The Spatial Profile of Macular Pigment in Subjects From a Singapore Chinese Population

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Purpose. To examine the spatial profile of macular pigment (MP) and its relationship with serum concentrations of lutein (L) and zeaxanthin (Z) in subjects from a Singapore Chinese population.

Methods. In this cross-sectional study, the following details were recorded in 95 healthy subjects: sociodemographic, lifestyle information, body mass index (BMI), visual acuity, MP spatial profile using a macular densitometer, and serum L and Z.

Results. The mean (SD) age of the population was 42.40 (±13) years, ranging from 21 to 68 years. Females demonstrated significantly lower MP optical density (MPOD) than males (MPOD: females = 0.52 ± 0.17; males = 0.61 ± 0.21, $P = 0.03$). MP spatial profile was typical and atypical with central dip in 68 (85%) and 12 (15%) subjects, respectively. Age and BMI were found to be significant predictors for atypical MP spatial profile (age: odds ratio, OR = 1.06, 95% confidence interval, CI = 1.01–1.13, $P = 0.04$; BMI: OR = 1.17, 95% CI = 1.01–1.34, $P = 0.03$). A positive relationship was observed between MPOD and serum concentrations of L and Z, but only the latter relationship reached statistical significance (serum L: $r = 0.12$, $P = 0.30$; serum Z: $r = 0.26$, $P = 0.02$).

Conclusions. A central dip in MP spatial profile was observed with older age and higher BMI, the two known risk factors for AMD, suggesting that atypical MP spatial profile may be associated with an increased risk of AMD. Further studies with larger sample sizes are required to confirm these observations.

Keywords: macular pigment, zeaxanthin, lutein

The primate macula has a distinctive yellow pigment, known as macular pigment (MP), which is composed of three carotenoids, lutein (L), zeaxanthin (Z), and *meso*-zeaxanthin (*meso*-Z).[1,2] Lutein and Z cannot be synthesized in vivo in humans and are derived from the dietary intake of these carotenoids.[3] *Meso*-zeaxanthin is generally not found in a typical human diet and its high concentration at the macula is attributable to isomerization of L at the macula.[4]

Macular pigment carotenoids by nature of their biochemical structure and functions are believed to protect the retina from oxidative damage.[5] Macular pigment acts as an antioxidant both passively and actively, the former mechanism being dependent on its ability to limit photo-oxidative damage by filtering short wavelength light and the latter being attributable to its capacity to quench reactive oxygen intermediates (protective hypothesis).[6,7] Macular pigment may also influence the quality of visual performance by means of selective blue light absorption at a pre-receptorial level prior to photoreceptor light capture, thereby attenuating the effects of chromatic aberration and light scatter (optical hypothesis).[8]

There is increasing evidence that MP levels in the retina can be augmented following increased intake of dietary L and Z in healthy and diseased retinas,[9–11] suggesting the possibility that therapeutic intervention in the form of dietary modification or nutritional supplementation may modulate the risk of diseases associated with a relative lack of MP. In fact, this was borne out in the Age-Related Eye Disease Study 2 where L plus Z demonstrated a protective effect for progression to advanced AMD, when exploratory subgroup analyses of the treatment effects were limited to participants in the lowest quintile of dietary L plus Z (hazard ratio, 0.74; CI, 0.59–0.94; $P = 0.01$).[12] Unlike other antioxidants, MP can be easily quantified in the human eye in vivo using subjective or objective non-invasive methods. Heterochromatic flicker photometry (HFP), a subjective psychophysical technique, is the most frequently used method for measuring MP optical density (MPOD) in humans.

Several previous studies have reported MPOD and its constituent carotenoids L and Z in serum and diet in Western populations.[13–17] A few studies have measured MPOD among Asians, including the Chinese population[18–25]; however, there is a paucity of data describing the spatial profile of MP among Asians. The spatial profile of MP, constituted by MPOD at the foveal center and at three different degrees of retinal eccentricities, provides a more complete and accurate representation of MP, enabling the correlation of MP distribution with retinal pathology. Furthermore, to date, no study has reported on the correlation between MPOD and serum levels of L and Z among ethnic Chinese. This study aims to examine the spatial profile of MP and its relationship with serum concen-

trations of L and Z in subjects from a Singapore Chinese population.

Materials and Methods

We recruited 95 healthy Singapore Chinese subjects in this cross-sectional study. The study was approved by the Research and Ethics Committee of the National Healthcare Group and the research procedures followed the tenets of the Declaration of Helsinki.

