Comparison of macular pigment and serum lutein concentration changes between free lutein and lutein esters supplements in Japanese subjects

Hiroko Yoshizako, MD, Katunori Hara, MD, Yasuyuki Takai, MD, PhD,

Sachiko Kaidzu, PhD, Akira Obana, MD, PhD and Akihiro Ohira, MD,PhD

Department of Ophthalmology, Shimane University School of Medicine, Izumo, Shimane, Japan

Correspondence:

Akihiro Ohira, MD, PhD, Department of Ophthalmology, Shimane University Faculty of Medicine, Enya 89-1, Izumo, Shimane, 693-8501, Japan

Tel: +81-853-20-2284

Fax: +81-853-20-2278

Email: aohira@med.shimane-u.ac.jp

ABSTRACT

Purpose: To compare changes in macular pigment optical density (MPOD) and serum lutein concentration between free lutein and lutein esters supplements in healthy Japanese individuals.

Methods*:* Twenty healthy subjects (age range, 22-47 years) were recruited into this prospective, randomized, doubled-blind comparative study. Individuals were evenly divided into two groups: free lutein group, supplementation with 10 mg of free lutein; or lutein esters group, supplementation with 20 mg of lutein esters equivalent to 10 mg of free lutein. Each participant took either type of oral lutein daily for 3 months. The serum lutein concentrations and MPOD levels were measured at baseline and 3 and 6 months after the start of supplementation.

Results: There were no significant differences in the serum lutein concentrations and MPOD levels at baseline between the groups. The increased serum lutein concentration and MPOD levels at 3 months were, respectively, 89% and 38% in the free lutein group and 97% and 17% in the lutein esters group. The serum lutein concentrations in both groups and MPOD levels in the free lutein group increased significantly ($p<0.05$) from baseline. No significant differences in serum lutein concentrations and MPOD levels were seen between the groups. Three months after supplementation ended, the serum

lutein concentration decreased; the MPOD remained elevated in both groups.

Conclusions: The serum lutein concentrations and MPOD levels increased significantly with either free lutein or lutein esters, and no significant differences were found between the two. Both were considered useful as lutein supplements.

Key words: free lutein -- lutein esters -- macular pigment optical density (MPOD) -- serum lutein concentration

Introduction

In 1980s, macular pigment was defined chemically as a mixture of two carotenoids, lutein and zeaxanthin (Bone et al. 1985; Snodderly et al. 1984), which are concentrated in the macula lutea, absorb blue light, and act as a filter that may attenuate photochemical damage caused by short-wavelength visible light (blue light). These carotenoids are also antioxidants that may protect against light-induced oxidative damage in the retina by quenching oxygen radicals (Sommerburg et al. 1999; Rapp et al. 2000).

Age-related macular degeneration (AMD) is a multifactorial disease, and oxidative stress caused by short wavelength blue light is considered an important factor in the disease (Nicolas et al. 1996). Since macular pigment protects against the blue light hazard, numerous studies of macular pigments and AMD have been undertaken (Puell et al. 2013; Tsika et al. 2011; Thurnham et al. 2015). Some studies have found that MPOD levels in AMD eyes were significantly lower than in normal eyes (Kaya et al. 2012; Beatty et al. 2001; Bernstein et al. 2002), and our previous study in a Japanese population suggested that lower MPOD levels may be a risk factor for AMD progression (Obana et al. 2008). The ability of lutein and zeaxanthin supplements to prevent AMD has been investigated (Krinsky & Johnson 2005; Krinsky et al. 2003;

Landrum & Bone 2001) and a large clinical study (Age-Related Eye Disease Study 2 Research Group, 2013) recommended antioxidant supplements containing lutein and zeaxanthin.

Humans cannot synthesize lutein in the body; it must be obtained from ingestion of vegetables and fruits or supplements. Lutein can be present in fruits and vegetables both in the free form and the more stable fatty acid esterified form (Dugo et al. 2008). In the case of lutein esters, ester is cleaved from lutein molecule and unesterified free lutein is generated during the absorption process in the intestines. Free lutein is incorporated into micelles and absorbed into blood via scavenger receptor class B type 1 (SR-B1) in the intestinal epithelial cells, where it binds mainly with high-density lipoprotein (HDL) (Li et al. 2010) and is transported in the blood vessels. Free lutein is taken into the retinal pigment epithelium by SR-B1 and then into the photoreceptor cells by inter-photoreceptor retinoid binding protein. In the retina, lutein binds steroidogenic acute regulatory domain protein 3 (Li et al. 2011) and is stored mainly in the inner and outer plexiform layers. Some investigators have suggested that the absorption rate in the intestine differs between free lutein and lutein esters, and some studies showed different serum lutein concentrations and MPOD levels after supplementation of free lutein and lutein esters. Bowen et al. (2002) reported that the

serum lutein concentrations in subjects taking lutein esters supplementation were higher than in those taking free lutein and concluded that the lutein esters form was more bioavailable than the free lutein. In contrast, Norkus et al. (2010) reported that the serum lutein response was higher with free lutein than with lutein esters. Few reports have been published about the response of the MPOD levels to lutein supplementation compared with each type of lutein (Landrum et al. 2012). Therefore, it remains unclear which type of lutein supplementation increases the MPOD levels. In the current study, we investigated the response in the serum lutein concentrations and MPOD levels to two forms of lutein in healthy Japanese individuals. This is not a study to prove the equivalence of both forms.

Methods

The Institutional Review Board of Shimane University Hospital approved the study. Each subject received a full explanation of the study and signed an informed consent form in compliance with the tenets of the Declaration of Helsinki.

Twenty healthy Japanese subjects who ranged in age from 22 to 47 years (8 men, 12 women) were recruited into this prospective, randomized, doubled-blind study and received either 10 mg of oral free lutein (n = 10) or 20 mg of lutein esters (n = 10) daily

for 3 months. The subjects were randomized to a supplement by a computer-generated table of random numbers. No subjects had taken lutein, zeaxanthin, or vitamins before this study. No subjects had a history of smoking. Based on an interview at examination, no subject missed taking lutein supplements during the 3-month period.

