Association of Macular Pigment Density with Plasma Omega-3 Fatty Acids: The PIMAVOSA Study

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Purpose. To assess the correlation between macular pigment optical density and plasma levels of lutein, zeaxanthin, and fatty acids, especially omega-3 polyunsaturated fatty acids (PUFAs).

Methods. The PIMAVOSA study is an observational study of 107 healthy volunteers, aged 20 to 60 years and born in southwest France, without histories of ocular disease. Macular pigment optical density (MPOD) was measured using the two-wavelength autofluorescence method with a modified scanning laser ophthalmoscope. Plasma measurements (lutein, zeaxanthin, and fatty acids) were performed from fasting blood samples collected on the day of the eye examination.

Results. MPOD within 6° correlated with plasma levels of lutein and zeaxanthin ($r = 0.35$, $P < 0.001$, and $r = 0.30$, $P < 0.005$, respectively). MPOD also significantly correlated with total plasma omega-3 PUFAs ($r = 0.22$, $P < 0.05$). Among the different omega-3 PUFAs, docosapentaenoic acid (DPA) had the highest correlation with MPOD ($r = 0.31$, $P < 0.001$), whereas correlation with eicosapentaenoic acid (EPA) was moderate ($r = 0.21$, $P < 0.05$) and did not reach statistical significance for docosahexaenoic acid ($r = 0.14$, $P = 0.14$).

Conclusions. In the present study, macular pigment density was associated not only with plasma lutein and zeaxanthin but also with omega-3 long-chain PUFAs, particularly with EPA and DPA. Further studies will be needed to confirm these findings and to identify the underlying mechanisms. (*Invest Ophthalmol Vis Sci.* 2012;53:1204–1210) DOI:10.1167/iovs.11-8721

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Age-related macular degeneration (AMD) is the leading cause of blindness in developed countries.[1–4] With the increased longevity of the aging population, it has become a disease of significant public health importance. AMD is a multifactorial disease that results from the combination of nonmodifiable factors (genetics, sex, age) and identified modifiable factors (nutritional status, smoking status, or both).[5] Controlling these modifiable factors may be a way of preventing a significant percentage of AMD cases. Evidence from both epidemiologic and laboratory studies have demonstrated an inverse association between dietary intake of xanthophyll carotenoids—lutein (L) and zeaxanthin (Z)— or omega-3 long-chain polyunsaturated fatty acids (LCPUFAs) and risk for advanced AMD.[6–15]

L and Z exclusively derive from dietary intake.[16,17] They are lipid soluble, and their metabolism is therefore strongly interlinked with lipids.[18,19] High dietary intake of L and Z, or their oral supplementation, is known to result in an increase of their plasma concentrations and, in turn, of their specific accumulation within the macula in which they form the macular pigment.[20–24] Macular pigment exhibits neuroprotective functions against oxidative stress and inflammation, both implicated in the pathogenesis of AMD.[25] The precise mechanism of macular pigment accumulation still remains to be determined but, as proposed recently, may rely on specific carotenoid binding proteins.[26,27]

On the other hand, omega-3 LCPUFAs, notably docosahexaenoic acid (DHA), are abundant in the human retina in which they exert some identified structural, functional, and neuroprotective roles.[28,29] In AMD, their neuroprotective role has been demonstrated by a number of epidemiologic studies that observed a decreased risk for AMD in subjects with high intake of omega-3 LCPUFAs.[10–14,28,29] Among other potential mechanisms supporting neuroprotection, it has been suggested that dietary intake of omega-3 LCPUFAs may favor the retinal accumulation of L and Z and thus increases macular pigment density.[19,20]

In the present study, we investigated, in a homogeneous population of healthy volunteers, the interrelations among macular pigment, plasma lutein and zeaxanthin, and plasma omega-3 LCPUFAs (and other fatty acids). This may foster better understanding of the factors influencing the accumulation of macular pigment and may identify some properties of omega-3 LCPUFAs in the retina, which might be implicated in their potential protective role in AMD.

Subjects and Methods

Study Aims

The PIMAVOSA (PIgment MAculaire chez le VOlontaire SAin [macular pigment in the healthy volunteer]) Study is an observational study aimed

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at assessing the associations between macular pigment and lutein, zeaxanthin, and fatty acids (polyunsaturated omega-3 and omega-6, monounsaturated, and saturated) determined from plasma measurements.