Study subjects were identified from healthy hospital staff, their relatives, and relatives accompanying patients to the hospital. All subjects underwent a comprehensive eye examination including slit-lamp biomicroscopy for anterior and posterior segment examination. Inclusion criteria for the study participants were as follows: Singaporean, ethnic Chinese, aged 21 years and older, either sex, best-corrected visual acuity 20/40 and better, and absence of any eye diseases. Subjects with a history of major psychiatric illness and/or poor cognitive function precluding the giving of informed consent and subjects who were not able to perform MPOD measurements despite repeated practice trials were excluded from the study.

Written informed consent was obtained from the subjects after explanation of the purpose, procedure, and possible consequences of the study. The following details were collected for each study participant: demographic data, lifestyle information, height (meters), weight (kilograms), body mass index (BMI, kilograms divided by meters squared), and best-corrected visual acuity.

Measurement of Macular Pigment Optical Density

Macular pigment optical density was measured psychophysically using a macular densitometer (Macular Metrics Densitometer; Macular Metrics Corp., Rehoboth, MA, USA),[26] a state-of-the-art instrument for measuring MPOD that utilizes the principle of customized HFP.

Heterochromatic flicker photometry is based on the principle of matching the luminance of two flickering light sources, one blue (458 nm, maximally absorbed by MP) and one green (560 nm, not absorbed by MP), at the fovea and then at the parafovea. If the green light remains at constant luminance while the luminance of the blue light is varied, a point of minimum flicker is achieved when the luminance of the two light sources are matched. The logarithm ratio of the luminance of blue light required to achieve this end point for foveal and parafoveal readings is a measure of the optical density of MP. The reason for this is because MP is optically undetectable at an eccentricity of 7°, and has peak absorption at 458 nm, corresponding to blue light.

Macular pigment optical density measurements were performed strictly in accordance with the procedure laid out in a standard operating procedure. The instrument was calibrated at the beginning of each day using a standard calibration procedure. An instructional video followed by a few practice trials on the actual instrument was given to each study subject prior to recording the MPOD readings. As a preliminary test, optimal flicker frequencies that facilitate good subject performance and reduce measurement error were estimated following determination of individual critical flicker frequency measurements (customized HFP). Selecting the best flicker rate for each subject enables one to accommodate the variation in flicker sensitivity due to factors such as age.

For MPOD measurements at the fovea, subjects were asked to look at a flickering blue/green light in the central field (stimulus no 1, 0.25^0) and to adjust the knob to find a zone of no/minimal flicker (null zone). Subjects were constantly instructed to blink several times during the procedure and to continue adjusting the knob until blinking no longer allowed the flickering sensation in the test targets to resume. The "perfectionist" adjustments were strongly discouraged, and in the majority of subjects, real measurements were easily made after a few nonrecorded trials. Then, the task of finding the null zone by adjusting the knob was repeated for stimuli numbers 2, 3, 4, and 5 corresponding to 0.50^0, 1.0^0, 1.75^0, and 7^0 (the reference point with negligible levels of MP) eccentric location to measure the spatial profile of MP. After each reading was recorded, the investigator set the luminance control to some new arbitrary position so that the subject could not learn how far to adjust the dial to obtain a match. Five radiance values were recorded for each of the five stimuli, and final MPOD at each stimulus was represented by the mean of the 5 values. Subjects whose test results had a standard deviation of more than 0.10 suggestive of wide variation in the radiance values were not included in the study.

The spatial profile of each subject was categorized into one of the two profile types based on individual MPOD results: The "typical MP spatial profile" describes a steady nearly exponential decline in MPOD from the center (0.25°) to periphery (7°), with each successive MPOD lower than the previous one. The "atypical MP spatial profile" describes a dip in the central MP (0.25°), followed by an increase in MPOD at 0.5°, and finally a steady decline to the periphery. In other words, the central MPOD (i.e., 0.25°) was lower than at 0.5° in subjects with atypical MP spatial profile.[27]

For assessment of test-retest variability of MPOD, we recorded MPOD in 15 healthy subjects during two sessions separated in time by at least 24 hours but not more than 2 weeks.

Serum Analysis of L and Z

Two 5-mL vacutainer tubes containing 4.5 U sodium or lithium heparin per milliliter of whole blood were used to collect 6 to 8 mL of blood sample. We have collected fasting samples for estimation of serum concentrations of L and Z where possible. The samples were centrifuged at 5000 rpm for 10 minutes within 8 to 10 hours. The separated layer of serum was then aliquoted into two light-sensitive microcentrifuge tubes and stored at −70°C until the time of analysis.