Each capsule of free lutein supplement contained lutein equivalent to 10 mg of free lutein. One capsule of free lutein contained 50 mg of 20% free lutein suspension (Katra Phytochem India Pvt. Ltd., Bangalore, India) in a safflower oil suspension. Each capsule of lutein esters supplement contained 25 mg of the esterified form of lutein (Lutein-P80, Oryza Oil & Fat Chemical Co. Ltd., Aichi, Japan), which was equivalent to 10 mg of free lutein in safflower oil suspension. Both supplements, each capsule of which weighed 200 mg, were prepared by Biyon Co. Ltd and supplied free of charge. The contents of each supplement are shown in Table 1.

No subjects had ocular or systemic pathologies. The best-corrected logarithm of the minimum angle of resolution visual acuity (logMAR VA) and refractive error of each individual were measured at baseline and 3 and 6 months after the start of supplementation. Subjects underwent contrast and glare sensitivity testing, using a contrast glare-tester (Model CGT-2000, Takagi, Nagano, Japan) at the three time points. With the CGT-2000, contrast threshold values were assessed at six visual angles (sizes)

of the target (6.3, 4.0, 2.5, 1.6, 1.0, and 0.64 degrees) under mesopic (10 candelas/square meter) conditions. The thresholds also were assessed under glare (40,000 candelas/square meter) conditions using the same target sizes. MPOD levels were measured using a resonance Raman spectroscopy (RRS) at the three time points. The RRS device and measurement procedures were described previously (Bernstein et al. 2002; Ermakov et al. 2004). Blood samples were obtained from each subject at the three time points, and measurements of serum lutein concentration were conducted with high-performance liquid chromatography using methods previously described (Obana et al. 2015) by Oryza Oil & Fat Chemical Co. Ltd.

Statistical analysis

Statistical analyses were performed using JMP version 11 software (JMP Statistical Discovery, Cary, NC, USA). Subject age, logMAR VA, spherical equivalent refractive error, contrast and glare sensitivity, MPOD levels, and serum lutein concentrations between the two supplementation groups were compared using the Mann-Whitney U-test. Sex was compared using Fisher’s exact probability test. VA, contrast and glare sensitivity, MPOD levels, and serum lutein concentrations were measured at baseline and 3 and 6 months in each individual and compared using the Wilcoxon signed-rank

test. $p < 0.05$ was considered significant.

Results

Table 2 shows the demographic data of the subjects at the baseline examination. Subject age, sex, spherical equivalent refractive error, serum lutein concentrations, and MPOD levels did not differ significantly between the groups. The mean logMAR VA was -0.08 ±1.46 in free lutein group and -0.13±0.08 in lutein ester group, which did not differ significantly. The logMAR VA was stable throughout the study and no significant changes were seen in both groups 3 and 6 months after the start of supplementation.

Figure 1 shows the contrast and glare sensitivities in both groups. Three and 6 months after the start of supplementation, there were no significant differences in contrast and glare sensitivities across all targets, except for the glare sensitivity at 4.0 degrees ($p = 0.04$) in the lutein esters group 6 months after supplementation.

The mean baseline serum lutein concentrations were 3.7±1.05 μmol/L in the free lutein group and 3.2±1.21 μmol/L in the lutein esters group, which did not differ significantly. Figure 2 shows the changes in the mean serum lutein concentrations. Three months after the start of supplementation, the serum lutein concentration increased to 6.4 ±2.98 μmol/L in the free lutein group and to 5.7±1.63 μmol/L in the

lutein esters group, both of which differed significantly from baseline. At 6 months, i.e., 3 months after the end of supplementation, the serum lutein concentrations decreased to 4.2 ± 1.02 μmol/L in the free lutein group and to 4.0 ± 1.30 μmol/L in the lutein esters group, but both were still significantly higher than baseline. Table 3 shows the increasing serum lutein concentrations in both groups. At 3 months, the rate was 89% in the free lutein group and 97% in the lutein esters group, which did not differ significantly.

The baseline MPOD levels were 1,322.9 ± 568.8 (Raman counts) in the free lutein group and 1,604.0 ± 372.5 in the lutein esters group, which did not differ significantly. Figure 3 shows the changes in the MPOD levels. At 3 months, the MPOD level increased to 1,660.6 ± 583.1 in the free lutein group, which differed significantly from baseline. At 6 months, i.e., 3 months after the end of supplementation, the MPOD level increased to 1,755.8 ± 556.1, which differed significantly from baseline. In the lutein esters group, the mean MPOD level at 3 months was 1,815.9 ± 209.6 and did not differ significantly from baseline, but the mean MPOD level was 2,301.7 ± 744.5 at 6 months. This was significantly higher than at baseline and 3 months. Table 4 shows the increasing MPOD levels in both groups. The increasing MPOD levels in the free lutein group were 38% at 3 months and 47% at 6 months. The increasing MPOD levels in the

lutein esters group were 17% at 3 months and 50% at 6 months. The increase in the lutein esters group at 3 months was lower than that of free lutein, but there was no significant difference at 6 month.

Discussion

The serum lutein concentrations increased 3 months after supplementation in both the free lutein and lutein esters group and the increasing levels did not differ between the two groups. The bioavailability of free lutein and lutein esters is not fully understood, and some studies have reported different effects on the increases in the serum lutein concentrations. In the current study, however, there was no significant difference between the two supplements that contained the same amount of free lutein. This result suggested that esterification did not affect intestinal absorption. Three months after cessation of the supplements, the serum lutein concentrations decreased in both groups. Landrum et al. (1997) also reported this rapid decrease. Lutein is generally stored in adipose tissue but not in the blood.

