Original Article

Effect of lutein supplementation on visual function in nonproliferative diabetic retinopathy

Ping-Chuan Zhang MD[1], Chang-Rui Wu MD[2], Zhi-Lun Wang MD[1], Li-Yuan Wang MSc[1], Yue Han MSc[1], Shu-Liu Sun MSc[1], Qing-Shan Li MSc[1], Le Ma MD[1,3]

[1]*School of Public Health, Xi'an Jiaotong University Health Science Center, Xi'an, Shaanxi, China*
[2]*The First Affiliated Hospital, Xi'an Jiaotong University, Xi'an, Shaanxi, China*
[3]*Key Laboratory of Environment and Genes Related to Diseases (Xi'an Jiaotong University), Ministry of Education of China, Xi'an, Shaanxi, China*

Background and Objectives: The purpose of this study was to determine whether supplementation with lutein improved visual function in patients with nonproliferative diabetic retinopathy (NPDR). **Methods and Study Design:** In this randomized, double-blind, placebo-controlled trial, 31 patients with NPDR were assigned randomly to 10 mg/d of lutein or identical placebo for 36 weeks. Visual performance indices, including visual acuity (VA), contrast sensitivity (CS) and glare sensitivity (GS) at four different spatial frequencies, were measured at baseline, week 18 and 36. **Results:** At 36 weeks, a slight improvement in VA was found in the lutein group. A significant association was observed between the changes in VA and the corresponding baseline values in treatment group (r=-0.53; p=0.04). At 36 weeks, the lutein treatment group increased CS at four spatial frequencies, and the improvement achieved statistical significance at 3 cycles/degree (p=0.02). The changes in CS at 3 cycles/degree for the lutein group was marginally significantly greater than those for the placebo group (p=0.09). There was also a slight increase in GS in the lutein group up to week 36, however, no significant changes were found over time in any cycles/degree. **Conclusions**: In patients with NPDR, supplementation with lutein resulted in potential improvements in CS at low spatial frequency. Further studies are required to determine the possibility that such intervention could be used as an adjunct therapy to prevent vision loss in diabetic patients.

Key Words: lutein, visual acuity, contrast sensitivity, diabetic retinopathy; randomized controlled trial

INTRODUCTION

Diabetic retinopathy (DR) is retinal damage, specifically to blood vessels in the retina, caused by complications of diabetic mellitus (DM).[1] It is the major cause of adult vision impairment and blindness in industrialized countries.[2] The disorder includes the presence of microaneurysms, hemorrhages, hard exudates, and cotton wool spot; these events subsequently cause fibrovascular proliferation and then retinal detachment.[3] Although visual dysfunction is found to be initiated in early DR, no permanent cure is currently available.[4] Therefore, effective treatments are needed to help in preventing or delaying the development and progression of the diabetes-induced visual dysfunction.

It has been suggested that oxidative stress is involved in the pathogenesis of DM as well as diabetic complications.[5] Sustained hyperglycemia results in greater production of reactive oxygen species (ROS) that contribute to oxidative stress in diabetes.[6] If the production of free radicals overwhelms the capacity of the antioxidant defenses, increased free radical activities disrupts the normal cellular metabolism that leads to the development of retinopathy.[7] Antioxidants with free-radical scavenging ability provide significant protection against oxidative stress and therefore may play a preventive role in development of DR. Lutein is one of the dietary xanthophyll carotenoids that are specifically concentrated in the macula, indicating that it may be crucial in protecting the retina from oxidative stress-induced damage.[8] Diabetic patients have significantly lower serum and retinal levels of lutein than did the control subjects.[9] Previous studies have shown that administration of lutein prevents diabetes-induced oxidative stress and inhibits the onset of retinopathy in diabetic rats; however, no intervention studies have been conducted concerning lutein in the prevention or treatment of DR at an early stage to date.[10,11] Meanwhile, most of the evidence on the protective role of lutein in eye health has been focussed on AMD,[12] and little is known concerning the effects of this carotenoid on the improvement in visual function in nonproliferative DR (NPDR).

The aim of this study is to evaluate the potential effects of supplementation with lutein on visual function in the patients with NPDR.