We used a system (HP 1090 LC; Hewlett-Packard, Palo Alto, CA, USA) with photodiode array detection at 292, 325, and 450 nm under computer control (Chem Station software; Agilent Technologies, Inc., Santa Clara, CA, USA). A 5-μm analytical/preparative 4.6 × 250 mm specialty reverse phase column (201 TP; Vydac, Hesparia, CA) was used with an inline guard column. The mobile phase, consisting of 97% methanol and 3% tetrahydrofuran, was degassed using an inline degasser. The flow rate was 1 mL/min. Hoffmann-La Roche provided the standards for HPLC analysis.

A 30-μL aliquot of plasma was deproteinized with equal volumes of ethanol-tert-butanol (EB) solution (4:1, vol/vol) and internal standard (echinenone, 0.4 mg/L) in an amber microcentrifuge tube. This was extracted with 100 μL of n-hexane for 2 minutes. After centrifugation, 160 μL of supernatant was transferred into another amber microcentrifuge tube and dried under a stream of nitrogen. The dried residue was reconstituted into 60 μL of EB solution and 30 μL was used for HPLC analysis. The three mobile phase solutions used for gradient separation were: pure acetonitrile, pure methanol, and a mixture of ethanol and tert-butanol (8:2, vol/vol). Using a Waters Alliance 2695 separation module, the gradient profile of mobile phase (A:B:C) was set at 75:25:0 from 21 to 30 minutes. During the first 8 minutes of chromatographic analysis, L that

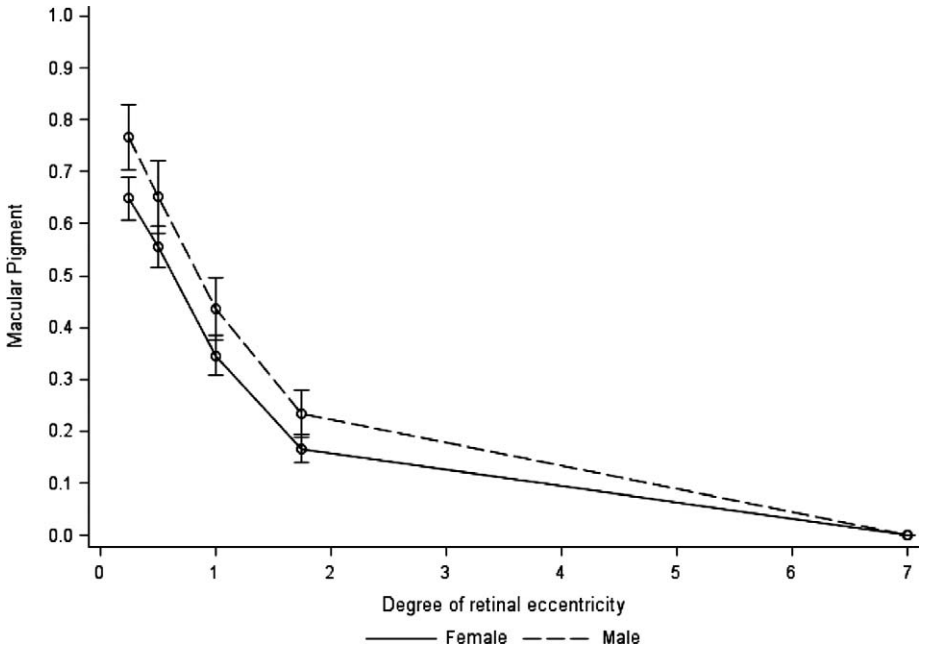

FIGURE 1. Sex variation in macular pigment optical density at different degrees of retinal eccentricities from the foveal center.

coeluted with Z on column 1 (Agilent Zobrax SB-C_{18}, 4 μm, 150 × 3.9 mm, I.D. Maidstone, UK, 25°C) was directed to column 2 (Whatman Partisphere-5C_{18}, 5 μm, 110 × 4.7 mm, I.D. Maidstone, 4°C). After the last elution of less-polar compounds from column 1 from 8 to 24 minutes, the flow was redirected to column 2 to separate L and Z, which eluted from 27 to 30 minutes. The peak heights were monitored at λ450 nm using a photodiode array detector.