MPOD levels increased 38% with 3 months supplementation of free lutein; in contrast, the increase was 17% in the lutein ester group, but the MPOD levels in the lutein ester group at 6 months, i.e., 3 months after cessation of supplementation

increased 50%, which was equivalent to 47% in the free lutein group. The interpretation of this delayed increase in the lutein ester group was uncertain, but it is unrealistic that there is any difference in the uptake of the two supplements from the blood to the retina, because lutein esters are converted to free lutein in the intestine, and free lutein binding with HDL and other lipoproteins is transported to the choriocapillaris. Therefore, we speculated that the low increase in the lutein esters level at 3 months may have been due to the small number of subjects. In this study, we did not repeat the measurement of MPOD levels before the supplements were stopped. Another study is needed to interpret the current results.

The MPOD levels at 6 months were higher than at 3 months in both supplement groups, although the serum lutein concentrations decreased. These results suggested that the MPOD levels keep increasing for some period after supplementation stopped. Several studies have reported the tendency for a post-supplementation increase in the MPOD levels (Landrum et al. 1997; Hammond et al. 1997; Trieschmann et al. 2007). Wang et al. (2007) reported that lutein was selectively retained in the retina of chicks receiving a xanthophyll-free diet for 28 days; in contrast, the lutein concentrations in the plasma and other tissues decreased up to 90% of their original level. Some mechanisms have been considered. Landrum et al. (1997) suggested a very slow turnover of

carotenoids in the retina and a possible specific mechanism to maintain the MPOD levels in the retina. Li et al. (2014) reported that the binding affinities between human β, β-carotene-9', 10'-dioxygenase (BCO2) and lutein, zeaxanthin, and meso-zeaxanthin were 10- to 40-fold weaker in humans than in mice (in vitro). BCO2 is a xanthophyll carotenoid cleavage enzyme. The inactivity of BCO2 in humans may induce lengthy preservation of lutein in the retina. Generally, adipose tissue is a major storage organ of carotenoids (Parker 1989; Kaplan et al. 1990). Johnson et al. (2000) examined the relationships among the lutein concentration in serum and adipose tissue and the MPOD levels in subjects with addition of spinach (60 g/day) and corn (150 g/day) to the diet for 15 weeks. After cessation of the dietary modification, lutein concentration in the adipose tissue decreased, while the MOPD levels remained high. The authors suggested that macular pigment in the retina might be supplied from lutein stored in adipose tissue.

There are several methods to measure the MOPD levels, such as heterochromatic flicker photometry (HFP), fundus autofluorescence imaging (AFI), fundus reflectance imaging, and RRS. HFP is used most widely and the consensus is that the method is accurate. However, since HFP is subjective, the MPOD levels cannot be measured in some subjects due to patient misunderstanding or poor response skills and it takes a

relatively long time to achieve measurement. RRS that is used only in approved clinical studies is an objective method, and MPOD levels can be measured in several seconds. In a study using RRS, the MPOD levels increased 24% after 3-months supplementation of 10 mg/day of free lutein in healthy Japanese subjects (Tanito et al 2012). The increased rate in the current study was higher (38%); however, the small number of subjects makes it impossible to reach a conclusion. Obana et al. (2015) failed to show an increase in the mean MPOD levels by RRS with 6-months supplementation with 10 mg/day of free lutein. However, those authors reported three response patterns in the increase in the MPOD levels and serum lutein concentrations, i.e., “retinal responders” who had an increases in both the MPOD levels and serum lutein concentrations, “retinal non-responders” who had only increased serum concentrations and no change in the MPOD levels, and “retinal and serum non-responders” who had no increases in either the MPOD level or plasma concentration. In the current study, the small number of subjects made it difficult to examine the response patterns. In reports using a measurement technique other than RRS, the MPOD levels increased by 32% by HFP with 18 to 24 weeks of supplementation of 10 mg/day lutein, although seven patients with AMD were included among the 13 subjects (Koh et al. 2004). This value was similar to the current one. Trieschmann et al. (2007) reported an 11% increase in MPOD

levels measured by AFI with 24 weeks of supplementation with 12 mg/day lutein and 1 mg/day zeaxanthin supplementation mostly in patients with AMD. The increase in the MPOD level may be lower in patients with AMD than in healthy subjects.

MPOD levels and serum lutein concentrations were affected by many factors, such as race (Rock et al. 2002; Gruber et al. 2004), age (Obana et al. 2014), sex (Hammond Jr. et al. 1996), smoking habits (Rock et al. 2002; Gruber et al. 2004), axial length (Tong et al. 2013; Obana et al. 2014), refractive error (Tanito et al. 2012), iris color (Hammond Jr. et al. 1996), body fat and BMI (Bovier et al. 2013; Hammond Jr. et al. 2002; Nolan et al. 2004), serum lipid concentration (Renzi et al. 2012; Loane et al. 2010), dietary intake, and genetic background (Liew et al. 2005). In the current study, we investigated the refractive errors and smoking habits but not the other factors. No subjects mentioned marked changes in dietary habits during the study in the final interview, but the absence of dietary information and other factors such as serum lipid concentration and genetic background limit the relevance of this study. Further, this study was not designed to determine the equivalence of the two lutein supplements. A more detailed investigation with more subjects is needed to prove the effects of free lutein or lutein esters.

In the current study, serum lutein concentrations increased significantly 3 months after supplementation with either free lutein or lutein esters, and no significant

differences were detected between the two. The MPOD levels significantly increased 6 months after supplementation began with both free lutein or lutein esters. Both forms of lutein were considered useful for supplements to increase macular pigments that are useful to prevent development of AMD.

References

Age-Related Eye Disease Study 2 Research Group. (2013): Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. JAMA 309: 2005-2015. doi: 10.1001/jama.2013.4997.

Beatty S, Murray IJ, Henson DB, Carden D, Koh H & Boulton ME (2001): Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. Invest Ophthalmol Vis Sci 42:439–446.

