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# Sex Differences in Macular Pigment Optical Density: Relation to Plasma Carotenoid Concentrations and Dietary Patterns

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**Sex differences in macular pigment (MP) optical density (measured psychophysically) were examined. Concentrations of lutein and zeaxanthin (L and Z) (non-separated) and beta-carotene (BC) in the blood were determined using reverse phase high-performance liquid chromatography. Dietary intake of L and Z, BC, fat, and iron were estimated by questionnaire. Males had 38% higher MP density than females ($P < 0.001$) despite similar plasma carotenoid concentrations and similar dietary intake (except for fat). Dietary intake of carotenoids, fat and iron, as well as plasma concentrations of L and Z were positively related to MP density in males. Conversely, only plasma L and Z was related to MP density for females, and dietary fat was negatively related to MP density. Sex differences in protection of the retina by MP and in the relationship between the retina, blood and diet could be a factor in the incidence of retinal diseases, especially age-related macular degeneration.** 

Macular pigment  Carotenoids  Psychophysics  Chromatography  Macular degeneration

## INTRODUCTION

Data from retinal studies are rarely segregated by sex. This practice persists despite evidence for sex differences in color vision anomalies, in normal visual processes, and in susceptibility to retinal diseases. For example, males typically have lower acuity thresholds than females (Burg, 1966; McGuinness, 1976; Brabyn & McGuinness, 1979). Furthermore, females tend to have increased susceptibility to retinal complications due to Type II diabetes (Armstrong *et al.*, 1992) and may have a higher risk of neovascular age-related macular degeneration (AMD) (Klein *et al.*, 1992). The basis for sex differences in visual performance and in disease susceptibility is unresolved, and it lends interest to results from both psychophysical and anatomical studies showing that the retina may exhibit more sex-related differences than generally appreciated (Eisner *et al.*, 1987; Werner & Steele, 1988; de Monasterio *et al.*, 1985).

In this paper we report on the yellow macular pigment (MP). The MP is composed primarily of the lipid-soluble xanthophylls, lutein (L) and zeaxanthin (Z) (Bone *et al.*, 1985), which are most concentrated in the central 1 mm of the primate fovea (Snodderly *et al.*, 1984a; Bone *et al.*, 1988). Psychophysical studies have shown that individuals differ widely in the optical density of their MP, varying by more than a factor of ten (Werner *et al.*, 1987; Pease *et al.*, 1987; Hammond & Fuld, 1992). These *in vivo* measures are consistent with *in vitro* studies that have found differences in MP concentrations ranging from 5 to 80 ng in the central retina of human donors (Bone *et al.*, 1988). Here we present evidence that some of this variation is attributable to sex differences.

In the present study, MP density was measured with a 1 deg test field in a large ($n = 88$) sample. This sample size was necessary to achieve sufficient statistical power to differentiate reliably between the sexes. We also measured total L and Z in the blood since little information is available on the relationship between blood and tissue concentrations of specific carotenoids. Blood concentrations or dietary intake of carotenoids are usually the only measures available in epidemiologic

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studies of disease incidence, including diseases of the retina (EDCC, 1992, 1993; Seddon *et al.*, 1994). If protection by carotenoids is conferred locally (i.e., within tissue), a deeper understanding of the epidemiology will be achieved by knowing the relationship between tissue and blood carotenoid concentrations. Handelman *et al.* (1991) have shown that the amount of MP can be estimated accurately from measurements of its optical density. In our case, we measured MP optical density psychophysically and compared the density with concentrations of carotenoids in the blood.

Although beta-carotene (BC) is not found in the retina under normal conditions (Handelman *et al.*, 1988, 1992), we measured plasma BC concentrations because it is the only carotenoid approved for human nutritional supplementation. Moreover, it is often the only carotenoid measured in epidemiology studies examining the protective function of carotenoids in retinal disease (e.g., West *et al.*, 1994; Hyman *et al.*, 1992).

If tissue concentrations are a function of long-term dietary intake (Prince & Frisoli, 1993), individual differences in long-term patterns may contribute to differences in MP density. To investigate this possibility, we estimated dietary intake of L and Z, BC, fat and iron using a questionnaire (Block *et al.*, 1986) that reflects long-term dietary intake. Epidemiologic studies (e.g., Seddon *et al.*, 1994) typically use dietary questionnaires of this type to assess the relationship of long-term dietary intake to chronic retinal diseases such as AMD.

Dietary fat was evaluated because Dimitrov *et al.* (1988) and Prince & Frisoli (1993) have shown that a certain amount of dietary fat is necessary to absorb BC into the blood. Like BC, L and Z are lipid-soluble and dietary fat may facilitate absorption through the gut. Dietary iron might also enhance gut absorption of BC (Swanson & Parker, 1993) and, through a similar mechanism, enhance absorption of L and Z into the blood and show a positive relationship to MP density. The possible effect of other dietary components on MP density was not assessed.

## MATERIALS AND METHODS

### *Subjects*

MP optical density measures were obtained for 88 nonsmoking subjects, 48 women (ages 19–79 yr; 93% Caucasian) and 40 men (ages 19–83 yr; 97% Caucasian). Results for smokers have been reported elsewhere (Hammond *et al.*, 1995b). Subjects were in good general health and no ocular problems were reported. These data were collected in two different labs using similar stimulus conditions. MP optical density measures for 45 subjects were obtained at the University of New Hampshire (Durham, NH, U.S.A.) using the procedures and stimulus conditions detailed in Hammond & Fuld (1992). The remaining MP measures (43 subjects) were obtained in a nearly identical manner at the Schepens Eye Research Institute (Boston, MA, U.S.A.). The procedures and stimulus conditions for the MP measures obtained in Boston are provided in this paper. The analyses explained in the Results section were conducted on both sets of data separately and then the data were combined. The separate results are similar to the amalgamated results*, except that combining the two data sets provides sufficient statistical power for a clear interpretation of the data.