Statistical Analyses

Data analyses were performed using statistical software (SAS package 9.2; SAS, Carey, NC, USA). We reported MPOD for the right eye only because there was good degree of interocular agreement in MPOD within an individual ($r = 0.84$; $P < 0.0001$). Descriptive statistics were computed for all the collected variables. Continuous variables were summarized as either mean (±SD), or median (range), as appropriate. Pearson correlations were estimated for evaluating the relationship between two continuous variables. Wilcoxon rank sum tests were used to compare the distribution of a continuous variable between two groups. Fisher's exact tests were used to compare proportions among groups. Multiple linear regression with MPOD as dependent variable was performed to evaluate its relationship with each independent variable while controlling for others. Logistic regressions with atypical spatial profile of MP as events were performed to investigate what factors can predict MP spatial profile. For test-retest, the Bland-Altman plots were generated, and Cronbach's alpha coefficients were estimated to evaluate the repeatability of the MPOD measures by the same rater. Statistical significance level was set at 0.05.

RESULTS

In this cross-sectional study, 95 healthy Singapore Chinese were recruited over a period of 18 months. The mean (±SD) age of the study population was 42.40 (±13) years, ranging from 21 to 68 years. There was a preponderance of female participants, comprising 70.5% of the study population. Myopia, defined as a spherical equivalent (SE) ≤ –0.50 diopters (D), was present in 60% of the study population with mean SE of –3.65 D and a maximum of –11.00 D.

Macular Pigment Optical Density

The mean (±SD) MPOD in the right eye at 0.50° foveal eccentricity was 0.55 (0.19), ranging from 0.16 to 0.99. There was a good degree of interocular agreement in MPOD within an individual ($r = 0.84$, CI = 0.69–0.92, $P < 0.0001$), with a median right-left eye difference of 0 (range, –0.18, 0.25).

Female subjects demonstrated significantly lower levels of MPOD when compared with male subjects (MPOD: females = 0.52 ± 0.17; males = 0.61 ± 0.21, $P = 0.03$, Fig. 1). When the study population was stratified into two age groups with cutoff age as 40 years, sex differences persisted in subjects aged ≤40 years, but was attenuated to nonsignificant levels in subjects aged >40 years (age ≤ 40 years: females = 0.52 ± 0.15; males = 0.67 ± 0.20, $P = 0.02$; age > 40 years: females = 0.51 ± 0.18; males = 0.57 ± 0.21, $P = 0.36$). Sex differences also persisted in normal weight subjects (females = 0.52 ± 0.17; males = 0.63 ± 0.21, $P = 0.02$) but not in overweight subjects (females = 0.54 ± 0.15; males = 0.55 ± 0.26, $P = 0.90$).

Macular pigment optical density did not demonstrate any meaningful relationship with age and BMI in our study population when these variables were taken as continuous variables (age: $r = –0.06$, $P = 0.55$; BMI: $r = –0.01$, $P = 0.91$). No significant difference in MPOD was observed in subjects with positive history of smoking (current and past smokers) when compared with subjects with no history of smoking (MPOD: smokers = 0.62 ± 0.20; nonsmokers = 0.53 ± 0.18, $P = 0.13$).

In multiple linear regression analysis, sex differences in MPOD persisted after controlling for age and BMI (sex, $P = 0.04$; age, $P = 0.59$; BMI, $P = 0.78$). Smoking was confounded with sex and therefore was not included in the model (smokers: males = 71.4%; nonsmokers: males = 19.2%, $P < 0.0001$). MPOD in females was 0.09 unit lower (95% CI 0.007–0.18) when compared with males after controlling for age and BMI.