Bernstein PS, Zhao D-Y, Wintch SW, Ermakov IV, McClane RW & Gellermann W (2002): Resonance Raman measurement of macular carotenoids in normal subjects and in age-related macular degeneration patients. Ophthalmology 109: 1780–1787.

Bone RA, Landrum JT & Tarsis SL (1985): Preliminary identification of the human macular pigment. Vision Res 25: 1531–1535.

Bovier ER, Lewis RD & Hammond BR Jr. (2013): The relationship between lutein and zeaxanthin status and body fat. Nutrients 5: 750-757.

Bowen PE, Herbst-Espinosa SM, Hussain EA & Stacewicz-Sapuntzakis M (2002): esterification does not impair lutein bioavailability in humans. J Nutr 132: 3668-3673

Dugo P, Herrero M, Kumm T, Giuffrida D, Dugo G & Mondello L (2008): Comprehensive normal-phase x reversed-phase liquid chromatography coupled to photodiode array and mass spectrometry detection for the analysis of free carotenoids and carotenoid esters from mandarin. J Chromatogr A 1189: 196-206.

Ermakov IV, Ermakova MR, Gellermann W & Bernstein PS (2004): Macular pigment Raman detector for clinical applications. J Biomed Optics 9: 139–148.

Gruber M, Chappell R, Millen A, LaRowe T, Moeller SM, Iannaccone A, Kritchevsky SB & Mares J (2004): Correlates of serum lutein + zeaxanthin: findings from the Third National Health and Nutrition Examination Survey. J Nutr 134: 2387-2394.

Hammond BR Jr, Curran-Celentano J, Judd S, Fuld K, Krinsky NI, Wooten BR & Snodderly DM (1996): Sex differences in macular pigment optical density: relation to plasma carotenoid concentrations and dietary patterns. Vision Res 36: 2001-2012.

Hammond BR Jr., Fuld K & Snodderly DM (1996): Iris color and macular pigment optical density. Exp Eye Res 62: 293-297.

Hammond BR Jr, Johnson EJ, Russell RM, Krinsky NI, Yeum KJ, Edwards RB & Snodderly DM (1997): Dietary modification of human macular pigment density. Invest Ophthalmol Vis Sci 38: 1795–1801.

Hammond BR Jr, Ciulla TA & Snodderly DM (2002): Macular pigment density is reduced in obese subjects. Invest Ophthalmol Vis Sci 43: 47-50.

Johnson EJ, Hammond BR, Yeum KJ, Qin J, Wang XD, Castaneda C, Snodderly DM & Russell RM (2000): Relation among serum and tissue concentrations of lutein and zeaxanthin and macular pigment density. Am J Clin Nutr 71: 1555-1562.

Kaplan LA, Lau JM & Stein EA (1990): Carotenoid composition, concentrations, and relationships in various human organs. Clin Physiol Biochem 8: 1-10.

Kaya S, Weigert G, Pemp B, Sacu S, Werkmeister RM, Dragostinoff N, Garhöfer G, Schmidt-Erfurth U & Schmetterer L (2012): Comparison of macular pigment in patients with age-related macular degeneration and healthy control subjects - a study using spectral fundus reflectance. Acta Ophthalmol 90: e399-e403.

Koh HH, Murray IJ, Nolan D, Carden D, Feather J & Beatty S (2004): Plasma and macular responses to lutein supplement in subjects with and without age-related maculopathy: a pilot study. Exp Eye Res 79: 21–27.

Krinsky NI, Landrum JT & Bone RA (2003): Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. Annu Rev Nutr 23: 171–201.

Krinsky NI & Johnson EJ (2005): Carotenoid actions and their relation to health and disease. Mol Aspects Med 26: 459–516.

Landrum JT, Bone RA, Joa H, Kilburn MD, Moore LL & Sprague KE (1997): A one year study of the macular pigment: the effect of 140 days of a lutein supplement. Exp Eye Res 65: 57-62.

Landrum JT & Bone RA (2001): Lutein, zeaxanthin, and the macular pigment. Arch Biochem Biophys 385: 28–40.

Landrum J, Bone R, Mendez V, Valenciaga A & Babino D (2012): Comparison of dietary supplementation with lutein diacetate and lutein: a pilot study of the effects on serum and macular pigment. Acta Biochim Pol 59: 167-169.

Li B, Vachali P & Bernstein PS (2010): Human ocular carotenoid-binding proteins. Photochem Photobiol Sci 9: 1418-1425.

Li B, Vachali P, Frederick JM & Bernstein PS (2011): Identification of StARD3 as a lutein-binding protein in the macula of the primate retina. Biochemistry 50: 2541-2549.

Li B, Vachalia PP, Gorusupudia A, Shena Z, Sharifzadeha H, Bescha BM, Nelsona K, Horvatha MM, Fredericka JM, Baehra W & Bernsteina PS (2014): Inactivity of human β,β-carotene-9′,10′-dioxygenase (BCO2) underlies retinal accumulation of the human macular carotenoid pigment. PNAS 111: 10173–10178.

Liew SH, Gilbert CE, Spector TD, Mellerio J, Marshall J, van Kuijk FJ, Beatty S, Fitzke F & Hammond CJ (2005): Heritability of macular pigment: a twin study. Invest Ophthalmol Vis Sci 46: 4430-4436.

Loane E, Nolan JM & Beatty S (2010): The respective relationships between lipoprotein profile, macular pigment optical density, and serum concentrations of lutein and zeaxanthin. Invest Ophthalmol Vis Sci 51: 5897-5905.

Nicolas MG, Fujiki K, Murayama K, Suzuki MT, Shindo N, Hotta Y, Iwata F, Fujimura T, Yoshikawa Y, Cho F & Kanai A (1996): Studies on the mechanism of early onset macular degeneration in cynomolgus monkeys. II. Suppression of metallothionein synthesis in the retina in oxidative stress. Exp Eye Res 62: 399-408.