Study Sample

Inclusion criteria in the PIMAVOSA Study for healthy volunteers were as follows; age range, 20 to 60 years; born in southwest France with a mother also born in the southwest of France (to optimize the homogeneity of nutritional habits); phakia, with visual acuity ≥20/25; absence of chronic disease with significant ocular consequences; and absence of myopia exceeding 4 diopters. Data collected during the examination included age, sex, use of vitamins or supplements, eye examination results, macular pigment optical density measurement in both eyes, and fasting plasma measurements of lutein, zeaxanthin, and fatty acids (polyunsaturated omega-3 and omega-6, monounsaturated and saturated).

This research followed the tenets of the Declaration of Helsinki. Participants gave written consent for participation in the study. The study design was approved by the Ethical Committee of Bordeaux (Comité de Protection des Personnes Sud-Ouest et Outre-Mer III) in March 2008.

Eye Examination and Macular Pigment Density Measurements

Eyes were examined in the Department of Ophthalmology of the University Hospital of Bordeaux. All subjects underwent comprehensive ocular examination that included, in both eyes, a measure of best-corrected visual acuity, refraction, and, after pupil dilatation with eyedrops containing 0.5% tropicamide, two 45° color retinal photographs (one centered on the macula, the other centered on the optic disc), fundus autofluorescence imaging, and MPOD measurement. Retinal imaging was performed with a high-resolution digital nonmydriatic retinograph (TRC NW6S; Topcon, Tokyo, Japan). Photographs were interpreted in duplicate by two specially trained graders; any observed abnormalities were exclusion criteria. MPOD measurements were obtained with the modified confocal scanning laser ophthalmoscope (mpHRA; Heidelberg Engineering, Heidelberg, Germany)[30] using autofluorescence images obtained at two wavelengths based on the pioneering work of Delori et al.[31,32] Subjects were positioned in front of the tabletop and were instructed to look straight ahead and to remain steady. Autofluorescence images (20°) were then obtained at excitation wavelengths of 488 nm and 514 nm of the posterior pole, with a high-pass filter transmitting at a wavelength greater than 530 nm. MPOD was quantified by calculating an MPOD map and comparing foveal and parafoveal autofluorescence at 488 nm and 514 nm. Density maps were processed to estimate MPOD within a circle centered on the fovea at different degrees of eccentricities (0.5°, 1°, 2°, and 6°), using the software provided by the manufacturer of the device. Correlation of MPOD values between both eyes was greater than 0.8 for all types of measurement. For each volunteer, the studied value of MPOD, expressed in optical density units, was the mean of MPOD measurement in both eyes.

Plasma Lutein and Zeaxanthin Measurements

Plasma lutein (L) and zeaxanthin (Z) measurements were performed at DSM Nutritional Products (Kaiseraugst, Switzerland). Their concentrations were determined by reversed-phase high-performance liquid chromatography, using dedicated analytical methods.[33] Plasma samples were analyzed for zeaxanthin (sum of all-E and Z-isomers) and lutein (sum of all-E and Z-isomers). The xanthophylls were extracted from plasma (100 μL) with a 20% mixture of n-hexane and chloroform (1100 μL) after dilution with water (100 μL) and proteins precipitation with ethanol (200 μL). After centrifugation, an aliquot (800 μL) of the clear supernatant fluid was dried under nitrogen at room temperature. The dried residue was quantitatively redissolved in the mobile phase (200 μL n-hexane and acetone; 19%, by volume). The resultant solution was injected (100 μL) into a normal-phase HPLC system equipped with an autosampler (15°C), a column oven (40°C), an HPLC pump, and an ultraviolet-visible detector. Data were analyzed with a data acquisition system (Atlas; Thermo Labsystems, Helsinki, Finland). The separation was performed on a polar column (Lichrosorb, Si60, 5 mm, 250 × 4 mm; Stagroma, Reinach, Switzerland) with a mixture of n-hexane and acetone (19%, by volume) at a flow rate of 1 mL/min. Xanthophylls were detected at a wavelength of 452 nm. The method is a standard one and is regularly checked for accuracy and precision by attending to interlaboratory studies organized by the National Institute of Standard and Technologies in the United States and by the Société Francophone Vitamine et Biofacteurs (SFVB) in the European Union. To assess the daily and long-term laboratory performance of the HPLC plasma analytics, dedicated control plasma was used. The control samples were analyzed four times a day during the study. Because of technical failure, L and Z plasma levels were available in only 99 subjects (62 women, 37 men) aged 20.1 to 60.9 years (mean, 39.1 ± 12.2 years). No one involved in plasma carotenoid determination had access to eye clinical findings at any time during the study.