Corresponding Author: Dr Le Ma, School of Public Health, Xi'an Jiaotong University Health Science Center, 76 Yanta West Road, Xi'an, Shaanxi, 710061, China.
Tel: +8629-82655105; Fax: +8629-82655032
Email: male@mail.xjtu.edu.cn
Manuscript received 12 November 2015. Initial review completed 19 January 2016. Revision accepted 15 February 2016.
doi: 10.6133/apjcn.032016.13

METHODS AND MATERIALS

Study population

Participants were recruited from nephrology and diabetes clinics. All subjects received a complete ophthalmologic examination to detect the presence of retinal disease. This included slit lamp, ophthalmoscopy, and stereoscopic fundus photographs of the macula. Those participants eligible were aged 40 to 85 years and had type 2 diabetes and a clinical diagnosis of NPDR in either mild or moderate stage, defined as the presence of microaneurysms, hemorrhages, or hard exudates, according to modification of the Airlie House Classification system.[13]

Patients were excluded from the study if they had proliferative DR or other eye disorders other than NPDR, including macular degeneration, diabetic macular edema, retinal detachment, ocular trauma or glaucoma requiring treatment; had a previous history of intraocular inflammation or laser treatment for retinal diseases; had any cardiovascular event within the past year, type 1 diabetes, and unstable chronic illness; were taking medication that would affect visual function for at least the previous 3 months; were vegetarian; and consumed vitamin, carotenoids or mineral supplements within the previous 6 months.

The research protocol was approved by the medical ethics committee of the Xi'an Jiaotong University. Participants gave written informed consent prior to study participation.

Study design

The study was a 36-week randomized, double-blind, placebo-controlled intervention. All participants were randomly assigned to one of two study groups in a 1:1 ratio according to a computer-generated randomization schedule in blocks of 4: Group Lutein, who received 10mg of lutein once a day for 36 weeks; and Group Placebo, who received a placebo capsule. Treatment allocation was blinded to the participants, investigators and staff directly involved in conduct of the study. All capsules were manufactured by Lutein Pharmaceutical Co Ltd (Guangzhou, China). Placebo and active capsules were identical in size and color.

During the study, the participants were requested to maintain their usual diet and physical activity and to avoid excessive intakes of food items rich in xanthophylls. Nutritional status was assessed using food-frequency questionnaires. Participants were instructed to take their supplements with meals, and treatment compliance was checked by returned capsule counts at monthly intervals. Visual performance indices were performed at baseline, week 18 and 36, including visual acuity (VA), contrast sensitivity (CS), and glare sensitivity (GS).

Visual performance indices

VA was measured with an Early Treatment Diabetic Retinopathy Study chart. The test distance was 4 m and the acuity chart was retroilluminated with automatic calibration to 130 cd/m^2. Visual acuity was scored by the total number of letters identified correctly with full spectacle correction and expressed in logarithm of the minimum angle of resolution (logMAR).[14]

CS was assessed by using the CSV 1000 contrast sensitivity test system (Vector Vision, Dayton, OH, USA). The system provides a fluorescent luminance source that retroilluminates the chart. The test was administered at the distance of 2.5 m under controlled room illumination (85 cd/m^2). Four different spatial frequencies of 3, 6, 12 and 18 cycles/degree are measured, and each spatial frequency are graded on a scale from 1 (high contrast) to 8 (low contrast). The subjects were asked to recognize the grating pattern in each column monocularly with their best correction after subjective refraction. Contrast threshold for each cycle/degree is determined by the contrast level of the last correct response.[15] After turning on the glare light, GS was assessed using the same method. The luminance intensity of glare was 350 cd/m^2.[16]

Statistical analyses

Sample size estimation was based on the change of VA from baseline, a 2-sided significance level of 0.05, and a power of 80%. To achieve the calculated power, 13 patients had to be enrolled in each treatment group. All analyses were performed using the intent-to-treat population. Data were checked for normal distribution using the Kolmogorov-Smirnov test. Differences of baseline characteristics among groups were tested with chi-square test or t test as appropriate. Changes between the final visit and baseline for continuous measures were assessed using the paired t test and the between-group differences in change among treatment groups were tested by analysis of covariance (ANCOVA) adjusted for baseline values. Changes in those same variables over time were further assessed using a repeated-measure ANOVA, with the baseline values as covariates. The Pearson tests were used for assessing the relationships between the baseline values and their changes during 36 weeks. All the calculations were conducted with SPSS 11.0 for Windows (SPSS Inc., Chicago, USA). Any differences showing a p value of less than 0.05 were considered to be statistically significant.