Nolan J, O'Donovan O, Kavanagh H, Stack J, Harrison M, Muldoon A, Mellerio J & Beatty S (2004): Macular pigment and percentage of body fat. Invest Ophthalmol Vis Sci 45: 3940-3950.

Norkus EP, Norkus KL, Dharmarajan TS, Schierle J & Schalch W (2010): Serum lutein response is greater from free lutein than from esterified lutein during 4 weeks of supplementation in healthy adults. J Am Coll Nutr 29: 575-585.

Obana A, Hiramitsu T, Gohto Y, Ohira A, Mizuno S, Hirano T, Bernstein PS, Fujii H, Iseki K, Tanito M & Hotta Y (2008): Macular carotenoid levels of normal subjects and age-related maculopathy patients in a Japanese population. Ophthalmology 115: 147-157.

Obana A, Gohto Y, Tanito M, Okazaki S, Gellermann W, Bernstein PS & Ohira A (2014): Effect of age and other factors on macular pigment optical density measured with resonance Raman spectroscopy. Graefes Arch Clin Exp Ophthalmol 252:1221-1228.

Obana A, Tanito M, Gohto Y, Okazaki S, Gellermann W & Bernstein PS (2015): Changes in macular pigment optical density and serum lutein concentration in Japanese subjects taking two different lutein supplements. PLoS One Oct 9;10(10):e0139257. doi: 10.1371/journal.pone.0139257. eCollection 2015.

Parker RS (1989): Carotenoids in human blood and tissues. J Nutr 119: 101-104.

Puell MC, Palomo-Alvarez C, Barrio AR, Gómez-Sanz FJ & Pérez-Carrasco MJ (2013): Relationship between macular pigment and visual acuity in eyes with early age-related macular degeneration. Acta Ophthalmol 91: 298-303.

Rapp LM, Maple SS & Choi JH (2000): Lutein and zeaxanthin concentrations in rod outer segment membranes from perifoveal and peripheral human retina. Invest Ophthalmol Vis Sci 41: 1200–1209.

Renzi LM, Hammond BR Jr, Dengler M & Roberts R (2012): The relation between serum lipids and lutein and zeaxanthin in the serum and retina: results from cross-sectional, case-control and case study designs. Lipids Health Dis 29: 11:33. doi: 10.1186/1476-511X-11-33.

Rock CL, Thornquist MD, Neuhouser ML, Kristal AR, Neumark-Sztainer D, Cooper DA, Patterson RE & Cheskin LJ (2002): Diet and lifestyle correlates of lutein in the blood and diet. J Nutr 132: 525S-530S.

Snodderly DM, Brown PK, Delori FC & Auran JD (1984): The macular pigment. I. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. Invest Ophthalmol Vis Sci 25: 660–673.

Sommerburg OG, Siems WG, Hurst JS, Lewis JW & van Kuijk FJGM (1999): Lutein and zeaxanthin are associated with photoreceptors in the human retina. Curr Eye Res 19:491–495.

Tanito M, Obana A, Gohto Y, Okazaki S, Gellermann W & Ohira A (2012): Macular pigment density changes in Japanese individuals supplemented with lutein or

zeaxanthin: quantification via resonance Raman spectrophotometry and autofluorescence imaging. Jpn J Ophthalmol 56:488–496.

Thurnham DI, Nolan JM, Howard AN & Beatty S (2015): Macular response to supplementation with differing xanthophyll formulations in subjects with and without age-related macular degeneration. Graefes Arch Clin Exp Ophthalmol 253: 1231-1243.

Tong N, Zhang W, Zhang Z, Gong Y, Wooten B & Wu X (2013): Inverse relationship between macular pigment optical density and axial length in Chinese subjects with myopia. Graefes Arch Clin Exp Ophthalmol 251: 1495–1500.

Trieschmann M, Beatty S, Nolan JM, Hense HW, Heimes B, Austermann U, Fobker M & Pauleikhoff D (2007): Changes in macular pigment optical density and serum concentrations of its constituent carotenoids following supplemental lutein and zeaxanthin: the LUNA study. Exp Eye Res 84: 718-728.

Tsika C, Tsilimbaris MK, Makridaki M, Kontadakis G, Plainis S & Moschandreas J (2011): Assessment of macular pigment optical density (MPOD) in patients with unilateral wet age-related macular degeneration (AMD). Acta Ophthalmol 89: 573-578.

Wang Y, Connor SL, Wang W, Johnson EJ& Connor WE (2007): The selective retention of lutein, meso-zeaxanthin and zeaxanthin in the retina of chicks fed a xanthophyll-free diet. Exp Eye Res 84: 591-598.

Table 1. Contents of supplements tested in the current study

	Free lutein	Lutein esters
Weight of contents in one capsule (mg)	200	200
Lutein contained in one capsule (mg)	10	10 (as free lutein)
Zeaxanthin contained in one capsule (mg)	0	0
Suspension	safflower oil	safflower oil

Table 2. Demographic data

	Free lutein	Lutein ester	P value
No. subjects	10	10	
Age			
Mean ± SEM	33.8 ± 7.4	30.7± 7.1	0.3516*
Range	22 to 47	22 to 41	
Sex			
Men	3 (30%)	5 (50%)	0.3613†
Women	7(70%)	5 (50%)	
Spherical equivalent (D)			
Mean ± SEM	-3.1 ± 1.8	-2.6 ± 2.5	0.6355*
Range	-0.5 ~ -6.0	0.25 ~ -7.75	
Serum lutein concentration at baseline (μmol/L)			
Mean ± SEM	3.7 ± 0.6	3.2 ± 0.7	0.3551*
Range	2.16 ~ 5.79	1.73 ~ 5.06	
MPOD at baseline (Raman counts)			
Mean ± SEM	1322.9 ± 179.9	1604.0 ± 117.8	0.2076*
Range	508 ~ 2317	1139 ~ 2364	

SEM: standard error of the mean

*Comparison between the free and esters groups by the unpaired t-test.

