, Y., Tsubota, K., Umezawa, K., Ohno, S., Oike, Yuichi, & Ishida, S. (2007) Macular pigment lutein is anti-inflammatory in preventing choroidal neovascularization. Arterioscler Thromb Vasc Biol. 27, 2555–2562.
20. Machalińska, A., Mozolewska-Piotrowska, K., Paczkowska, E., & Lubiniski, W. (2014) Increased expression of endothelin-1—a novel diagnostic marker for early AMD detection? Klin Oczna. 116, 16–20.
21. Bernstein, P.S., Zhao, D.Y., Wintch, S.W., Ermakov, I.V., McClane, R.W., & Gellermann, W. (2002) Resonance Raman measurement of macular carotenoids in normal subjects and in age-related macular degeneration patients. Ophthalmology. 109, 1780–1787.
22. Richer, S., Stiles, W., Statkute, L., Pulido, J., Frankowski, J., Rudy, D., Pei, K., Tsipursky, M., & Nyland, J. (2004) Double-masked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: the Veterans LAST study (Lutein Antioxidant Supplementation Trial). Optometry. 75(4), 216–230.
23. Rinsky, B., Hagbi-Levi, S., Elbaz-Hayoun, S., Grunin, M., & Chowers, I. (2017) Characterizing the effect of supplements on the phenotype of cultured macrophages from patients with age-related macular degeneration. Mol Vis. 23, 889–899.
24. Metelitsina, T.I., Grunwald, J.E., DuPont, J.C., Ying, G.S., Brucker, A.J., & Dunaief, J.L. (2008) Foveolar choroidal circulation and choroidal neovascularization in age-related macular degeneration. Invest Ophthalmol Vis Sci. 49(1), 358–363.
25. Tobe, L.A., Harris, A., Hussain, R.M., Eckert, G., Huck, A., Park, J., Egan, P., Kim, N.J., & Siesky, B. (2015) The role of retrobulbar and retinal circulation on optic nerve head and retinal nerve fiber layer structure in patients with open-angle glaucoma over an 18-month period. Br J Ophthalmol. 99(5), 609–612.
26. Galassi, F., Sodi, A., Ucci, F., Renieri, G., Pieri, B., & Baccini, M. (2003) Ocular hemodynamics and glaucoma prognosis: a color Doppler imaging study. Arch Ophthalmol. 121(12), 1711–1715.
27. Martinez, A., & Sanchez, M. (2005) Predictive value of color Doppler imaging in a prospective study of visual field progression in primary open-angle glaucoma. Acta Ophthalmol Scand. 83(6), 716–722.
28. Moore, N.A., Harris, A., Wentz, S., Verticchio Vercellin, A.C., Parekh, P., Gross, J., Hussain, R.M., Thieme, C., & Siesky, B. (2017) Baseline retrobulbar blood flow is associated with both functional and structural glaucomatous progression after 4 years. Br J Ophthalmol. 101(3), 305–308.
29. Choi, J., & Kook, M.S. (2015) Systemic and Ocular Hemodynamic Risk Factors in Glaucoma. Biomed Res Int.141905.
30. Stefánsson, E., Geirsdóttir, Á., & Sigurdsson, H. (2011) Metabolic physiology in age related macular degeneration. Prog Retin Eye Res. 30(1), 72–80.
31. Bohn, T. (2017) Bioactivity of Carotenoids - Chasms of Knowledge. Int J Vitam Nutr Res.1–5. 2017 Feb 10
32. Gust, J.L., & Logomarsino, J.V. (2016) The Association Between Cartenoid Status and Body Composition in Children 2–18 Years of Age - A Systematic Review. Int J Vitam Nutr Res. 86(3–4), 91–120.

Conflict of Interest
The authors declare that there are no conflicts of interests.

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