RESULTS

A total of 31 participants were recruited for the study and randomly assigned to receive 10mg lutein (n=15), or placebo (n=16). The baseline characteristics of the two groups are presented in Table 1. The mean (SD) age of study population was 60.2 (10.3) years, and the mean (SD) BMI was 25.0 (2.1), and about 27% were women. Demographic variables and baseline characteristics of participants were equally distributed among the groups. All participants completed the protocol, except one participant in the placebo group dropped out for personal reasons. More than 95% of participants took at least 80% of their capsules, and there was no difference in adherence between groups (p>0.05).

The changes from baseline in VA over time are presented in Figure 1. LogMAR of VA decreased by means (SD) of 0.08 (0.22) in the lutein group and increased by 0.02 (0.19) in the placebo group at 36 week. Relative to baseline, there was a slight but non-significant improvement in VA in lutein group throughout the treatment period (p=0.11). No significant differences were found between groups in changes in VA from baseline to 36

Table 1. Baseline characteristics of study participants[†]

Characteristics	Placebo group (n=15)	Lutein group (n=15)	*p* value[‡]
Age, y	62.8 (12.0)	58.6 (8.9)	0.22
Women, n (%)	3 (20.0)	5 (33.3)	0.68
Education, y	11.7 (4.3)	13.3 (3.1)	0.25
Body mass index[§], kg/m^2	24.7 (2.1)	25.2 (2.0)	0.38
Waist circumstance, cm	89.8 (10.8)	93.0 (9.9)	0.40
Family history of DM, n (%)	2 (13.3)	2 (13.3)	>0.99
Smoking, n (%)	9 (60.0)	5 (33.3)	0.27
Drinking, n (%)	9 (60.0)	7 (46.7)	0.72
Blood pressure, mmHg			
Systolic	130 (12.0)	134 (17.4)	0.50
Diastolic	83.6 (11.9)	84.1 (13.4)	0.93

DM: diabetic mellitus.
[†]Value expressed as mean (standard deviation) unless otherwise noted.
[‡]*P*-values for any difference in groups derived from t test for continuous variables or the χ^2 test for categorical variables.
[§]Body mass index calculated as weight in kilograms divided by height in meters squared.

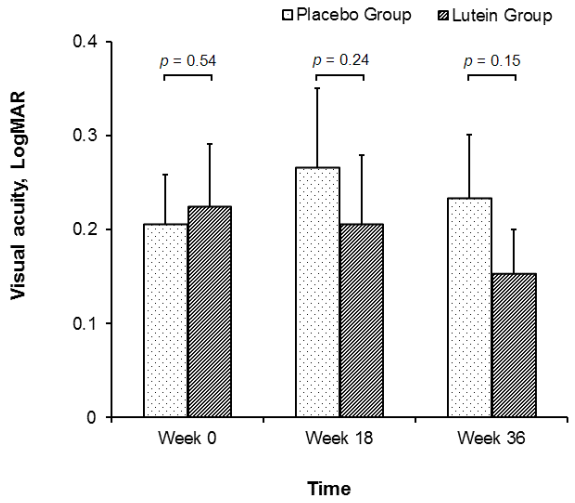

Figure 1. Change in visual acuity for two treatment groups of patients with nonproliferative diabetic retinopathy after lutein supplementation. Error bars indicate standard error of the mean.

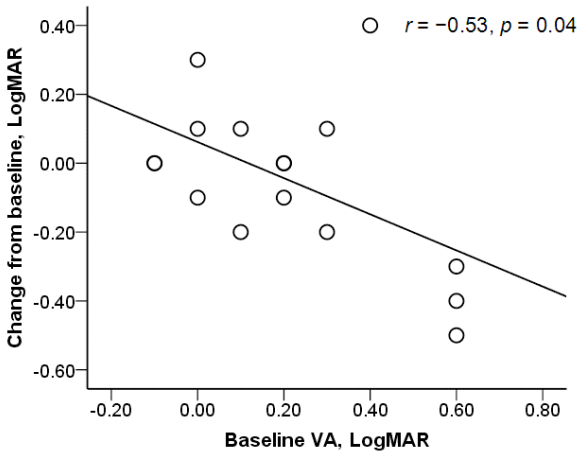

Figure 2. The relationship between the values of baseline VA and the changes in VA from baseline in the lutein group. Abbreviations: LogMAR, logarithm of the minimum angle of resolution; VA, visual acuity

weeks (p>0.05). The relationships between the values of baseline VA and the amount of their improvements over the study period are shown in Figure 2. The changes in logMAR of VA from baseline to 36 weeks were inversely correlated with the corresponding baseline values for active treatment group (correlation coefficients r=-0.53; p=0.04).