†Comparison between the free and esters groups by Fisher's exact probability test.

Table 3. Increasing serum lutein concentrations in both groups

Lutein type	3 months	6 months
Free	1.89 ± 1.11*	1.20 ± 0.32
Ester	1.97 ± 0.79*	1.40 ± 0.63†

The serum lutein level after 3 or 6 months/serum lutein level at baseline.

The data are expressed as the mean ± standard deviation.

*$p<0.05$ vs baseline by the Wilcoxon signed-rank test.

†$p<0.05$ vs. 3 months by the Wilcoxon signed-rank test.

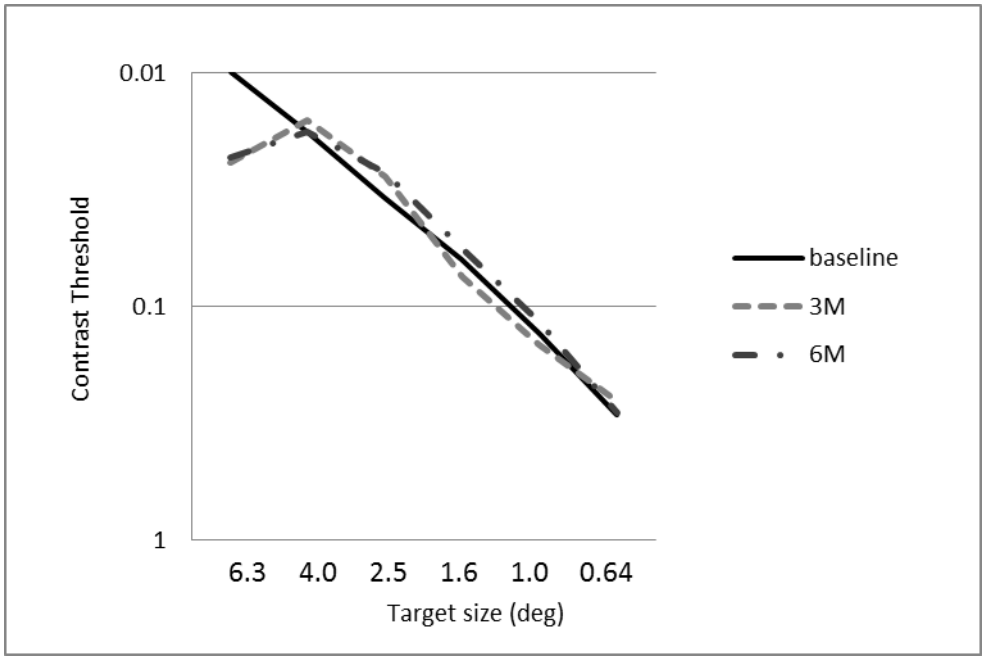
Table 4. Increasing MPOD levels in both groups

Lutein type	3 months	6 months
Free	1.38 ± 0.68*	1.47 ± 0.58*
Ester	1.17 ± 0.24	1.50 ± 0.66*†

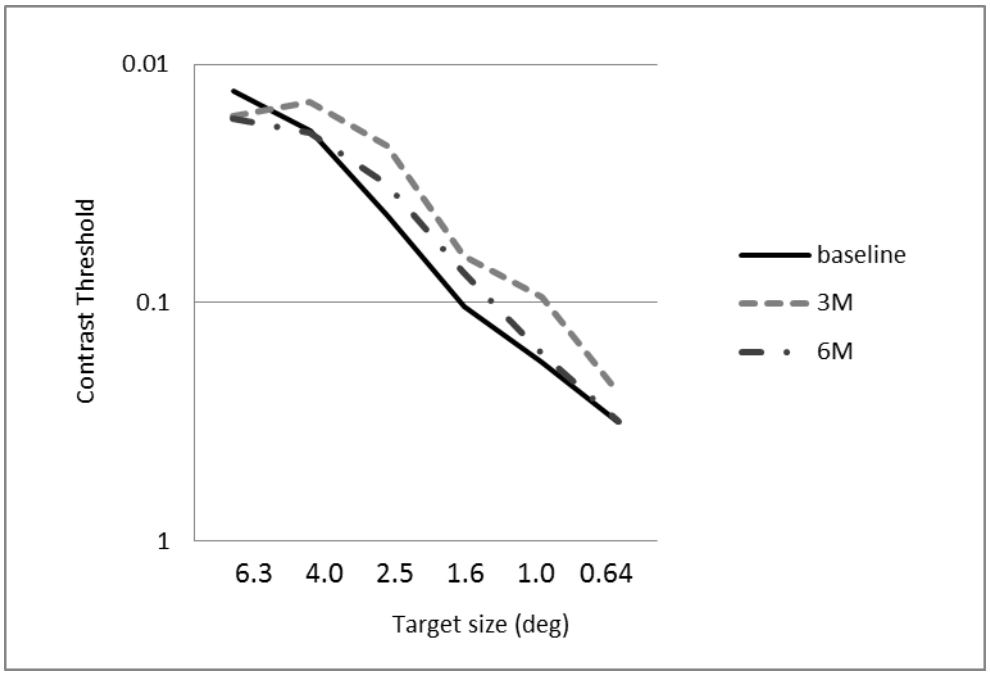
The MPOD levels after 3 or 6 months/MPOD levels at baseline.
The data are expressed as the mean ± standard deviation.
*$p<0.05$ vs. baseline by the Wilcoxon signed-rank test.
†$p<0.05$ vs. 3 months by the Wilcoxon signed-rank test.