Plasma Phospholipid Fatty Acids Measurements

Lipid assays were performed in the Department of Nutrition Metabolism and Health at the French Institute for Fats and Oils in Bordeaux, France (ITERG). Total lipids were extracted from plasma according to the Folch procedure.[34] Total phospholipids were separated from neutral lipids by thin layer chromatography (Kieselgel 60 H; Merck, Fontenay-sous-Bois, France) using the solvent mixture ether/acetone (60: 20, vol/vol). The phospholipid fraction was transesterified using boron trifluoride in methanol according to the method of Morrison and Smith.[35] Fatty acid methyl esters (FAME) were analyzed by gas chromatography on a chromatograph (FOCUS GC; Thermo Electron Corporation) equipped with a split injector and a flame ionization detector. Separation of FAME was performed with a BPX70 fused silica capillary column (60 m length × 0.25 mm internal diameter, 0.25 μm film thickness; SGE, Courtaboeuf, France). The hydrogen inlet pressure was 1 bar. Injector and detector temperatures were set to 250°C and 280°C, respectively, and the oven temperature was programed from 150°C to 190°C at 1.3°C/min with a 50-minute hold and then to 225°C at 20°C/min with a 10-minute hold. Plasma phospholipid fatty acids were expressed in a percentage of total fatty acids.

Statistical Analysis

Statistical analysis was performed (SAS software, version 9.1; SAS Institute Inc, Cary, NC). Correlations were estimated using Spearman's rank correlation coefficient because some variables departed from normality. $P < 0.05$ was considered statistically significant.

Results

Characteristics of the Population

One hundred seven healthy volunteers (64 women, 43 men) from 20.1 to 60.9 years of age (mean ± SD, 38.9 ± 12.1 years) were included in the study (Table 1); their mean best-corrected LogMAR visual acuity was −0.1 (± 0.1). Seven of 107 volunteers stated they took some vitamins or supplements.

MPOD Correlates Positively with Plasma Levels of Lutein and Zeaxanthin

We confirmed the expected correlation of the optical measurements of MPOD with plasma levels of L and Z (Table 2, Fig. 1A). As shown in Table 2, within 0.5° of eccentricity, no statistically significant correlations were observed between MPOD and plasma xanthophylls (L+Z) ($r = 0.16$; $P = 0.1$). However, beyond 1° of eccentricity, MPOD and plasma xanthophylls correlated positively ($r = 0.26$, $P < 0.05$ within 1°; $r = 0.33$, $P < 0.005$ within 2°; $r = 0.36$, $P = 0.0005$ within 6°). Similar data were obtained when L and Z were considered

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Table 1. Characteristics of the Studied Population

	Total (n = 107)	20–39 years (n = 53)	40–60 years (n = 54)	P
Age, y	38.9 (±12.1)	28.2 (±5.9)	49.3 (±5.5)	<0.0001
Sex, men	43	23	20	0.50
Best-corrected visual acuity, LogMAR units	−0.10 (±0.1)	−0.10 (±0.09)	−0.09 (±0.1)	0.48
MPOD, within 6° of eccentricity, optical density units	0.2 (±0.1)	0.2 (±0.0)	0.2 (±0.1)	0.11
Plasma phospholipid omega-3 PUFAs, % of total fatty acids				
Total	6.9 (±1.9)	6.5 (±1.8)	7.2 (±1.9)	0.05
ALA	0.2 (±0.1)	0.2 (±0.1)	0.2 (±0.1)	0.96
Long-chain omega-3 PUFAs				
Total	6.7 (±1.9)	6.3 (±1.8)	7.0 (±1.9)	0.05
EPA	1.2 (±0.7)	1.1 (±0.7)	1.4 (±0.7)	0.02
DPA	0.9 (±0.2)	0.9 (±0.3)	1.0 (±0.2)	0.08
DHA	4.5 (±1.2)	4.3 (±1.3)	4.7 (±1.2)	0.18
Plasma phospholipid omega-6 PUFAs, % of total fatty acids				
Total	34.8 (±2.4)	34.9 (±2.2)	34.7 (±2.5)	0.60
Linoleic acid	18.7 (±2.4)	18.5 (±2.6)	18.9 (±2.2)	0.40
Long-chain omega-6 PUFAs				
Total	15.3 (±2.1)	15.6 (±1.9)	15.0 (±2.2)	0.19
Eicosadienoic acid	0.3 (±0.1)	0.3 (±0.1)	0.3 (±0.1)	0.47
Dihomo-γ-linolenic acid	3.0 (±0.7)	3.1 (±0.7)	2.9 (±0.7)	0.11
Arachidonic acid	12.0 (±2.0)	12.1 (±2.0)	11.8 (±2.0)	0.43
Plasma phospholipid saturated fatty acids, % of total fatty acids	44.4 (±1.2)	44.3 (±1.3)	44.5 (±1.1)	0.35
Plasma phospholipid monounsaturated fatty acids, % of total fatty acids	13.0 (±1.4)	13.4 (±1.3)	12.7 (±1.4)	0.01
Plasma xanthophylls,* μg/L				
Plasma lutein*	150.1 (±58.9)	137.8 (±48.4)	161.8 (±65.8)	0.04
Plasma zeaxanthin*	40.9 (±20.2)	40.4 (±17.5)	41.3 (±22.6)	0.83
Plasma lutein + zeaxanthin*	191.1 (±75.4)	178.2 (±62.4)	203.1 (±84.8)	0.10

