Differential composition of DHA and very-long-chain PUFAs in rod and cone photoreceptors

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Abstract Long-chain PUFAs (LC-PUFAs; C20–C22; e.g., DHA and arachidonic acid) are highly enriched in vertebrate retina, where they are elongated to very-long-chain PUFAs (VLC-PUFAs; C ≥28) by the elongation of very-long-chain fatty acids-4 (ELOVL4) enzyme. These fatty acids play essential roles in modulating neuronal function and health. The relevance of different lipid requirements in rods and cones to disease processes, such as age-related macular degeneration, however, remains unclear. To better understand the role of LC-PUFAs and VLC-PUFAs in the retina, we investigated the lipid compositions of whole retinas or photoreceptor outer segment (OS) membranes in rodents with rod- or cone-dominant retinas. We analyzed fatty acid methyl esters and the molecular species of glycerophospholipids (phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine) by GC-MS/GC-flame ionization detection and ESI-MS/MS, respectively. We found that whole retinas and OS membranes in rod-dominant animals compared with cone-dominant animals had higher amounts of LC-PUFAs and VLC-PUFAs. Compared with those of rod-dominant animals, retinas and OS membranes from cone-dominant animals also had about 2-fold lower levels of di-DHA (22:6/22:6) molecular species of glycerophospholipids. Because PUFAs are necessary for optimal G protein-coupled receptor signaling in rods, these findings suggest that cones may not have the same lipid requirements as rods.—Agbaga, M-P., D. K. Merriman, R. S. Brush, T. A. Lydic, S. M. Conley, M. I. Naash, S. Jackson, A. S. Woods, G. E. Reid, J. V. Busik, and R. E. Anderson. Differential composition of DHA and very-long-chain PUFAs in rod and cone photoreceptors. J. Lipid Res. 2018. 59: 1586–1596.

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DHA (22:6n3) and arachidonic acid (AA; 20:4n6) are the major long-chain PUFAs (LC-PUFAs) in the retina and rod outer segment (ROS) membranes of all vertebrate species examined thus far (1, 2). DHA is also highly enriched in some, but not all, invertebrate retinas (3, 4). Both DHA and AA are obtained either from the diet or synthesized from dietary essential fatty acids [α-linolenic acid (18:3n3) and linoleic acid (18:2n6), respectively]. DHA is essential for normal development of rod function in the rat retina, first demonstrated by our laboratory in 1973 and 1975 (5, 6). In these studies, rats deficient in PUFAs had reduced electroretinographic responses (5). Feeding diets containing

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Abbreviations: AA, arachidonic acid; AMD, age-related macular degeneration; ROS, cone outer segment; ELOVL4, elongation of very-long-chain fatty acids-4; FAME, fatty acid methyl ester; LC-PUFA, long-chain PUFA; NPD1, neuroprotectin D1; Nrl, neural leucine zipper transcription; OS, outer segment; GC, glycerophosphatidylcholine; PE, glycerophosphatidylethanolamine; PS, glycerophosphatidylserine; ROS, rod outer segment; RPE, retinal pigment epithelium; STGD3, Stargardt-like macular dystrophy; TLGS, thirteen-lined ground squirrel; VLC-PUFA, very-long-chain PUFA.

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and AA are essential for normal brain and retina development. The Bernstein group also reported significantly lower VLC-PUFAs in the human retina, and found that the macula had less DHA than the periphery. Their analyses did not include VLC-PUFAs and PUFAs (15, 16). Our laboratory cloned and expressed ELOVL4 and identified its products as saturated fatty acids and PUFAs of 28 carbons or greater in length (17, 18). We subsequently showed that the STGD3 mutant protein did not have enzymatic activity and exerted a dominant negative effect on the activity of the WT enzyme in vitro (19, 20), consistent with reports that the mutant formed dimers with the WT protein (21–23).

STGD3 is a juvenile-onset macular dystrophy that primarily affects the cones in the macula of the retina, starting as early as the first decade of life (24, 25). Because the mutation is in an enzyme that utilizes LC-PUFA precursors to make VLC-PUFAs, there is considerable interest in knowing the size and nature of the precursor pool in human cones and the VLC-PUFA products generated from them. Van Kuijk and Buck (26) were the first to compare the fatty acid compositions of peripheral and macular regions of the human retina, and found that the macula had less DHA than the periphery. Their analyses did not include VLC-PUFAs. More recently, Bernstein’s group showed that while there were no age-related changes in DHA in total lipids from whole human retinas, there was a significant reduction in DHA in retinas from age-matched patients with age-related macular degeneration (AMD) (27). Interestingly, there was no change in AA in these same retinas. The Bernstein group also reported significantly lower VLC-PUFAs in the whole retina lipids compared with age-matched control values (27). In a later study, using GC-MS technology to analyze the lipids in 4 mm trephine retinal punches, the Bernstein group found that human macula lipids contained less VLC-PUFAs than peripheral lipids, although the levels of DHA were the same (28). An interesting finding from the Bernstein study was that mouse retinas contain almost 10 times the level of VLC-PUFAs and almost twice the level of DHA as do normal human retinas.