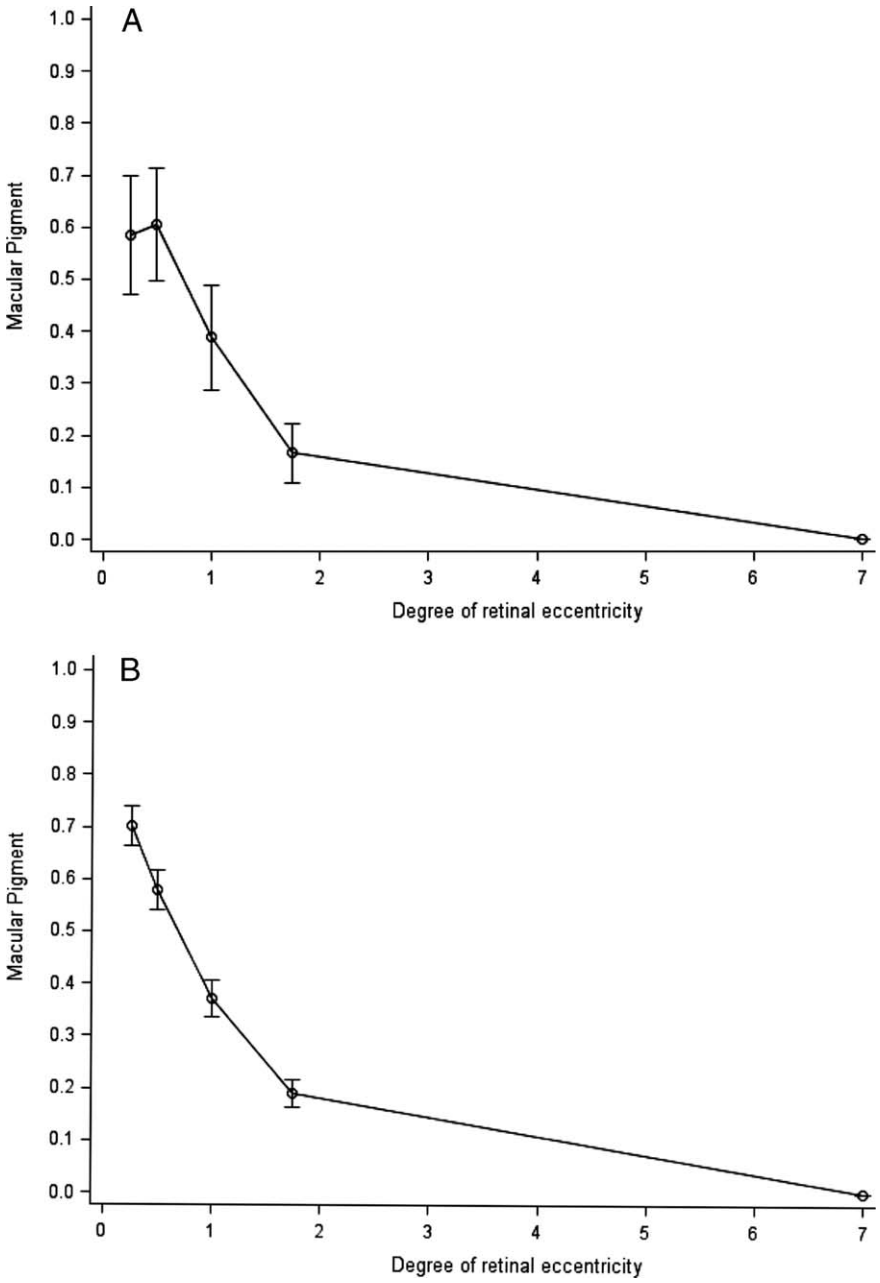

FIGURE 2. Spatial profile of macular pigment. (**A**) An atypical macular pigment spatial profile with a dip at the foveal center. (**B**) A typical macular pigment spatial profile with peak density in the foveal center and exponential decline with increasing eccentricity from the foveal center.

Spatial Profile of MP

The mean ± SD (range) MPOD in the right eye was 0.65 ± 0.19 (0.17, 1); 0.55 ± 0.19 (0.16, 0.99); 0.37 ± 0.15 (0.09, 0.69); and 0.19 ± 0.11 (0, 0.49) at 0.25°, 0.50°, 1.00°, and 1.75° foveal eccentricities, respectively. A "classic" exponential decline in MPOD was seen in 68 (85%) subjects (typical profile, Fig. 2B) whereas a dip in the central MP was observed in 12 (15%) subjects (atypical profile, Fig. 2A). A statistically significant difference in MPOD was observed at 0.25° between typical and atypical profiles (MPOD: typical profile = 0.70 ± 0.15; atypical profile = 0.59 ± 0.18, $P = 0.02$). However, no difference in MPOD was observed at other foveal eccentricities between typical and atypical profiles (0.50°, $P = 0.60$; 1.00°, $P = 0.72$; 1.75°, $P = 0.48$). There was a good degree of symmetry in the spatial profile of MP between right and left eye within an individual ($n = 32$, Fig. 3).

In multivariate logistic regression analysis, age and BMI were found to be predictors for MP spatial profile whereas sex was not (age: OR = 1.06, 95% CI 1.01–1.13, $P = 0.04$; BMI: OR = 1.17, 95% CI 1.01–1.34, $P = 0.03$; sex: OR = 0.32, 95% CI 0.05–2.04, $P = 0.23$). Smoking was confounded with sex and therefore not included in the model (smokers: males = 71.4%; nonsmokers: males = 19.2%, $P < 0.0001$). As age increased by 1 year and BMI increased by 1 unit, odds of atypical profile increased by 6% and 17%, respectively.