Data are the mean (±SD); n = 107 unless specified otherwise. ALA, alpha-linolenic acid.
* Because of technical failure, L and Z plasma measurements were available in only 99 subjects.

separately, as follows: no significant association within 0.5° of eccentricity (r = 0.16, P = 0.12 for L; r = 0.15, P = 0.15 for Z), positive correlation within 1° (r = 0.25, P < 0.05 for L; r = 0.24, P < 0.05 for Z), 2° (r = 0.32, P < 0.005 for L; r = 0.29, P < 0.005 for Z), and 6° (r = 0.35, P < 0.001 for L; r = 0.30, P < 0.005 for Z). These results were not affected by exclusion of the seven subjects declaring use of dietary supplements (data not shown).

MPOD Correlates Positively with Plasma Phospholipid Omega-3 LCPUFAs

As shown in Table 3 and in Figure 1B, total omega-3 PUFAs correlated positively with MPOD whatever the degree of eccentricity considered (r = 0.19, P < 0.05 within 0.5°; r = 0.21, P < 0.05 within 1°; r = 0.20, P < 0.05 within 2°; r = 0.22, P < 0.05 within 6°). By contrast, α-linolenic acid, the precursor of omega-3 LCPUFAs, did not correlate with MPOD (r = 0.0035, P = 0.97 within 0.5°; r = −0.0011, P = 0.99 within 1°; r = −0.00,074, P = 0.99 within 2°; r = 0.0016, P = 0.98 within 6°). Among the omega-3 LCPUFAs (Table 3, Fig. 1C), correlation of eicosapentaenoic acid (EPA) with MPOD was statistically significant from 1° to 6° of eccentricity (r = 0.18, P = 0.06 within 0.5°; r = 0.21, P < 0.05 within 1°; r = 0.20, P < 0.05 within 2°; r = 0.21, P < 0.05 within 6°). Correlation of docosapentaenoic acid (DPA) with MPOD was even stronger whatever the degree of eccentricity (Table 3, Fig. 1D; r = 0.33, P < 0.001 within 0.5°; r = 0.32, P < 0.001 within 1°; r = 0.30, P < 0.005 within 2°; r = 0.31, P = 0.001 within 6°). DHA did not show any statistically significant correlations with macular pigment (r = 0.13, P = 0.18 within 0.5°; r = 0.14, P = 0.16 within 1°; r = 0.12, P = 0.23 within 2°; r = 0.14, P = 0.14 within 6°).

MPOD Is Not Correlated with Plasma Phospholipid Monounsaturated and Saturated Fatty Acids

The association of MPOD with the other fatty acids was then analyzed. Table 3 shows that neither saturated nor monounsaturated fatty acids correlated in any way with MPOD (r = −0.12, P = 0.2, and r = −0.06, P = 0.5, within 6°, respectively).