### *Measurement of macular pigment optical density*

*Apparatus.* A three-channel, Maxwellian view optical system was used. Two channels were combined by a rotating sectored mirror to provide a test stimulus, alternating between a measuring and a reference field. A third channel provided a background field. The fixation point was provided by placing a small black dot (6') on transparent glass in the path of the light that formed the background field. The subject's head position was stabilized by an adjustable dental-impression bite-bar and a head-rest assembly. A viewing system with a calibrated reticle was used to align the subject's pupil. This system was also used periodically to ensure that the stimuli always entered the center of the subject's pupil. Illumination from a xenon arc was rendered monochromatic by a grating monochromator (nominal half bandwidth of 8 nm; Bausch and Lomb, Model No. HD426); blocking filters eliminated stray light. Ditric Optics interference filters (half-bandwidth of 7 nm) were used to select the desired wavelength bands of light in the other two channels.

*Test stimulus.* The stimulus consisted of a 1 deg test field, which was composed alternately of a 460 nm measuring field and a 550 nm, 2.63 log Td, reference field. The measuring and reference fields were superposed and presented out of phase (in square wave alternation) at a temporal rate of 12–15 Hz. Since thresholds for critical flicker frequency vary with age and other factors (Curran, 1990), the temporal rate of the test stimulus was varied for each individual to optimize performance of the task. Axial chromatic aberration was minimized by adjusting the lateral and vertical position of an achromatizing lens to achieve the best correction for each subject. For measuring MP optical density, the test field was presented near the center of a 460 nm, 10 deg, 3 log Td background. Measurements made at this locus were compared to measurements made in the parafovea by placing a fixation point at 5.5 deg eccentricity. In the foveal condition, subjects were instructed to fixate on the center of the test field. In the parafoveal condition,

*MP optical densities for the New Hampshire (NH) laboratory were as follows: males, $n = 15$, mean = 0.36, SD = 0.15; females, $n = 29$, mean = 0.27, SD = 0.16. MP optical densities for the Boston laboratory were: males, $n = 26$, mean = 0.39, SD = 0.24; females, $n = 21$, mean = 0.20, SD = 0.13. The wide differences in sample size between the two laboratories make a direct comparison between the blood measures difficult. Nonetheless, the Boston males and females ($n = 12$) had 46% higher plasma L and Z concentrations than the NH males and females ($n = 40$). However, the dietary intake of L and Z for the Boston group also exceeded the NH group by 65%. Since the dietary questionnaires were identical, we concluded that this difference was due to differences in the composition of the sample and in their diets, rather than differences in technique.

TABLE 1. Descriptive statistics for the retinal, plasma, and diet measures

| Variable | Sample size ($n$) | | Mean ($\pm$ SD) | | |
|---|---|---|---|---|---|
| | Males | Females | Males | Females | $P$ values |
| | | | Raw values | | |
| MPOD | 40 | 48 | 0.38 (0.216) | 0.24 (0.159) | $P < 0.001^{**}$ |
| Plasma L and Z ($\mu$mol/l) | 20 | 32 | 0.356 (0.174) | 0.376 (0.181) | $P < 0.66$ |
| Dietary L and Z ($\mu$g/day) | 24 | 31 | 3125 (2618) | 2091 (1973) | $P < 0.11$ |
| Plasma BC ($\mu$mol/l) | 19 | 27 | 0.511 (0.467) | 0.361 (0.227) | $P < 0.21$ |
| Dietary BC ($\mu$g/day) | 24 | 31 | 4667 (4689) | 3032 (2147) | $P < 0.12$ |
| Iron (mg/day) | 24 | 31 | 25.7 (22) | 15.8 (7) | $P < 0.05^{*}$ |
| Total fat (g/day) | 24 | 31 | 132.3 (121) | 71.1 (36) | $P < 0.03^{*}$ |
| | | | Adjusted dietary values | | |
| Dietary L and Z ($\mu$g)/1 kcal | 24 | 31 | 1239 (1397) | 1013 (752) | $P < 0.48$ |
| Dietary L and Z ($\mu$g)/lb | 24 | 31 | 18.4 (16) | 15.6 (12) | $P < 0.48$ |
| Dietary BC ($\mu$g)/1 kcal | 24 | 31 | 1632 (1584) | 1555 (1129) | $P < 0.84$ |
| Dietary BC ($\mu$g)/lb | 24 | 31 | 27.6 (28) | 23.2 (15) | $P < 0.49$ |
| Iron (mg)/1 kcal | 24 | 31 | 7.7 (2.8) | 7.9 (2.1) | $P < 0.75$ |
| Iron (mg)/lb | 24 | 31 | 0.15 (0.13) | 0.12 (0.04) | $P < 0.28$ |
| Total fat (g)/1 kcal | 24 | 31 | 39.9 (7.7) | 33.7 (10) | $P < 0.01^{*}$ |
| Total fat (g)/lb | 24 | 31 | 0.856 (0.74) | 0.541 (0.25) | $P < 0.05^{*}$ |

A Student's $t$-test was used to assess the statistical significance of the difference between males and females. Asterisks next to significant $P$ values represent the overall Bonferroni-adjusted significance levels ($^{**}P < 0.01$, $^{*}P < 0.05$).The $P$ values used in the table are for $t$-tests without this correction. The abbreviations used in the following analysis are: MPOD, peak macular pigment optical density; Plasma L and Z, total plasma lutein and zeaxanthin; Plasma BC, plasma beta-carotene. The remaining abbreviations refer to those variables measured using the food frequency questionnaire and are expressed in amounts per day: Dietary L and Z, total lutein and zeaxanthin; Dietary BC, beta-carotene; Total fat, total fat; Iron, iron. "Adjusted" dietary values are expressed in units per 1000 calories (/1 kcal) and per one pound of body weight (/lb).

subjects were instructed to fixate the fixation point located at the edge of the background field while attending to the test stimulus which would now be located 5.5 deg in their periphery.

*Procedure*

Subjects were tested in one to five sessions. Approximately 50% of the subjects were tested for five repeated sessions, 15% were tested over three sessions, 10% were tested over two sessions, and 25% were tested in one session. Past studies have shown a high degree of intersession reliability (i.e., a cross-session variance of 0.05–0.07; Hammond & Fuld, 1992; Hammond *et al.*, 1995a), suggesting that the small number of sessions for some subjects was adequate.