Test-Retest Data

The mean difference in MPOD at 0.25° between the first and repeat session was 0.024 (± 0.11) with a 95% confidence

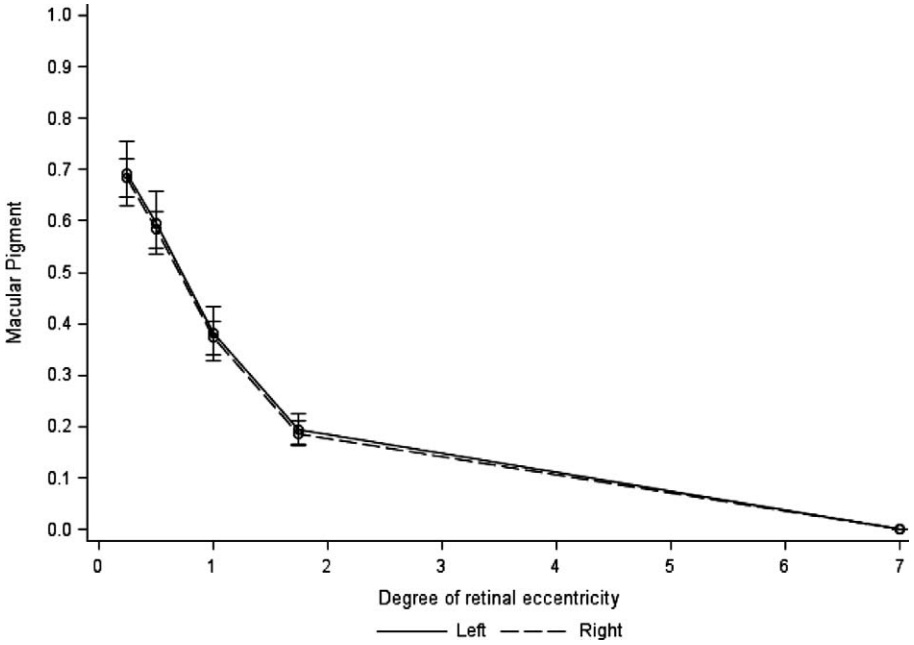

FIGURE 3. Symmetrical macular pigment spatial profile between the right and left eye within an individual.

interval of –0.186 to 0.234. The mean difference in MPOD at 0.50° between the first and repeat session was –0.002 (±0.09) with a 95% confidence interval of –0.182 to 0.178. The mean difference in MPOD at 1.00° between the first and repeat session was –0.015 (± 0.125), with a 95% confidence interval of –0.259 to 0.229. The mean difference in MPOD at 1.75° between the first and repeat session was 0.009 (± 0.129) with a 95% confidence interval of –0.243 to 0.262. Cronbach's alpha value for the test-retest sessions were 0.92, 0.95, 0.81, and 0.24 at 0.25°, 0.50°, 1.00°, and 1.75° foveal eccentricities, respectively.

MPOD and Serum Concentrations of L and Z

The mean (±SD) serum concentrations of L and Z in our study population were 0.14 (±0.05) and 0.06 (±0.02) μg/mL, respectively. No sex differences in serum concentrations of L and Z were observed in our study population (serum L: females = 0.14 ± 0.05, males = 0.13 ± 0.05 , $P = 0.30$; serum Z: females = 0.06 ± 0.02, males = 0.06 ± 0.02, $P = 0.74$). A positive relationship was observed between MPOD and serum concentrations of L and Z, but only the latter relationship reached statistical significance (serum L: $r = 0.12$, $P = 0.30$; serum Z: $r = 0.26$, $P = 0.02$, Fig. 4).

DISCUSSION

In this study, we measured the spatial profile of MP and serum concentrations of L and Z in 95 healthy Singapore Chinese subjects. A typical exponential decline in MP spatial profile was observed in 85% of the subjects while 15% of them