Figure 1

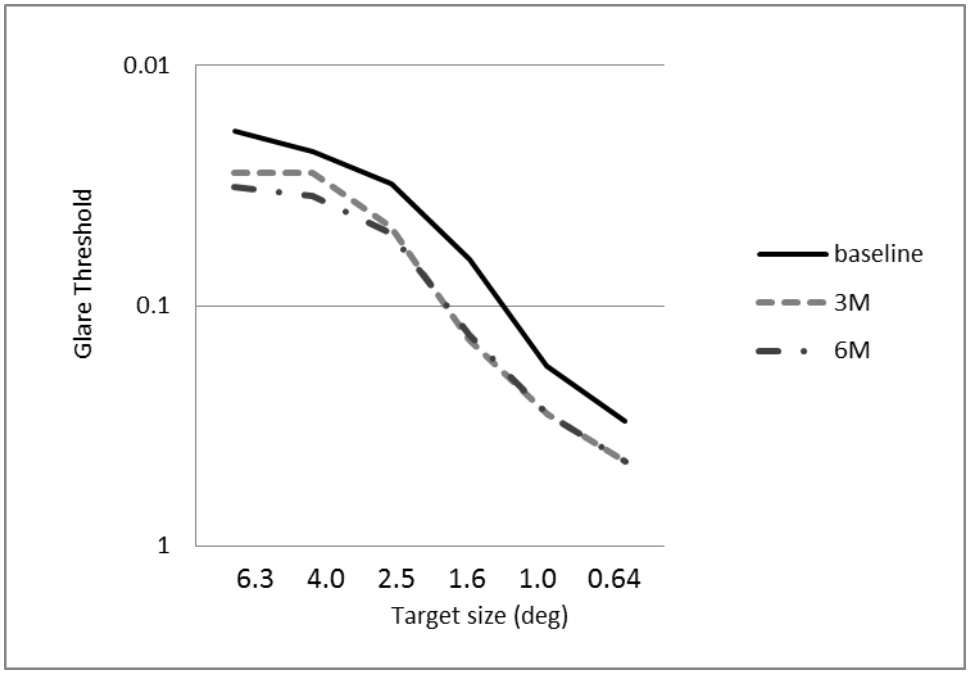
A


0.01
0.1
1
Contrast Threshold
6.3
4.0
2.5
1.6
1.0
0.64
Target size (deg)
baseline
3M
6M

B


0.01
0.1
1
Contrast Threshold
6.3
4.0
2.5
1.6
1.0
0.64
Target size (deg)
baseline
3M
6M

C



D

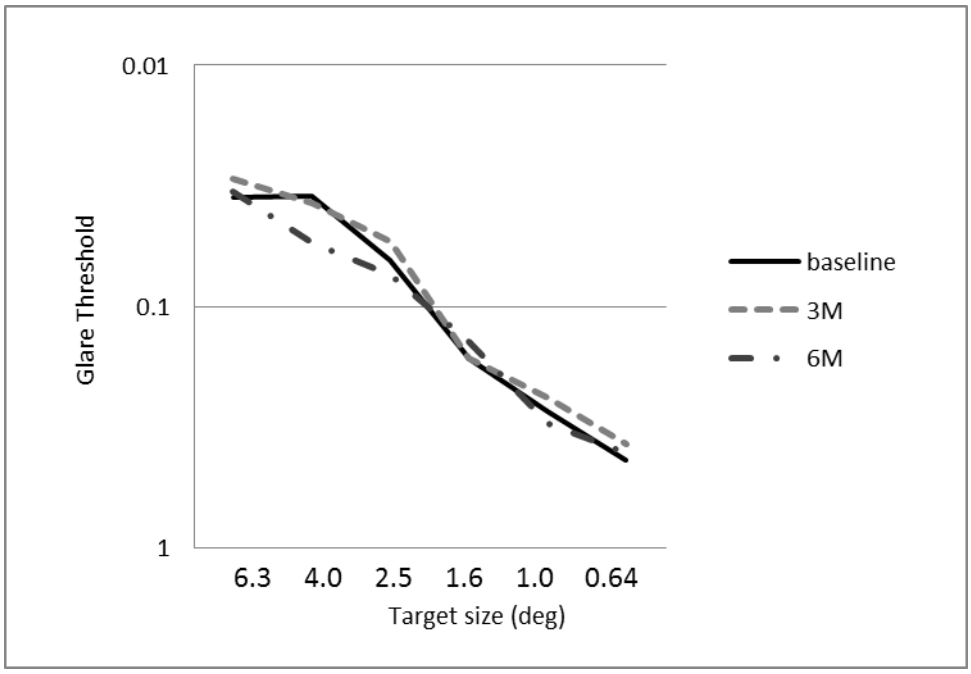


Fig. 1. Contrast and glare sensitivity. A, B, There are no significant differences in contrast sensitivity across all targets 3 and 6 months after the start of supplementation. C, D, Glare sensitivities in both groups 3 and 6 months after supplementation do not significantly change at all targets except for 4.0 degrees ($p = 0.04$) in the lutein esters group 6 months after supplementation. Deg = degrees; 3M = 3 months; 6M = 6 months. A and B, respectively, show measurements of the contrast sensitivity in the

free lutein group and lutein esters group. C and D, respectively, show measurements of the glare sensitivity in the free lutein group and lutein esters group.