MPOD Correlates Negatively with Some Plasma Phospholipid Omega-6 LCPUFAs

Table 3 further displays the results of the correlation analyses between MPOD and omega-6 PUFAs. Total omega-6 LCPUFAs did not show any relationships with MPOD (r = −0.092, P = 0.3 within 6°). However, when detailing the different omega-6, we found that two of them did correlate negatively with MPOD: eicosadienoic acid (Table 3, Fig. 1E; r = −0.30, P = 0.001 within 0.5°; r = −0.25, P < 0.01 within 1°; r = −0.22,

Table 2. Correlation of MPOD with Plasma Lutein and Zeaxanthin Levels

	MPOD within 0.5°	MPOD within 1°	MPOD within 2°	MPOD within 6°
Lutein + zeaxanthin	0.16 (0.1)	**0.26 (0.01)**	**0.33 (0.001)**	**0.36 (0.0005)**
Lutein	0.16 (0.1)	**0.25 (0.01)**	**0.32 (0.001)**	**0.35 (0.0006)**
Zeaxanthin	0.15 (0.1)	**0.24 (0.02)**	**0.29 (0.005)**	**0.30 (0.003)**

n = 99. Results are expressed as r (P). Data in bold are significant.

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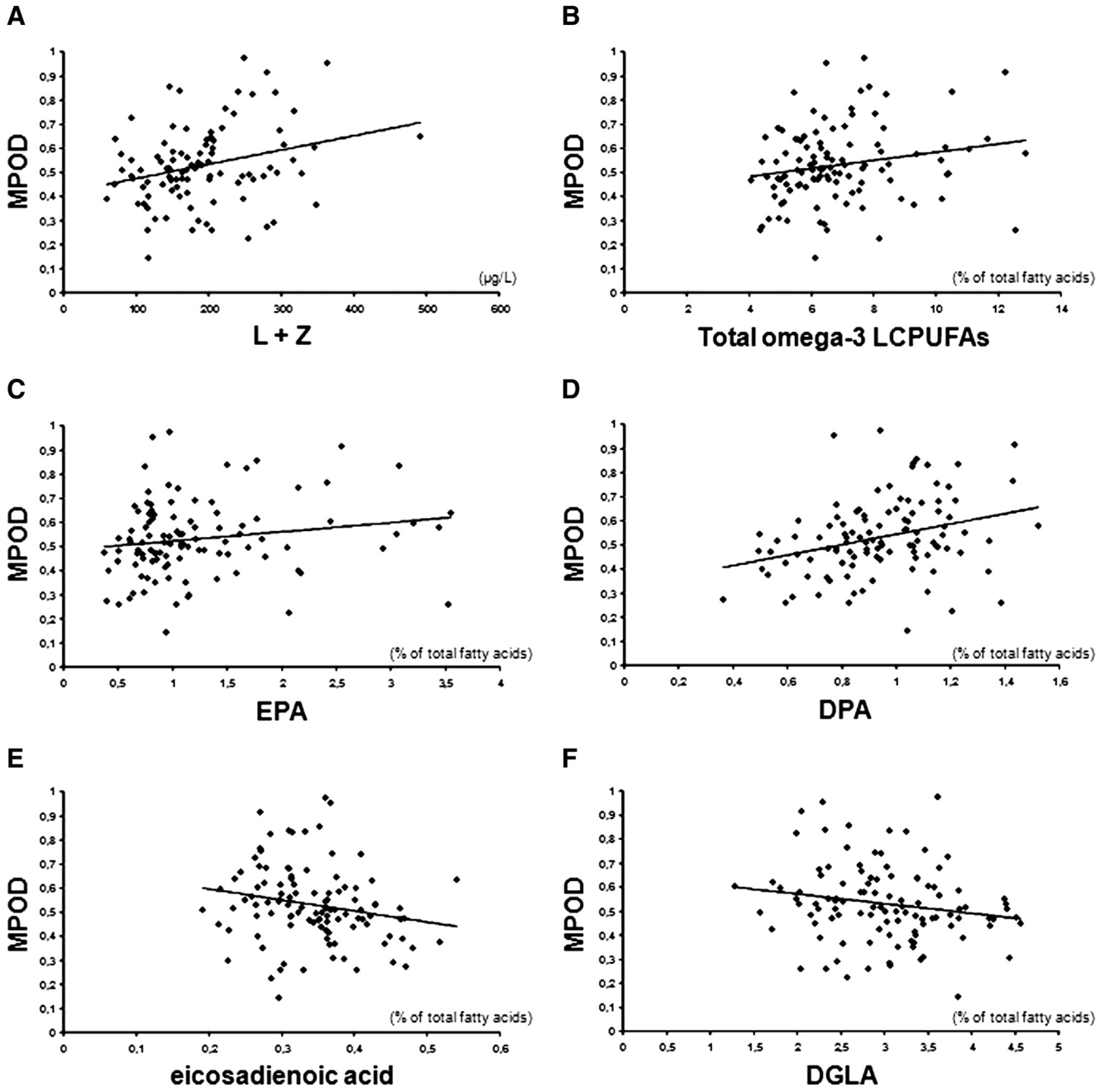

FIGURE 1. Scatterplots depicting the correlations between MPOD at 1° of eccentricity with (**A**) L+Z, (**B**) total omega-3 LCPUFAs, (**C**) EPA, (**D**) DPA, (**E**) eicosadienoic acid, and (**F**) DGLA.