The changes in CS at four spatial frequencies were not significant for both groups at 18 weeks (p>0.05). At 36 weeks, the lutein treatment group increased CS at 3 cycles/degree by 0.16, and the improvement achieved statistical significance (p=0.02, Table 2). An ANCOVA analysis showed a marginally significant difference was noted between groups in changes in CS at 3 cycles/degree from baseline to 36 weeks (p=0.09). CS at 6 cycles/degree increased from a baseline value of 1.28 to 1.38 in participants assigned to the lutein group, and increased from 1.20 to 1.22 in participants assigned to placebo. The magnitude of changes was diminished in CS at 12 and 18 cycles/degree. In contrast with the significant change in CS at 3 cycles/degree, no significant changes in CS at the other three cycles/degrees were found over time in any group. Repeated-measure analyses of CSs at four spatial frequencies demonstrated a significant time effect, but not intervention effect, for CS at 3 cycles/degree (p =0.04).

The pattern of changes in GS at different spatial frequencies between groups loosely paralleled the changes in CS (Table 2). Although participants assigned to the lutein group had slight improvements from baseline in GSs, the within-group differences at 36 weeks did not achieve statistical significance. Similarly, GS changes at different spatial frequencies between groups were not statistically significant over time (all p>0.05).

DISCUSSION

In this study, we demonstrated that supplementation with lutein led to improvements in CS at low spatial frequencies for the patients with NPDR. Furthermore, the magnitudes of improvements in VA were inversely associated with their corresponding baseline levels. These findings provide evidence that lutein supplementation may exert beneficial effects on the visual function for diabetic subjects.

The retina is particularly susceptible to oxidative damage by ROS. Hyperglycemia, high oxygen fluctuation and high metabolic activity in the retina also induce the for-

Table 2. Change in contrast sensitivity and glare sensitivity from baseline

Outcome	Placebo group			Lutein group			p value†
	Baseline, mean (SD)	Mean (SD) change	p value (vs baseline)	Baseline, mean (SD)	Mean (SD) change	p value (vs baseline)	
Contrast sensitivity, log							
3 cyc/d	1.08 (0.29)	0.02 (0.26)	0.79	1.05 (0.25)	0.16 (0.24)	0.02	0.09
6 cyc/d	1.20 (0.35)	0.03 (0.31)	0.73	1.28 (0.31)	0.10 (0.23)	0.12	0.13
12 cyc/d	0.87 (0.30)	-0.02 (0.26)	0.84	0.98 (0.34)	0.08 (0.25)	0.24	0.36
18 cyc/d	0.41 (0.25)	-0.05(0.31)	0.59	0.40 (0.31)	0.07 (0.30)	0.39	0.58
Glare sensitivity, log							
3 cyc/d	1.02 (0.30)	-0.01 (0.30)	0.90	0.98 (0.22)	0.07 (0.38)	0.49	0.55
6 cyc/d	1.18 (0.32)	-0.11 (0.22)	0.16	1.18 (0.33)	0.04 (0.36)	0.71	0.27
12 cyc/d	0.87 (0.31)	-0.05 (0.24)	0.45	0.80 (0.30)	0.07 (0.39)	0.49	0.37
18 cyc/d	0.43 (0.32)	0.08 (0.24)	0.36	0.39 (0.31)	0.04 (0.37)	0.71	0.74

SD: standard deviation.
†p values for between-group difference in change from baseline derived from analysis of covariance analysis adjusting for baseline value.