Macular pigment density was determined by comparing the spectral sensitivity of the fovea (where MP is most dense) and the parafovea (where MP is optically undetectable) on the basis of heterochromatic flicker photometry (HFP). Measurements were taken at the peak of the absorption spectrum (*c.* 460 nm) and at a wavelength where absorption by the macular pigment is negligible (in this case, 550 nm). Isolation of the middle- and long-wave cone systems was achieved by selective adaptation to our background field and by use of a temporal frequency of 12–15 Hz that is above the critical flicker frequency for the short-wave sensitive cone system under our test conditions (Brindley *et al.*, 1966). The resultant spectral sensitivities are assumed to be mediated by the middle- and long-wave sensitive cones. There is good evidence that these cones are represented in equal ratios in the fovea and parafovea (Nerger & Cicerone, 1992; Cicerone & Nerger, 1989) and that the sensitivity of the foveal and parafoveal middle- and long-wave sensitive cone system is similar (Wooten & Wald, 1976). The absorption spectrum measured with our technique is similar to MP measured *in vitro* (Snodderly *et al.*, 1984b), which argues well for the validity of the method.

Subjects adjusted the radiance of the 460 nm measuring field by setting that channel's neutral density wedge to achieve minimum flicker with the 550 nm reference field. Before each setting was made, the wedge was turned to a new starting value in a pseudo-random sequence by the experimenter. For the optical density measurement, subjects made ten determinations at the fovea and ten determinations at 5.5 deg in the parafovea. For repeated measures, sessions were conducted on separate days.

*Calibration*

Before every experimental session the relative spectral energy of the measuring field was measured by placing a calibrated photodetector in the path of the light from the reference field while all other sources of light in the system were blocked. These values were used to ensure system stability.

*Plasma carotenoid analysis*

Plasma carotenoid measures were obtained for 52 subjects, 32 women and 20 men. These measures were obtained in two different laboratories using reverse-phase high-performance liquid chromatography. Blood concentrations for 40 subjects were measured at the

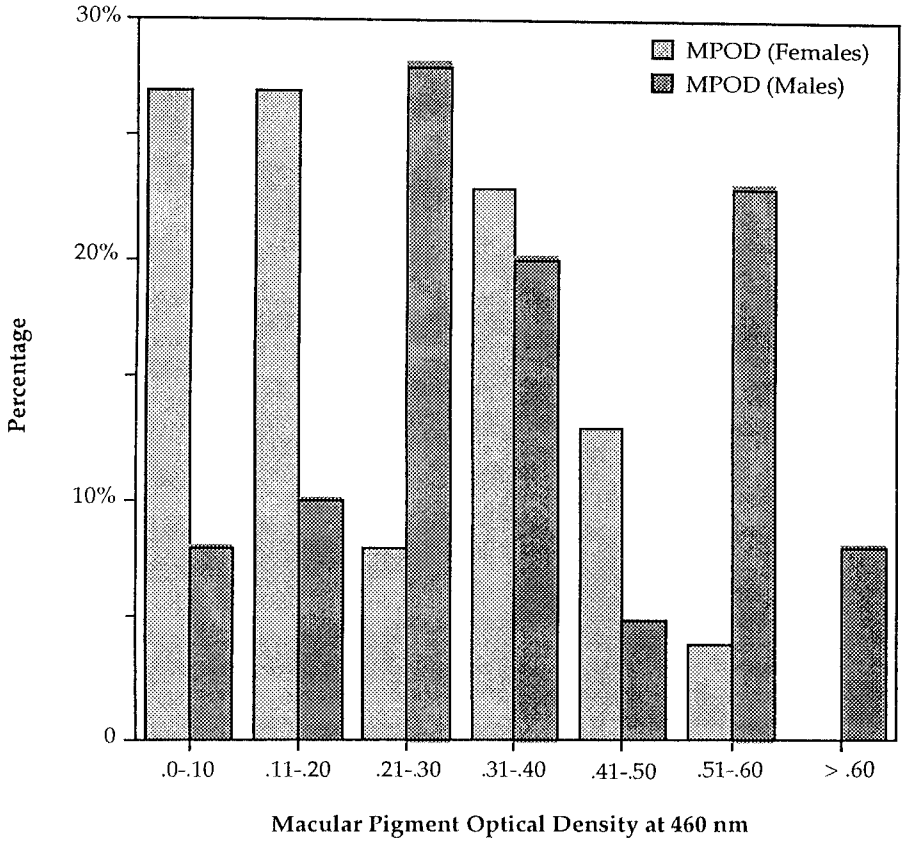


FIGURE 1. Frequency distribution of macular pigment optical density for males and females.

University of New Hampshire using the materials and methods explained in Hammond *et al.* (1995a). Blood carotenoid concentrations for 12 subjects were determined using a similar technique at Tufts University School of Medicine (Boston, MA, U.S.A.) as previously described (Krinsky *et al.*, 1990). In these data sets, L and Z were not separated in order to minimize any small differences that might be due to analyzing the blood in two different laboratories. Since we were unable to make a direct comparison by analyzing the same blood samples at both locations, effects due to small differences in technique cannot be ruled out.

*Dietary assessment of usual carotenoid intake*

Dietary information was gathered using the Health Habits and History questionnaire (HHHQ) (Block *et al.*, 1986). This questionnaire contains over 100 questions pertaining to different food items. Two responses are required for each question, frequency and serving size. The frequency a given item is ingested is indicated by the number of servings per day, week, month or year (e.g., four apples per week). Serving size is determined as small, medium or large. The exact size of a medium serving is given for each food item (e.g., a medium serving of broccoli is 1/2 cup). Supplement use is also assessed. The responses to these items were analyzed using the recently revised version of the HHHQ Diet System Analysis Software (Block *et al.*, 1994) to optimize our quantification of carotenoid intake. In most cases, subjects completed this questionnaire in the presence of the experimenter.