$P < 0.05$ within 2°; $r = -0.21$, $P < 0.05$ within 6°) and dihomo-γ-linolenic acid (Table 3, Fig. 1F; $r = -0.21$, $P < 0.05$ within 0.5°; $r = -0.20$, $P < 0.05$ within 1°; $r = -0.19$, $P = 0.05$ within 2°; $r = -0.19$, $P = 0.05$ within 6°). No correlations with arachidonic acid were observed ($r = 0.02$, $P = 0.8$ within 0.5°; $r = 0.022$, $P = 0.8$ within 1°; $r = 0.0083$, $P = 0.9$ within 2°; $r = 0.0075$, $P = 0.9$ within 6°).

Plasma Phospholipid Omega-3 LCPUFAs Do Not Correlate with Plasma Levels of Lutein and Zeaxanthin

Because MPOD correlated positively with both plasma phospholipid omega-3 LCPUFAs and plasma xanthophylls (L and Z), we looked at a potential relationship between omega-3 and carotenoids in the plasma. As described in Table 4, we did not observe any correlations between any types of omega-3 and any carotenoids, as illustrated by the absence of association between total omega-3 LCPUFAs and L+Z ($r = 0.14$, $P = 0.2$).

DISCUSSION

Supplementation or high dietary intake of L and Z are now well known to increase macular pigment density and may reduce the risk for advanced AMD.[6–9,19–23,35–37] Because macular pigment plays a pivotal role against oxidative stress damage and inflammation within the central neurosensory retina,[25] identification of the mechanisms underlying the specific macular

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Table 3. Correlation of MPOD with Plasma Phospholipid Omega-3 PUFA Levels and Other Plasma Phospholipid Fatty Acids

	MPOD within 0.5°	MPOD within 1°	MPOD within 2°	MPOD within 6°
Omega-3 PUFAs				
Total	**0.19 (0.04)**	**0.21 (0.03)**	**0.20 (0.04)**	**0.22 (0.02)**
ALA	0.0035 (0.97)	−0.0011 (0.99)	−0.00074 (0.99)	0.0016 (0.98)
Omega-3 LCPUFAs				
Total	**0.20 (0.04)**	**0.22 (0.02)**	**0.20 (0.04)**	**0.22 (0.02)**
EPA	0.18 (0.06)	**0.21 (0.04)**	**0.20 (0.04)**	**0.21 (0.03)**
DPA	**0.33 (0.0006)**	**0.32 (0.0007)**	**0.30 (0.002)**	**0.31 (0.001)**
DHA	0.13 (0.18)	0.14 (0.16)	0.12 (0.23)	0.14 (0.14)
Other fatty acids				
Saturated fatty acids	−0.15 (0.1)	−0.11 (0.2)	−0.11 (0.3)	−0.12 (0.2)
Monounsaturated fatty acids	−0.04 (0.7)	−0.09 (0.3)	−0.08 (0.4)	−0.06 (0.5)
Omega-6 PUFAS				
Total	−0.07 (0.5)	−0.064 (0.5)	−0.076 (0.5)	−0.092 (0.3)
Linoleic acid	0.02 (0.8)	0.014 (0.9)	0.0088 (0.9)	−0.027 (0.8)
Omega-6 LCPUFAs				
Total	−0.07 (0.5)	−0.064 (0.5)	−0.076 (0.5)	−0.092 (0.3)
Eicosadienoic acid	**−0.30 (0.001)**	**−0.25 (0.008)**	**−0.22 (0.02)**	**−0.21 (0.03)**
DGLA	**−0.21 (0.03)**	**−0.20 (0.04)**	**−0.19 (0.05)**	**−0.19 (0.05)**
Arachidonic acid	0.02 (0.8)	0.022 (0.8)	0.0083 (0.9)	0.0075 (0.9)

$n = 107$. Results are expressed as r (P). Data in bold are significant.

accumulation of xanthophylls represents a key step toward a better understanding of macular physiology and disease.