mation of ROS which can initiate lipid peroxidation, thereby leading to oxidative damage to various cell structures.[17] Cumulative oxidative stress has been implicated in etiology of diseases such as AMD and DR.[18] As the main component of macular pigment, lutein can quench singlet oxygen, scavenge free radicals, and protect retinal cells from oxidative damage.[19] In animal models of DR, lutein has been demonstrated to be capable of normalizing the diabetes-induced histological modifications and maintaining mitochondrial homeostasis, suggesting that lutein supplementation may reverse the vision loss in early DR patients.[11,20] Previous studies had suggested supplementation with xanthophylls found in the macular might have beneficial effects on improving visual function. Richer et al demonstrated that lutein and antioxidant supplementation in atrophic AMD patients induced a 5.4-letter increase in Snellen equivalent VA.[21] In a 9-month supplementation study by Cangemi, subjects with atrophic AMD receiving lutein supplement gained an average of one-half of a line of VA at 6 months.[22] In accordance with these studies, the results of the study conducted by Dawczynski et al also indicated the significant improvement in VA was observed after supplementation with macular xanthophylls and co-antioxidants in persons with dry AMD.[23] However, only a trend toward increase in VA was observed in active treatment group in our study. This difference in the amount of VA improvement across studies can be attributed partly to differences in subject populations. Compared with the dry AMD patients in most previous studies, the subjects with early DR in this study had good baseline VA; and therefore the magnitude of improvement in VA for these subjects was smaller than that for the patients with late AMD. This hypothesis was also supported by the finding from our study, which demonstrated significant relationships between the improvements in VA and the corresponding baseline level. Consequently, this result indicated that individuals with relatively reduced acuity might benefit more from supplementation.

VA at normal illumination level (100%) only relates to the ability to resolve details of maximum contrast, and increased VA is not always associated with the improvements in quality of vision under photopic and mesopic conditions.[24-25] Individuals with normal VA can have reduced CS and may experience trouble in identifying objects at night or in dimly lit places. As evaluating visual capacity comprehensively, CS is considered a more accurate and reliable predictor of acuity improvement.[26] In contrast to no significant change in VA during 36 weeks, the significant improvements in CS at low illumination levels were observed in the present study, suggesting that macular carotenoids play the functional roles in the human eye. The blue-light filtration effects of these xanthophylls combined with their ability to minimize chromatic aberration may serve to enhance fine detail distinction, increase contrast, and improve visual sensitivity.[26-27] In addition, recent findings demonstrated that the ability to withstand glare decreased in people with low macular pigment levels.[28] The present study showed that lutein supplementation led to a slight improvement in GS at different spatial frequencies in the patients with NPDR. The mechanism by which macular xanthophylls increased GS may involve its capability of filtering short-wavelength light, thereby possibly decreasing glare.[29-30]

The findings of our study have important clinical and public health implications concerning DR prevention. DR remains a common cause worldwide of blindness and visual impairment that has been gaining increased health policy importance. Our results showed that high intake of lutein and zeaxanthin were expected to have an acuity improvement for NPDR patients. As CS and GS were correlated highly with reported poor quality of vision, our finding has important implications for improving vision-related quality of life in NPDR patients. It has been estimated that the mean intake of lutein from the diet by the entire American population is 1.71 mg/day, which is substantially lower than the recommended amounts.[31] In addition, consumption of dark-green leafy vegetables, which typically contain the highest concentration of lutein, tends to be quite low.[32] Clinically, ophthalmologists could recommend NPDR patients to increase consumption of dark green vegetables and lutein supplements. Given the high prevalence and extensive treatment costs of DR, such dietary modification by increasing the consumption of lutein-rich foods may bring considerable benefits in preventing or reducing progression of DR.

In conclusion, the results of the present study showed that lutein supplementation has the potential to improve visual function in the patients with NPDR, especially for CS at low spatial frequency. Up to now, no prospective

cohort studies have been conducted to determine whether dietary antioxidant carotenoids, in particular lutein, can play a role in preventing the onset or progression of DR. Therefore, well-designed large prospective cohorts are required to examine this relationship between lutein status and DR risk. Moreover, further large long-term RCTs will be necessary to determine the possibility that such intervention could be used as an adjunct therapy to prevent vision loss and inhibit progression of retinopathy in diabetic patients, which may be important for establishing preventive and treatment approaches for this disease.