## RESULTS

*Macular pigment*

As shown in Table 1, a comparison of males ($n = 40$) and females ($n = 48$) indicates statistically significant differences in MP density between the two groups. A Bonferroni correction was applied to the $t$-tests in Table 1 to prevent effects due to accumulated Type I error. These corrections are indicated by the asterisks in the table. As shown in the table, males had an average MP density of 0.38 (SD = 0.216), and females had an average density of 0.24 (SD = 0.159). Frequency distributions of the MP optical density measures for both groups are displayed graphically in Fig. 1. Differences in MP density between males and females are evident in the extremes of the distribution. A higher proportion of females (54%) had low densities of MP (0.00–0.20) compared to males (18%). In contrast, a higher proportion of males (32%) had high densities of MP (over 0.50) compared to females (4%). As shown in the figure, the distribution of MP measures for both males and females has a bimodal appearance. This bimodal tendency was not sufficient to require the use of nonparametric statistics (Shapiro & Wilk, 1965).

*Plasma and dietary lutein and zeaxanthin*

Descriptive and inferential statistics for plasma and

TABLE 2. Pearson product moment correlation matrices on raw values for males

| | Males (Raw values) | | | | | | |
|---|---|---|---|---|---|---|---|
| | MPOD | Plasma L and Z (μmol/l) | Dietary L and Z (μg/day) | Plasma BC (μmol/l) | Dietary BC (μg/day) | Total fat (g/day) | Iron (mg/day) |
| MPOD | 1.0 | | | | | | |
| Plasma L and Z (μmol/l) | 0.62* | 1.0 | | | | | |
| Dietary L and Z (μg/day) | 0.64* | 0.70* | 1.0 | | | | |
| Plasma BC (μmol/l) | 0.44* | 0.83* | 0.82* | 1.0 | | | |
| Dietary BC (μg/day) | 0.80* | 0.79* | 0.81* | 0.82* | 1.0 | | |
| Total fat (g/day) | 0.56* | −0.44* | 0.39* | −0.29 | 0.72* | 1.0 | |
| Iron (mg/day) | 0.67* | −0.41* | 0.57* | −0.29 | 0.78* | 0.91* | 1.0 |

Significant correlations ($P < 0.05$) are indicated by an asterisk. Abbreviations used in the following analysis are the same as those used in Table 1.

TABLE 3. Pearson product moment correlation matrices on values adjusted for caloric intake for males

| | Males (Values adjusted for calorific intake) | | | | | | |
|---|---|---|---|---|---|---|---|
| | MPOD | Plasma L and Z (μmol/l) | Dietary L and Z (μg/1 kcal) | Plasma BC (μmol/l) | Dietary BC (μg/1 kcal) | Total fat (g/1 kcal) | Iron (mg/1 kcal) |
| MPOD | 1.0 | | | | | | |
| Plasma L and Z (μmol/l) | 0.62* | 1.0 | | | | | |
| Dietary L and Z (μg/1 kcal) | 0.29 | 0.89* | 1.0 | | | | |
| Plasma BC (μmol/L) | 0.44* | 0.83* | 0.94* | 1.0 | | | |
| Dietary BC (μg/1 kcal) | 0.32* | 0.95* | 0.59* | 0.87* | 1.0 | | |
| Total fat (g/1 kcal) | 0.21 | −0.44* | −0.33* | −0.42* | −0.30 | 1.0 | |
| Iron (mg/1 kcal) | 0.34* | 0.21 | 0.53* | 0.17 | 0.00 | −0.06 | 1.0 |

Significant correlations ($P < 0.05$) are indicated by an asterisk. Abbreviations used in the following analysis are the same as those used in Table 1.

TABLE 4. Pearson product moment correlation matrices on raw values for females

| | Females (Raw values) | | | | | | |
|---|---|---|---|---|---|---|---|
| | MPOD | Plasma L and Z (μmol/l) | Dietary L and Z (μg/day) | Plasma BC (μmol/l) | Dietary BC (μg/day) | Total fat (g/day) | Iron (mg/day) |
| MPOD | 1.0 | | | | | | |
| Plasma L and Z (μmol/l) | 0.30* | 1.0 | | | | | |
| Dietary L and Z (μg/day) | −0.10 | 0.56* | 1.0 | | | | |
| Plasma BC (μmol/L) | −0.03 | 0.29* | 0.38* | 1.0 | | | |
| Dietary BC (μg/day) | 0.12 | 0.15 | 0.64* | 0.47* | 1.0 | | |
| Total fat (g/day) | −0.51* | −0.54* | 0.32* | −0.30* | 0.08 | 1.0 | |
| Iron (mg/day) | −0.21 | −0.12 | 0.63* | 0.00 | 0.63* | 0.62* | 1.0 |

Significant correlations ($P < 0.05$) are indicated by an asterisk. Abbreviations used in the following analysis are the same as those used in Table 1.

dietary L and Z are given in Table 1. The large difference in MP density for males and females was not reflected in a similar difference in the total concentration of L and Z in the blood ($P < 0.66$). An analysis of dietary intake indicated that males do have a higher mean intake of L and Z (3125 μg) per day than females (2091 μg). As shown in Table 1, this difference was minimized ($P < 0.48$) when values were adjusted for differences in body weight and caloric intake.

The relationship between MP optical density and plasma L and Z concentrations for males and females was also examined. This analysis is shown in the separate correlation matrices of Tables 2–5 and the results are displayed graphically in Fig. 2. MP optical density was significantly related to plasma L and Z for males ($r = 0.62$, $P < 0.005$) and females ($r = 0.30$, $P < 0.05$). Although the relationship was stronger for males than females, this difference was not statistically significant ($P < 0.22$). Plasma L and Z was significantly related to dietary L and Z for males ($r = 0.70$, $P < 0.0005$) and females ($r = 0.56$, $P < 0.005$).