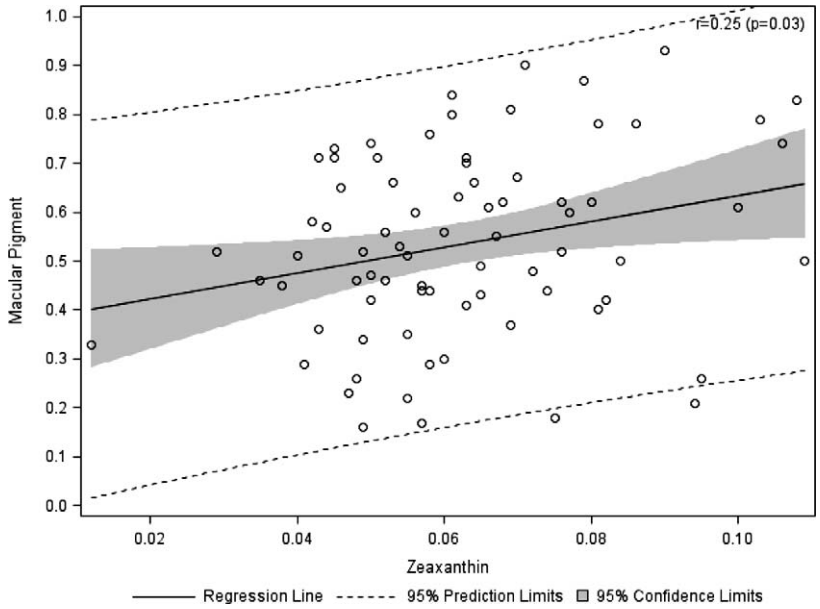

FIGURE 4. Scattergram showing a statistically significant and positive relationship between macular pigment optical density and serum zeaxanthin.

TABLE. List of Studies Showing Macular Pigment Optical Density Among Asian Population

Author (y)	Study Population	Sample Size	Instrument/Technique	MP Measurement	Age Range (Mean), y	Mean MP
Chen et al. (2001)	Chinese (Taiwan)	54	Reflectometry (objective)	Spatial profile	44 (22–83)	0.23
Chang et al. (2002)	Chinese (Taiwan)	55	Reflectometry (objective)	Spatial profile	22–83 (44)	0.23
Tang et al. (2004)*	Chinese (Hong Kong)	67	HFP (subjective), custom built	Center point	(18–23)	0.48
Lam et al. (2005)*	Chinese (Hong Kong)	92	HFP (subjective)	Spatial profile	39 (16–85)	0.43
Obana et al. (2008)	Japanese (Japan)	100	Raman spectroscopy (objective)	Center point	NA	600 RC
Raman et al. (2011)*	Indians (India)	161	HFP (subjective)	Spatial profile	(20–60)	0.64
Yu et al. (2012)*	Chinese (Mainland)	281	HFP (subjective),	Spatial profile	(17–85)	0.56
Howells et al. (2013)*	South Asians (UK)	120	HFP (subjective), MPS 9000	Central point	21 (18–30)	0.43

NA, not available; RC, Raman counts.
* Rows represent studies that have measured macular pigment optical density using HFP.

demonstrated an atypical MP spatial profile in the form of a central dip. Age and BMI were found to be predictors of atypical MP spatial profile. Furthermore, MPOD demonstrated a statistically significant and positive relationship with serum Z.

The mean MPOD in our population is similar to that obtained in past studies among ethnic Chinese subjects (Table),[21,24,25] but is relatively higher than that in Western populations using the HFP method.[26,28–30] Indeed, in a systematic review and hierarchical Bayesian meta-analysis, Europeans/whites were more likely to have AMD than Asians and Africans (Wong T, et al. IOVS 2013;54:ARVO E-Abstract 220). However, a higher prevalence of neovascular AMD was reported among Asian subjects when compared with whites.[31] In addition, polypoidal choroidal vasculopathy constitutes a major part of advanced AMD among Asian countries with prevalence rates of 30% to 50% in patients with presumed neovascular AMD.[32–35]

This observation is consistent with a recent study by Howell et al. where a statistically significant difference in MPOD was demonstrated between Asians and Caucasians.[20] The exact reason for MPOD variation among Asians and Caucasians is not entirely clear but may be attributable to ethnic differences in the consumption, absorption, transport and capture of L and Z. Future studies are warranted to elucidate the underlying mechanisms for the observed inter-ethnic differences in MP levels.

In our study, no age-related decline in MPOD was observed when MP and age were taken as continuous variables. Similarly, MPOD did not demonstrate any meaningful relationship with smoking and BMI when analyses were undertaken using these variables in continuous mode. However, age and BMI were found to be significant predictors of MP spatial profile in this study. Older subjects and subjects with higher BMI were more likely to demonstrate an atypical MP spatial profile in the form of a central dip when compared with younger subjects and subjects with lower BMI. Kirby et al. observed a similar association between age and spatial profile of MP with older subjects demonstrating a central dip in the MP spatial profile using customized HFP.[27] The authors suggested that atypical MP spatial profile in the form of a central dip is associated with AMD risk factors such as older age and smoking, and represents an undesirable distribution of MP.