AUTHOR DISCLOSURES

None of the authors have any conflicts of interest associated with this study. This study was partially supported by grants from the National Natural Science Foundation of China (NSFC-81202198); the Natural Science Foundation of Shaanxi Province of China (2013JQ4008); New-star Plan of Science and Technology of Shaanxi Province (2015LJXX-07); the China Postdoctoral Science Special Foundation (2015T81036); and the China Postdoctoral Science Foundation Funded Project (2014M560790).

REFERENCES

1. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. Lancet. 2010;376:124-36. doi: 10.1016/S0140-6736(09)62124-3.
2. Bourne RR, Stevens GA, White RA, Smith JL, Flaxman SR, Price H et al. Causes of vision loss worldwide, 1990-2010: a systematic analysis. Lancet Glob Health. 2013;1:e339-49. doi: 10.1016/S2214 -109X(13)70113-X.
3. Hassan SS, Bong DB, Premsenthil M. Detection of neovascularization in diabetic retinopathy. J Digit Imaging. 2012; 25:437-44. doi: 10.1007/s10278-011-9418-6.
4. Bressler NM, Beck RW, Ferris FL. Panretinal photocoagulation for proliferative diabetic retinopathy. N Engl J Med. 2011;365:1520-6. doi: 10.1056/NEJMct0908432.
5. Kaštelan S, Tomić M, Gverović Antunica A, Salopek Rabatić J, Ljubić S. Inflammation and pharmacological treatment in diabetic retinopathy. Mediators Inflamm. 2013;2013: 213130. doi: 10.1155/2013/213130.
6. Annadurai T, Thomas PA, Geraldine P. Ameliorative effect of naringenin on hyperglycemia-mediated inflammation in hepatic and pancreatic tissues of Wistar rats with streptozotocin- nicotinamide-induced experimental diabetes mellitus. Free Radic Res. 2013;47:793-803. doi: 10.3109/10715762.2013.823643.
7. Zhong Q, Mishra M, Kowluru RA. Transcription factor Nrf2-mediated antioxidant defense system in the development of diabetic retinopathy. Invest Ophthalmol Vis Sci. 2013;54:3941-8. doi: 10.1167/iovs.13-11598.
8. Böhm F, Edge R, Truscott TG. Interactions of dietary carotenoids with singlet oxygen (1O2) and free radicals: potential effects for human health. Acta Biochim Pol. 2012;59:27-30.
9. Brazionis L, Rowley K, Itsiopoulos C, O'Dea K. Plasma carotenoids and diabetic retinopathy. Br J Nutr. 2009;101: 270-7. doi: 10.1017/S0007114508006545.
10. Sasaki M, Ozawa Y, Kurihara T, Kubota S, Yuki K, Noda K, Kobayashi S, Ishida S, Tsubota K. Neurodegenerative influence of oxidative stress in the retina of a murine model of diabetes. Diabetologia. 2010;53:971-9. doi: 10.1007/s00125-009-1655-6.
11. Kowluru RA, Zhong Q, Santos JM, Thandampallayam M, Putt D, Gierhart DL. Beneficial effects of the nutritional supplements on the development of diabetic retinopathy. Nutr Metab (Lond). 2014;11:8. doi: 10.1186/1743-7075-11-8.
12. Liu R, Wang T, Zhang B, Qin L, Wu C, Li Q, Ma L. Lutein and zeaxanthin supplementation and association with visual function in age-related macular degeneration. Invest Ophthalmol Vis Sci. 2015;56:252-8. doi: 10.1167/iovs.14-15553.
13. Diabetic Retinopathy Study Group. Diabetic retinopathy study. Report Number 7. A modification of the Airlie House classification of diabetic retinopathy. Invest Ophthalmol Vis Sci. 1981;21:210-26.
14. Hong T, Mitchell P, Rochtchina E, Fong CS, Chia EM, Wang JJ. Long-term changes in visual acuity in an older population over a 15-year period: the Blue Mountains Eye Study. Ophthalmology. 2013;120:2091-9. doi: 10.1016/j.ophtha.2013.03.032.
15. Gil MA, Varón C, Cardona G, Vega F, Buil JA. Comparison of far and near contrast sensitivity in patients symmetrically implanted with multifocal and monofocal IOLs. Eur J Ophthalmol. 2014;24:44-52. doi: 10.5301/ejo.5000335.
16. Schmitz S, Dick HB, Krummenauer F, Schwenn O, Krist R. Contrast sensitivity and glare disability by halogen light after monofocal and multifocal lens implantation. Br J Ophthalmol. 2014;84:1109-12.
17. Wu Y, Tang L, Chen B. Oxidative stress: implications for the development of diabetic retinopathy and antioxidant therapeutic perspectives. Oxid Med Cell Longev. 2014;2014: 752387. doi: 10.1155/2014/ 752387.
18. Wang Y, Shen D, Wang VM, Yu CR, Wang RX, Tuo J, Chan CC. Enhanced apoptosis in retinal pigment epithelium under inflammatory stimuli and oxidative stress. Apoptosis. 2012;17:1144-55. doi: 10.1007/ s10495-012-0750-1.
19. Bian Q, Gao S, Zhou J, Qin J, Taylor A, Johnson EJ, Tang G, Sparrow JR, Gierhart D, Shang F. Lutein and zeaxanthin supplementation reduces photooxidative damage and modulates the expression of inflammation-related genes in retinal pigment epithelial cells. Free Radic Biol Med. 2012;53: 1298-307. doi: 10.1016/j.freeradbiomed.2012.06.024.
20. Kowluru RA, Menon B, Gierhart DL. Beneficial effect of zeaxanthin on retinal metabolic abnormalities in diabetic rats. Invest Ophthalmol Vis Sci. 2008;49:1645-51. doi: 10.1167/iovs.07-0764.
21. Richer S, Stiles W, Statkute L, Pulido J, Frankowski J, Rudy D. 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. 2004;75:216-30.
22. Cangemi FE. TOZAL Study: an open case control study of an oral antioxidant and omega-3 supplement for dry AMD. BMC Ophthalmol. 2007;7:3.
23. Dawczynski J, Jentsch S, Schweitzer D, Hammer M, Lang GE, Strobel J. Long term effects of lutein, zeaxanthin and omega-3-LCPUFAs supplementation on optical density of macular pigment in AMD patients: the LUTEGA study. Graefes. Arch Clin Exp Ophthalmol. 2013;251:2711-23. doi: 10.1007/ s00417-013-2376-6.
24. Koefoed VF, Baste V, Roumes C, Høvding G. Contrast sensitivity measured by two different test methods in healthy, young adults with normal visual acuity. Acta Ophthalmol. 2015;93:154-61. doi: 10.1111/ aos.12487.
25. Hutchinson CV, Ledgeway T. Spatial summation of first-order and second-order motion in human vision. Vision Res. 2010;50:1766-74. doi: 10.1016/j.visres.2010.05.032.
26. Rossi EA, Rangel-Fonseca P, Parkins K, Fischer W, Latchney LR, Folwell MA, Williams DR, Dubra A, Chung MM. In vivo imaging of retinal pigment epithelium cells in