### *Plasma and dietary beta-carotene*

Descriptive and inferential statistics for plasma and dietary BC are given in Table 1. One male subject and two female subjects were removed from these analyses because their plasma BC values exceeded the average by five standard deviations. The excluded male subject supplemented his diet with pure BC and the females had unusually high dietary intakes of BC (among the highest for both males and females) relative to other dietary components. For the remaining subjects, no significant sex difference was found in the concentration of BC in the blood ($P < 0.21$). Males had a higher mean intake of

TABLE 5. Pearson product moment correlation matrices on values adjusted for caloric intake for females

| | Females (Values adjusted for caloric intake) | | | | | | |
|---|---|---|---|---|---|---|---|
| | MPOD | Plasma L and Z ($\mu$mol/l) | Dietary L and Z ($\mu$g/1 kcal) | Plasma BC ($\mu$mol/l) | Dietary BC ($\mu$g/1 kcal) | Total fat (g/1 kcal) | Iron (mg/1 kcal) |
| MPOD | 1.0 | | | | | | |
| Plasma L and Z ($\mu$mol/l) | 0.30* | 1.0 | | | | | |
| Dietary L and Z ($\mu$g/1 kcal) | 0.07 | 0.68* | 1.0 | | | | |
| Plasma BC ($\mu$mol/l) | −0.03 | 0.29* | 0.45* | 1.0 | | | |
| Dietary BC ($\mu$g/1 kcal) | 0.25 | 0.26 | 0.50* | 0.53* | 1.0 | | |
| Total fat (g/1 kcal) | −0.31* | −0.46* | −0.39* | −0.45* | −0.69* | 1.0 | |
| Iron (mg/1 kcal) | 0.21 | 0.18 | 0.20 | 0.09 | 0.25 | −0.55* | 1.0 |

Significant correlations ($P < 0.05$) are indicated by an asterisk. Abbreviations used in the following analysis are the same as those used in Table 1.

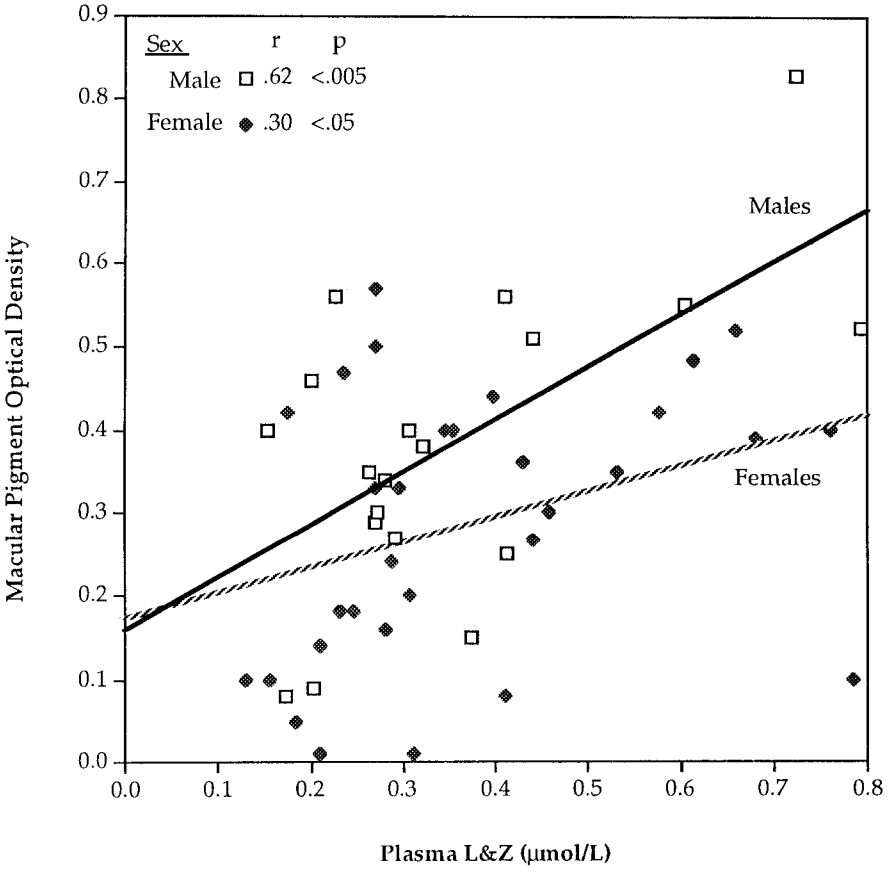


FIGURE 2. Relationship of macular pigment optical density to plasma lutein and zeaxanthin for males ($r = 0.62$) and females ($r = 0.30$).

BC, but this difference was not significant when values were adjusted for differences in body weight ($P < 0.49$) and caloric intake ($P < 0.84$).

The relationship between MP density and plasma BC for males and females is shown in Figs 3 and 4. One male and three females (indicated in the figures by open circles) were excluded from this analysis because their plasma BC values were over three standard deviations above the mean. Males showed a significant relationship between plasma BC and MP density ($r = 0.44$, $P < 0.025$). This relationship was reduced ($r = 0.18$) when the one male outlier was included in the analysis. The females did not show a relationship ($r = -0.03$). In fact, when the two female outliers were included in the correlational analysis, the relationship between MP density and plasma BC was slightly negative ($r = -0.20$). As shown in Figs 3 and 4, the relationship of MP density to plasma L and Z was stronger than the relationship of MP density to plasma BC; this difference was not statistically significant for males or females, probably because of the high correlation between plasma L and Z and plasma BC.