In the present work, we confirmed the correlation between L and Z plasma levels and MPOD within 1° and beyond (Table 2). MPOD within 0.5° was not found to be significantly correlated with plasma carotenoids. This could be secondary to interindividual variations in the spatial distribution of MPOD at the very center of the macula.[32,38] Beyond 0.5° of eccentricity, correlations of plasma L and Z (considered separately or together) with MPOD were similar, whatever the degree of eccentricity of the measurements. Because Bone et al.[39] reported a particular spatial distribution of xanthophyll carotenoids—with Z clearly dominant in the center of the fovea and a Z/L ratio decreasing peripherally—a stronger correlation of Z with MPOD within the lower degrees of eccentricity and of L with MPOD at larger degrees of eccentricity would have been expected but was not observed. However, our method of quantification of MPOD implied the calculation of the optical density within a circle; hence, the values at large eccentricities (2° or 6°) included measurements within the lower eccentricity circles (0.5° or 1°). Therefore, some spatial differences between the association of L or Z with MPOD might have been canceled out.

Among the different determinants of macular pigment concentration under focus, omega-3 LCPUFA dietary intakes, especially those in DHA (22:6 ω-3), have been proposed as key factors.[19,20] In the present study, analysis of the fatty acid composition of total plasma phospholipids was used as a valid biomarker of LCPUFA dietary intakes[40] and showed that high plasma levels of total omega-3 PUFAs are associated with high MPOD (Table 3). This observation was even stronger with total omega-3 LCPUFAs (Table 3). The mechanisms through which omega-3 LCPUFAs correlate with MPOD remain to be determined. These could be modulation of the gastrointestinal uptake of L and Z, their carriage by lipoproteins, or their highly selective concentration and further use in the macular area.[19,20,41] In our view, the first hypothesis would have implied a correlation between plasma levels of omega-3 LCPUFAs and xanthophyll carotenoids, which was not observed (Table 4). The two last hypotheses remain possible because an increase of HDL and LDL subfractions has been observed after omega-3 LCPUFA supplementation,[42–44] and an influence of omega-3 PUFAs on xanthophyll-binding proteins that may concentrate L and Z in the retina cannot be excluded.[26]

Table 4. Correlation of Plasma Carotenoids with Plasma Phospholipid Omega-3 PUFAs

	Lutein + Zeaxanthin	Lutein	Zeaxanthin
Total omega-3 PUFAs	0.14 (0.2)	0.15 (0.1)	0.07 (0.5)
Alpha-linolenic acid	0.03 (0.8)	0.008 (0.9)	0.05 (0.6)
Omega-3 LCPUFAs			
Total	0.14 (0.2)	0.15 (0.1)	0.06 (0.5)
EPA	0.15 (0.1)	0.16 (0.1)	0.09 (0.4)
DPA	0.08 (0.4)	0.12 (0.2)	−0.06 (0.6)
DHA	0.08 (0.4)	0.09 (0.4)	0.02 (0.8)

$n = 99$. Results are expressed as r (P).

In total plasma phospholipids, EPA (20:5 ω-3), DPA (22:5 ω-3), and DHA are the main assessable omega-3 LCPUFAs (EPA, DPA, and DHA, accounting for 1.2%, 0.9%, and 4.5% of total fatty acids, respectively; Table 1). Analysis of the association between their plasma levels and macular pigment density showed that EPA and DPA correlated significantly with MPOD, whereas DHA did not (Table 3). DHA, the major LCPUFA in structural lipids of the human retina (its overall percentage accounts for approximately 30% of total retinal fatty acids), is an essential structural component of retinal membranes and exhibits several essential neuroprotective properties.[28] In the present study, the lack of correlation between plasma DHA level and MPOD does not allow argument about DHA status within the retina and its role in relation to macular pigment concentration. However, consistent with our results, Johnson et al.[19] reported that DHA supplementation did not show a significant increase in total MPOD values, whereas it could influence MPOD distribution. EPA, the other major dietary omega-3 LCPUFA in plasma, is poorly accreted to the retina because it is quickly converted to DHA or eicosanoid biosynthesis. EPA undergoes oxidative metabolism by cyclooxygenases and lipooxygenases to produce eicosanoids with vasoregulatory and anti-inflammatory properties.[28] Contrary to DHA, a significant positive relationship was observed between plasma EPA level and MPOD (Table 3). The positive correlation