age related macular degeneration. Biomed Opt Express. 2013;4:2527-39. doi: 10.1364/BOE.4.002527.
27. Loskutova E, Nolan J, Howard A, Beatty S. Macular pigment and its contribution to vision. Nutrients. 2013;5:1962-9. doi: 10.3390/nu5061962.
28. Stringham JM, Garcia PV, Smith PA, McLin LN, Foutch BK. Macular pigment and visual performance in glare: benefits for photostress recovery, disability glare, and visual discomfort. Invest Ophthalmol Vis Sci. 2011;52:7406-15. doi: 10.1167/iovs.10-6699.
29. Subczynski WK, Wisniewska A, Widomska J. Location of macular xanthophylls in the most vulnerable regions of photoreceptor outer-segment membranes. Arch Biochem Biophys. 2010;504:61-6. doi: 10.1016/j.abb.2010.05.015.
30. Hammond BR, Fletcher LM, Elliott JG. Glare disability, photostress recovery, and chromatic contrast: relation to macular pigment and serum lutein and zeaxanthin. Invest Ophthalmol Vis Sci. 2013;54:476-81. doi: 10.1167/iovs.12-10411.
31. Institute of Medicine. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington, DC: National Academy Press; 2000.
32. O'Neill ME, Carroll Y, Corridan B, Olmedilla B, Granado F, Blanco I, Van den Berg H, Hininger I, Rousell AM, Chopra M, Southon S, Thurnham DI. A European carotenoid database to assess carotenoid intakes and its use in a five-country comparative study. Br J Nutr. 2001;85:499-507.