*Dietary fat and iron*

The difference between males and females in dietary intake of fat and iron is apparent in Table 1. A significant

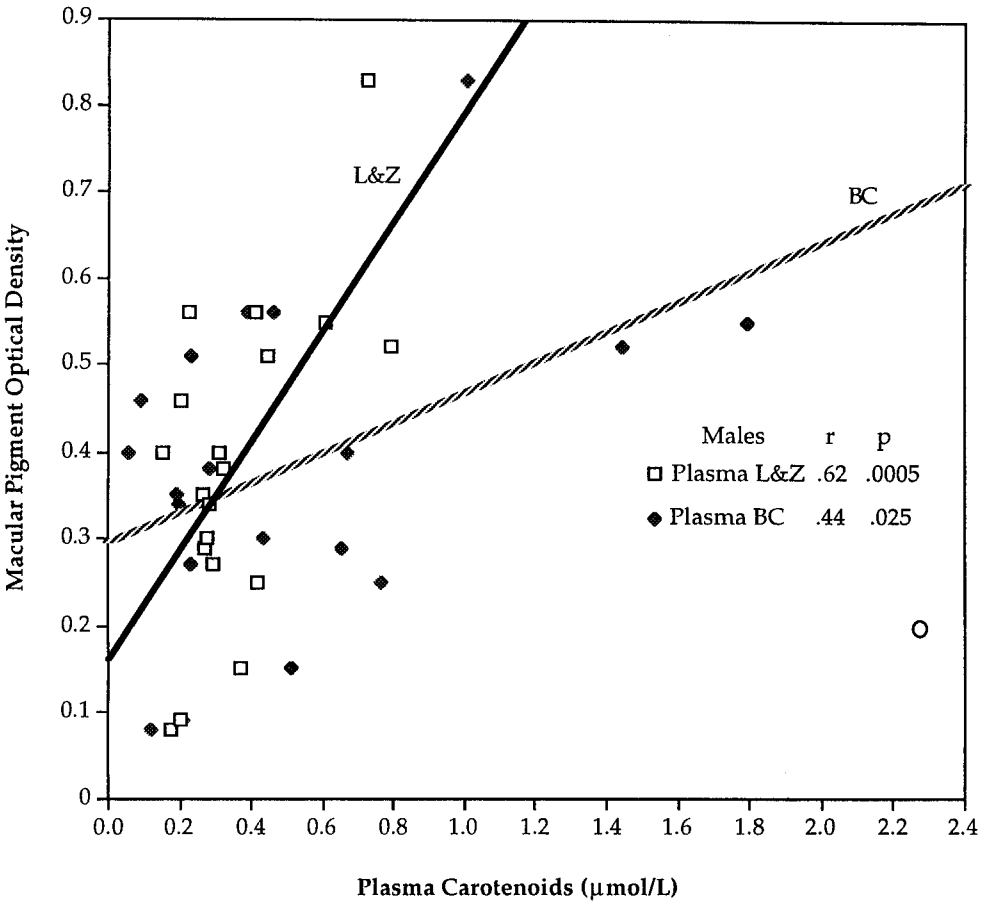


FIGURE 3. Comparison of the macular pigment and plasma lutein and zeaxanthin relationship (open squares) ($r$ = 0.62) with the macular pigment and plasma beta-carotene relationship (solid diamonds) ($r$ = 0.44) for males. The regression line for the macular pigment and plasma beta-carotene relationship was calculated without including the outlier (open square). The inclusion of this outlier reduces the strength of the correlation ($r$ = 0.18) and requires a different regression line ($y = 0.571x + 0.388$).

difference between males and females was found for iron intake ($P < 0.05$). This difference was removed, however, when values were adjusted for differences in body weight ($P < 0.28$) and caloric intake ($P < 0.75$). In contrast, a significant difference ($P < 0.03$) in total fat intake (males = 132.3 g; females = 73.5) was maintained, even after adjustment for body weight and total caloric intake. Since iron and fat were highly correlated for the males ($r$ = 0.91) and females ($r$ = 0.62), it is possible that the effect found for iron is spurious.

The relationship between MP density and dietary fat and iron for males and females was also examined. As shown in Tables 2–5, the relationship between MP, fat, and iron appears to be opposite between the sexes. Males have significant positive relationships between MP density, fat, and iron, whereas females have inverse relationships.

The different relationship between MP density and dietary fat for males and females is particularly apparent when the correlational analysis is applied to the adjusted dietary values. Whereas adjusting the dietary values for differences in body weight does not change the correlations more than 10% overall, adjusting the dietary values for differences in caloric intake has a larger effect that is different for males and females. For males, the caloric adjustment weakens the relationship of MP to dietary variables but increases the correlation of plasma carotenoids with the dietary variables. For females, the caloric adjustment decreases the correlations of both MP and the plasma carotenoids with the dietary variables. Since caloric intake is so highly related to fat intake (males, $r$ = 0.92; females, $r$ = 0.85), the difference in the effect of caloric adjustment is probably due to a sex difference in the relationship of MP to fat intake.

## DISCUSSION

Although large individual differences in MP density have been well documented, little information is available regarding the natural variation of MP among different groups in the normal population. The present study utilized a sample size large enough to assess differences in MP density between males and females. This sample size was also sufficient to allow a separate correlational analysis for males and females, in order to assess sex differences in the relationship of MP to carotenoids in the diet and blood and the relationship of MP to dietary fat and iron.