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was even more significant with DPA. DPA, a metabolic intermediary between EPA and DHA, is the second most abundant omega-3 LCPUFA found within the retina; its endogenous level is approximately one-tenth that of DHA in retinal lipids.[45] DPA is known to be the potential precursor of omega-3 very long chain polyunsaturated fatty acids (VLCPUFAs). Omega-3 VLCPUFAs are present in restricted mammalian organs such as retina, brain, testes, and thymus. Omega-3 VLCPUFAs, which are not present in normal human diet, can be synthesized from DPA through the consecutive enzymatic activities of elongases and D6- and D5-desaturases. More precisely, DPA is known to be the metabolically active precursor for the synthesis of 24:5 ω-3, the most abundant omega-3 VLCPUFA in the retina.[45] Its synthesis is an important metabolic step in the retina because 24:5 ω-3 plays a central role as a metabolic precursor in the synthesis of other omega-3 VLCPUFAs and is an obligatory intermediate in the synthesis of DHA.[46] Although identified early, the precise role of omega-3 VLCPUFAs has not been yet elucidated because of their great lengths and minor abundance, which makes them difficult to analyze. However, alterations in their biosynthesis have been shown to result in macular alteration. In particular, defects in the elongation of the very long chain fatty acids 4 (*ELOVL4*) gene are associated with dominant Stargardt macular dystrophy.[47] Recently, decreases in DPA, DHA, and some omega-3 VLCPUFAs (notably 24:5 ω-3) have been observed in early and intermediate AMD retinas compared with age-matched control retinas,[45] suggesting retinal vulnerability associated with decreased levels of omega-3 LCPUFAs and VLCPUFAs.

Imbalance between omega-6 and omega-3 LCPUFAs has also emerged as a potential risk factor for AMD.[45,48] Total plasma phospholipid omega-6 LCPUFAs did not display any association with MPOD measurements (Table 3). However, when the different omega-6 types were detailed, we observed that two minor omega-6 LCPUFAs, eicosadienoic acid (20:2 ω-6) and dihomo-γ-linolenic acid (DGLA; 20:3 ω-6), exhibited a negative relationship with MPOD (Table 3). In plasma, DGLA is almost exclusively localized in phospholipids and represents approximately 20% of omega-6 LCPUFAs. In the retina, DGLA accounts for $<$2.5% of total retinal fatty acids. DGLA is metabolized from linoleic acid through γ-linolenic acid (18:3 ω-6) and is further converted to arachidonic acid (20:4 ω-6). DGLA has been reported to have a notably anti-inflammatory action and to be a substrate for the production of eicosanoids, which are generally viewed as having anti-inflammatory properties that counteract the synthesis of proinflammatory and vasoconstrictive mediators derived from 20:4 ω-6.[49] Concerning eicosadienoic acid (20:2 ω-6), data about its potential role in human health are scarce. Eicosadienoic acid is a relatively minor metabolite of linoleic acid (LA; 18:2 ω-6) found in human plasma and red blood cells.[50] It has been suggested that LA, in some physiological (aging) and pathologic (diabetes) situations, could be metabolized through a route other than the PUFA desaturase-elongase pathway to form eicosadienoic acid. A recent in vitro study[51] reported a possible role of eicosadienoic acid or its metabolites (among them DGLA) in the modulation of the inflammatory response. Because inflammation is postulated to be involved in AMD, the negative relationship of plasma DGLA and eicosadienoic acid with MPOD we observed may suggest a reduced risk for AMD by metabolic use of these omega-6 LCPUFAs before modulation of the inflammatory status for the synthesis of eicosanoids with anti-inflammatory properties in the retina.

In conclusion, in the present study, macular pigment density was associated not only with plasma lutein and zeaxanthin but also with plasma phospholipid omega-3 LCPUFAs, particularly EPA and DPA. Further studies will be needed to confirm these findings and to identify the underlying mechanisms. Our results moreover suggest that xanthophylls and omega-3 PUFAs may act synergistically in the constitution of macular pigment. This may represent an additional motive for supplementation with both xanthophylls and omega-3 PUFAs for protection against AMD. Such supplementation is being tested in the ongoing Age-Related Eye Diseases Study 2 (www.areds2.org), which will give important insights into potential reduction in the incidence of AMD with supplementation with xanthophylls and omega-3 LCPUFAs.

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