Figure 2

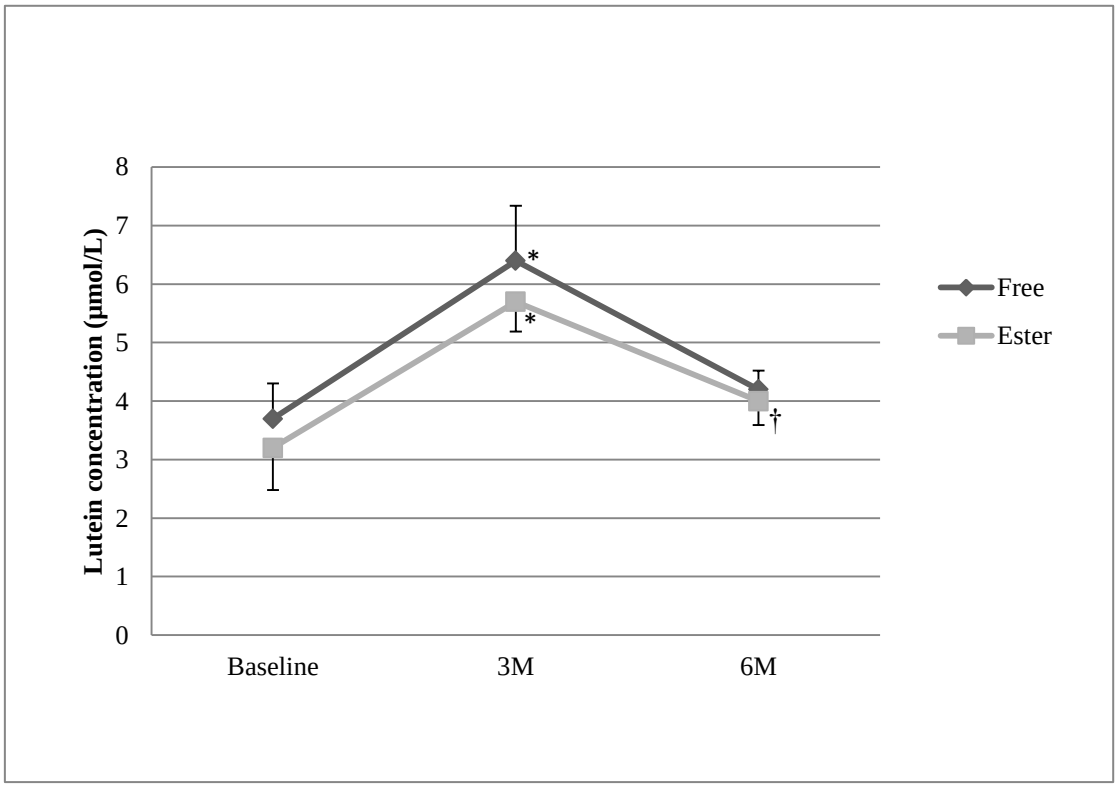


Fig. 2. Serum lutein concentration. Three months after the start of lutein supplementation, the serum lutein concentration significantly increases in both groups compared with baseline. Six months after the start of supplementation, the serum lutein concentration decreases in both groups. The data are expressed as the mean ± standard error of the mean (μmol/L). 3M = 3months; 6M = 6months. *$p<0.05$ vs. baseline by the Wilcoxon signed-rank test. †$p<0.05$ vs. 3 months by the Wilcoxon signed-rank test.

Figure 3

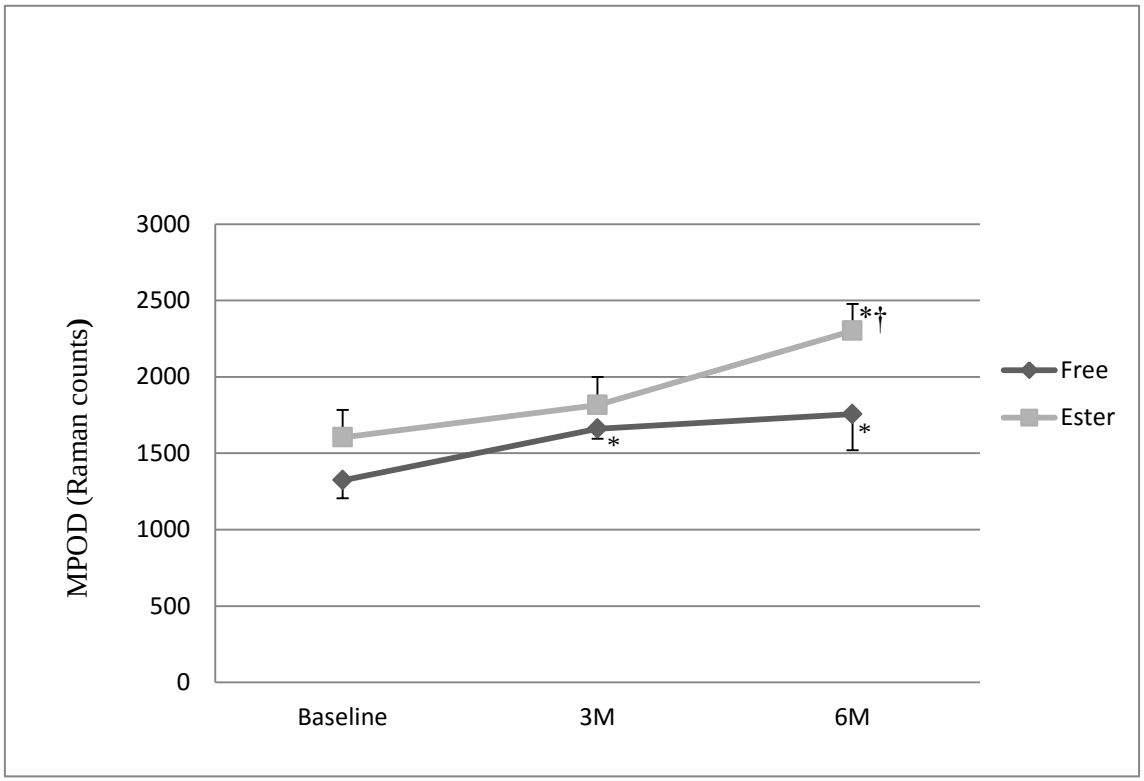


Fig. 3. Changes in the MPOD levels. Three months after the start of supplementation, the MPOD levels increased in both groups compared with baseline. After the end of supplementation, the MPOD levels remained elevated in both groups. The data are expressed as the mean ± standard error of mean (Raman counts). 3M = 3 months; 6M = 6 months. *p<0.05 vs. baseline by the Wilcoxon signed-rank test. †p<0.05 vs. 3 months by the Wilcoxon signed-rank test.