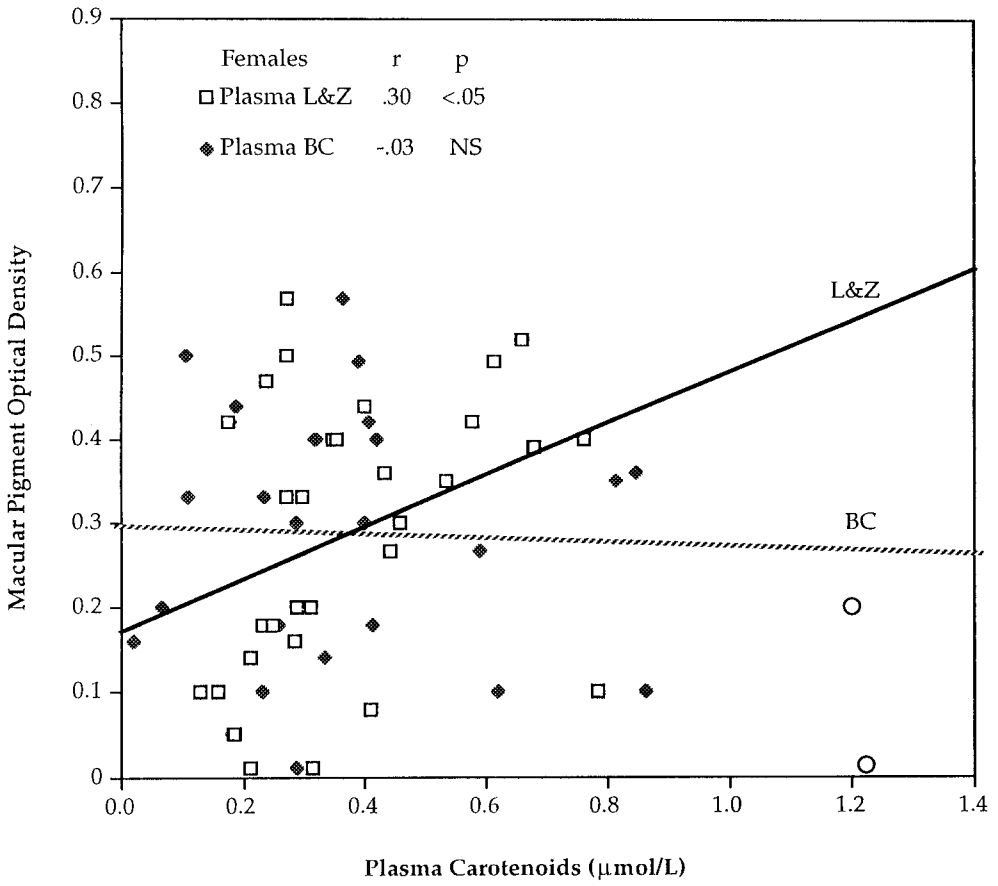


FIGURE 4. Comparison of the macular pigment and plasma lutein and zeaxanthin relationship (open squares) ($r = 0.30$) with the macular pigment and plasma beta-carotene relationship (solid diamonds) ($r = -0.03$) for females. The regression line for the macular pigment and plasma beta-carotene relationship was calculated without including the two outliers (open squares). The inclusion of these outliers increases the negative trend of the correlation ($r = -0.20$) and requires a different regression line ($y = -0.121x + 0.326$).

*Macular pigment*

The major finding in the present study was a statistically significant difference in MP density between males and females ($P < 0.001$). Macular pigment density for males was 38% higher than for females. This difference is particularly striking compared to the sex differences found in other neural structures, which are often less than half this much (LeVay, 1993). For example, a recent study (Witelson *et al.*, 1995) reports that the density of neurons in the granular layers of the posterior temporal cortex of women is 11% higher than the comparable region in men.

The only other study that has examined sex differences in MP density was conducted by Bone & Sparrock (1971). With 49 subjects, this study did not find any evidence for sex differences in MP density. These differences may have been obscured, however, because of the limitations of their method. For example, equivalent spectral sensitivity could not be assumed between their central and parafoveal conditions, since differences in spectral sensitivity owing to differences in short-wave cone populations were not eliminated. Further, as the authors pointed out, their parafoveal measures may have been contaminated by rod intrusion. Our method was designed to minimize the effects of these potential artifacts.

*Plasma and dietary lutein and zeaxanthin*

In contrast to the large sex differences we found in MP density, we did not find sex differences in plasma concentrations of L and Z (the carotenoids that compose MP) or the dietary intake of L and Z. Plasma concentrations of L and Z for males and females differed by only 5%. This finding is consistent with past reports (Olmedilla *et al.*, 1994; Ito *et al.*, 1991). Similarly, we did not find a significant difference in dietary intake of L and Z after these values were adjusted for sex differences in body weight and caloric intake. These results suggest that sex differences in MP density cannot be explained by differences in the dietary intake of L and Z or the concentrations of these carotenoids in the blood. There must be a sex difference in the way that carotenoids are metabolized by the retina.

We also examined the relationship between MP density and blood concentrations and dietary amounts of L and Z. Blood and diet measures of carotenoids were positively correlated for both males and females (see Tables 2–5), but, consistent with a past report (Ascherio

*et al.*, 1992), were more strongly related in males. The relationship between MP density and blood L and Z was significant for both males and females, although the relationship was stronger for males ($r = 0.62$, $P < 0.005$) than females ($r = 0.30$, $P < 0.05$).

*Plasma and dietary beta-carotene*

No differences were found in average intake or in plasma concentrations of BC for males and females. Sex differences were found, however, in the relationship of plasma and dietary BC to MP density. Males had a positive significant relationship between MP density and both plasma BC and dietary BC. Females showed no relationship between MP density and dietary intake of BC or blood concentrations of BC.

Since BC is not found in the retina under normal conditions (Handelman *et al.*, 1988, 1992), the relationship of MP density to plasma BC for males may be a function of the strong relationship of plasma BC to plasma L and Z ($r = 0.83$). The positive correlation between these plasma carotenoids is partially due to the fact that our male subjects did not supplement BC, and foods often contain mixtures of carotenoids. At present, it is not clear whether epidemiologic results showing a protective effect of plasma BC on retinal disease (e.g., West *et al.*, 1994; Hyman *et al.*, 1992; EDCC, 1992, 1993) may also be a consequence of the high covariance between L and Z and BC in the blood.

For some subjects, such as our excluded outliers, the possibility that L and Z and BC might interact is also a concern. A controlled nutritional study (Micozzi *et al.*, 1992) has indicated that BC supplementation lowers L and Z concentrations in human plasma. The possibility that supplementation of one carotenoid may inhibit another is supported by research on animals (White *et al.*, 1993; High & Day, 1951; Kelley & Day, 1950). The three subjects that we excluded as outliers because of extremely high plasma BC concentrations (five standard deviations above the mean for their group), had lower than average L and Z plasma concentrations and low MP density (see open circles in Figs 3 and 4). Since BC intake exceeded L and Z intake in these subjects, this pattern may reflect differences in dietary intake. It is also possible, however, that high BC intake may have interfered with the absorption and/or metabolism of L and Z. Since BC supplementation is under consideration for the prevention or treatment of retinal disease, the effects of supplemental BC on retinal carotenoids warrants further examination.

*Dietary fat and iron*

We also assessed sex differences in dietary intake of fat and iron. We selected these nutrients based on past studies of factors influencing BC absorption (Swanson & Parker, 1993; Dimitrov *et al.*, 1988; Prince & Frisoli, 1993). After adjusting for sex differences in body weight and caloric intake, we did not find a significant sex difference in the intake of iron. We did, however, find a significantly higher fat intake by males.