The spatial profile of MP, along with peak optical density and lateral extent, vary dramatically among individuals. Given that the spatial profile of MP may be affected by foveal architecture,[36] subjects with older age and higher BMI may have a unique foveal architecture that allow a central dip in the MP spatial profile. However, we did not examine the foveal architecture in our study population. Alternatively, a central dip in the MP spatial profile may be attributable to a lack of conversion of L to *meso*-Z secondary to oxidative stress as hypothesized by past investigators.[37] Indeed, an atypical MP profile with a central dip is associated with known risk factors of AMD, such as older age, smoking and higher BMI.[6] However, it remains unclear whether the atypical MP spatial profile represents a unique stable feature of the MP distribution within an individual or an unstable feature that has evolved secondary to processes that take place within an individual, such as oxidative stress. Longitudinal studies mapping the spatial profile of MP are necessary to address this question in the future.

Several studies in the past have reported deviations from the monotonic decline from the central fovea in the MP spatial profile[27,38–42]; however, terminology used to describe such deviations varied widely among studies. Of note, it has been confirmed that these atypical profiles are real and reproducible features of MP spatial profile, when measured using customized-HFP.[43] Furthermore, a study by Nolan et al. have reported that the typical central peak of MP can be attained in subjects demonstrating atypical MP spatial profile when supplemented with a preparation containing *meso*-Z, but not when supplemented with a formulation lacking this carotenoid.[44]

The association between MPOD and sex has been reported in several past studies; however, the results have been inconsistent. Some studies have reported that females have relatively lower levels of MPOD than males[45–47] while others have failed to demonstrate any sex-associated difference in MPOD.[13,24,29,48–50] In our study, female subjects demonstrated significantly lower MPOD at all degrees of retinal eccentricity measured when compared with male subjects. When the study population was stratified into two groups with the cutoff age as 40 years, the sex difference persisted in subjects with age $\leq$40 years but attenuated to nonsignificant levels in subjects aged older than 40 years.

Sex differences in the metabolism, transport, and accumulation of carotenoids have been documented.[45,46,51] For instance, experimental studies in animal models have suggested that uptake and storage of MP may differ with sex. Also, a hormonally controlled variation in the lipid transport system that is used by carotenoids and known to affect MP levels has been observed in female individuals.[30] Furthermore, female subjects have relatively higher percentage of body fat and higher circulating serum L when compared with males.[52] It is therefore possible that L contained in the adipose tissue and circulating in serum may act as a buffer against any decline in MPOD with increasing age. Indeed, in our population, we failed to observe sex variation in MPOD among older population ($P = 0.36$) and in subjects who were overweight ($P = 0.90$).

We demonstrated a positive association between MPOD and serum concentrations of L and Z but the relationship reached statistical significance only for serum Z. L and Z may have

different functions in the retina. L and Z are constitutional isomers but their biochemical structures differ in many subtle ways. Z predominates in the macula to a radial distance of 2.5 mm from the fovea whereas L is found in greater abundance in the peripheral retina.[53,54] Also, it has been proposed that a conversion mechanism may exist in the cones whereby L is isomerized to *meso*-Z and may thus explain the predominance of Z at the cone-enriched fovea. Furthermore, both carotenoids protect the lipid matrix from free radicals but Z has been shown to be a better photo-protector during prolonged exposure to ultraviolet radiation.[55]

It is believed that traditional Chinese food with higher concentrations of carotenoids may translate to higher MPOD among Chinese population.[56] Serum levels of L and Z in our population were relatively lower when compared to that of a native Chinese population from the greater Beijing area (serum L: our study = 0.25 μmol/L; past study = 0.53 μmol/L).[57] This could be related to dietary differences but the lack of dietary information is a major limiting factor in the present study.

This is the first study to describe MPOD (at foveal center and three different degrees of retinal eccentricities) among Singapore Chinese subjects and to correlate MPOD with its constituent carotenoids in serum. The main limitations of this study are small sample size and lack of dietary information. We also acknowledge the limitation of using BMI as a gauge of excessive body fat as high BMI does not equate to adiposity.[58] Furthermore, the test-retest variability was measured only in hospital staff for convenience reasons. Hospital staffs were on average younger than other study participants; however, we do not expect this to significantly bias the test-retest reliability because past studies have demonstrated that most subjects can reliably perform this task.[16,59] In addition, the hospital staffs involved were equally naïve about the purpose of the study as the rest of the group. Data from 13 subjects were not included in the statistical analyses. Of the 13 subjects, eight were unable to perform MP test reliably (SD $>$ 0.10), two subjects were unable to complete the test successfully due to concentration problem, two subjects found extremely difficult to perform peripheral task, and one subject was not able to understand the concept of minimum/no flicker zone.

In summary, a central dip in the MP spatial profile was seen with older age and higher body mass index, two known risk factors for AMD. This observation suggests that atypical MP spatial profile may be associated with an increased risk of AMD. Future studies with larger sample sizes are required to confirm our observations.

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