Unlike differences in absolute intake, both males and females had a similar negative relationship between dietary fat and iron intake and plasma BC and L and Z concentrations. This finding suggests that, although a sufficient amount of fat may be necessary for gut absorption of lipid-soluble carotenoids, high regular intake of fat and iron (perhaps indicating high meat consumption) may cause a decrease in plasma concentrations of BC or L and Z. This conclusion is consistent with results from a recent study by Bowen *et al.* (1993) of women with high plasma lipid concentrations. Bowen *et al.* report that a disproportionate number of these females were very poor at absorbing carotenoids into their blood.

More importantly, our data indicate that dietary intake of fat may have different effects on MP density for males and females. Most notably, the relationship between dietary fat and MP density was positive for males ($r = 0.56$, $P < 0.005$) and negative for females ($r = -0.51$, $P < 0.005$). A sex-specific difference in tissue uptake as a response to chronic fat intake may have implications for dietary studies of retinal disease that use calorie-adjusted dietary variables. Such studies attempt to control for systematic population differences in caloric intake (e.g., increased caloric intake due to differences in the body size of males and females) by either dividing nutrient values by total caloric intake (nutrient densities) or adjustment by regression analysis (Willett & Stampfer, 1986). Since the effects of caloric intake on MP density differ by sex, caloric adjustments of epidemiologic data that may be affected by MP would be most appropriate if the data were separated by sex. Even without separating males and females, epidemiologic studies on the effects of carotenoid intake on AMD (e.g., Seddon *et al.*, 1994) have shown a protective effect of calorie-adjusted high carotenoid intake. To estimate the magnitude of this effect most precisely, however, future studies will need to analyze both raw and adjusted dietary intake and separate these analyses by sex.

*Hormonal interactions*

The poor relationships between the retina, blood and diet for females suggest the existence of moderating variables. Although we have no data on what factors might be most important, we hypothesize that steroid hormones may play a role. One avenue could be hormonally controlled variations of the lipid transport systems utilized by carotenoids (HDL and LDL; Heiss *et al.*, 1980; Schaefer *et al.*, 1983; Basdevant, 1992). It is also possible that steroid hormones may affect the metabolism of L and Z directly, but no data are available on this point. Since estrogen use and parity alter the risk for neovascular AMD (EDCC, 1992), possible effects of hormones on protective systems in the retina should be evaluated.

*Clinical implications*

Given the putative protective role of MP (Snodderly, 1995), the finding that males and females differ in MP density may have clinical implications. It is often

suggested on the basis of experimental and clinical evidence (Lawwill *et al.*, 1977; Young, 1987; Young, 1988; Kirschfeld, 1982; Nussbaum *et al.*, 1981; Schalch, 1992) that MP protects the retina (Haegerstrom-Portnoy, 1988; Weiter *et al.*, 1988) and retinal pigment epithelium (Lawwill *et al.*, 1977; Ham *et al.*, 1978) from damage.

Protection by MP is also consistent with current epidemiologic data (Snodderly, 1995). Goldberg *et al.* (1988) originally reported that ingestion of fruits and vegetables rich in carotenoids and vitamin A were inversely related to AMD. The Eye Disease Case-Control Group (EDCC) refined this finding and showed that blood concentrations of carotenoids are preferentially related to a reduction of the neovascular form of AMD (EDCC, 1992; EDCC, 1993). Recently, a detailed dietary analysis of these patients (Seddon *et al.*, 1994) showed that dietary intake of L and Z, the carotenoids that compose MP, was more protective than dietary intake of other carotenoids found in the blood. Thus, the group who had low intake of L and Z had a 43% higher probability of developing neovascular AMD compared to the high intake group. Our results show that low plasma concentrations of L and Z (and dietary intake for males) are associated with reduced MP density. This suggests the possibility that reduced AMD incidence due to high carotenoid intake and higher blood concentrations of L and Z may be due to increased density of MP.

Consistent with this view, a reduction of MP density may partially explain how some risk factors increase the prevalence of AMD. For example, smoking is a major risk factor for neovascular AMD (Paetkau *et al.*, 1978; Hyman *et al.*, 1992; Klein *et al.*, 1993; Seddon *et al.*, 1994; Vingerling *et al.*, 1995). Recently, Hammond *et al.* (1995b) have shown a strong inverse relationship between smoking frequency and MP density. We suggest that smoking-induced reduction of MP density contributes to the increased risk of neovascular AMD in smokers.

### *Sex differences in disease susceptibility*

The finding of reduced MP density in females suggests that females may be at higher risk for AMD than males due to reduced macular pigmentation. Females may have greater risk of retinal damage, as evidenced by greater loss of short-wave cone sensitivity (specified at the retina) over time compared to males (Werner & Steele, 1988; Eisner *et al.*, 1987). A sex-specific change in the spatial profile of short-wave cone populations has also been noted in older Rhesus monkeys (de Monasterio *et al.*, 1985).

Sex-specific differences in normal visual structures may signal sex differences in susceptibility to visual disease. For example, female patients with non-insulin dependent diabetes mellitus have significantly higher hydroperoxide levels with associated retinal complications than males (Armstrong *et al.*, 1992). There is also evidence (Klein *et al.*, 1992; West *et al.*, 1994) that older females (75+ yr) have a significantly higher incidence of neovascular AMD compared to males, even after controlling for differences in age. This pattern has not been found for the atrophic forms of the disease. Not all epidemiologic studies have found a higher incidence of AMD in females (see the review by Hyman, 1991), but our results warrant further examination of sex differences in AMD, especially the neovascular form.

Treatment outcomes for AMD, particularly the neovascular form, are poor (Hampton & Nelsen, 1992). Consequently, a preventive approach would be especially valuable. The expeditious nature of psychophysical assessment of MP density (it is non-invasive and can be applied to most individuals) allows for population-based testing. This type of testing may help to identify high risk groups and individuals who might be candidates for nutritional counseling. We should now be able to examine the possibility that adequate MP densities could help to prevent neovascular AMD.

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