Given the differences in LC-PUFAs and VLC-PUFAs reported for peripheral versus central normal human retinas (26, 27), we undertook a systematic comparative analysis of lipids from several species of rodents whose retinas either contained a preponderance of cone photoreceptors or had been genetically manipulated to express cone-like photoreceptors. These rodents included mice with a global knock-out of the neural leucine zipper transcription (Nrl), which results in a nocturnal retina containing entirely cone-like photoreceptors and no rod photoreceptors, as previously characterized (29–32). Thirteen-lined ground squirrels (TLGSs) (Ictidomys tridecemlineatus), tree squirrels (Sciurus niger), and tree shrews (Tupaia belangeri) are visually guided cone-dominant diurnal mammals with photoreceptor distributions similar to the cone-rich foveal region within the primate macula (33). We performed comprehensive glycerophospholipid analyses on these rod- and cone-dominant retinas to define and compare their lipid compositions, which may account for the differences in their function. Here, we show that outer segment (OS) membranes from cone-dominant retinas have much lower levels of PUFAs and VLC-PUFAs than do rod-dominant retinas.

MATERIALS AND METHODS

Animal care and preparation of OS membranes

Nrl−/− mice were generously provided by Anand Swaroop, PhD (National Eye Institute, Bethesda, MD) and were housed and bred at the Dean McGee Eye Institute and the University of Oklahoma Animal Research Facility vivarium on 12 h light:12 h dark cycle. The Nrl−/− mice were bred with C57BL/6J mice purchased from Jackson Laboratories (Bar Harbor, ME) to generate Nrl−/− and WT C57BL/6J controls. Because the retinas of Nrl−/− mice undergo age-related retinal structural changes by about three months of age (29, 34), our studies were carried out in 1-month-old WT, Nrl−/−, and Nrl−/− animals. Sprague Dawley rats were raised at the Dean McGee Eye Institute vivarium and TLGSs (I. tridecemlineatus) were raised at the University of Wisconsin Oshkosh (Oshkosh, WI). Tree squirrels (S. niger) were obtained from local licensed trappers. Tree shrews were bred and raised at the Max Planck Florida Institute, Jupiter, FL, by Rebekah Corlew, PhD, who generously provided us with retinal tissues. All animals were handled and euthanized according to the guidelines outlined in the Statement for the Use of Animals in Ophthalmic and Vision Research, and the protocols were approved by the University of Oklahoma Health Sciences Center IACUC.

Prior to preparation of OS membranes, animals were dark-adapted overnight. Retinas were dissected under dim red light, flash-frozen in liquid nitrogen, and stored at −80°C until they were used. Cone OSs (COs) or ROs, collectively referred to as photoreceptor OSs, were prepared by discontinuous sucrose gradient ultracentrifugation according to protocols previously published (35, 36). Two interfaces were collected: the top band containing OS-enriched membranes (band I), the second band (band II) containing mostly broken rods and mitochondria, and the pellet at the bottom of the tube containing nuclei, melanin granules, and other non-OS membranes. The three preparations were washed with Krebs-Ringer phosphate buffer (pH 7.4) and the membranes were pelleted by centrifugation at 45,145 g for

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10 min (37). All procedures were carried out at 4°C under ambient room light.

**Retinal OS membrane preparation and validation**

An aliquot of membranes from each fraction was taken for protein determination by quantitative BCA assay in order to use equal amounts of membrane fractions for lipid and fatty acid analyses. For Western blotting, the membranes were lysed in a lysis buffer containing 20 mM Tris-HCl (pH 7.4), 100 mM NaCl, 1 mM EDTA, EDTA-free protease inhibitor tablets (Roche Applied Sciences, Indianapolis, IN), and 1% Triton X-100 (Sigma-Aldrich, St. Louis, MO). After brief sonication on ice, the lysates were incubated with gentle rocking at 4°C for 1 h, and centrifuged at 27,000 g for 30 min at 4°C. Supernatants were collected and protein concentrations determined using the Pierce BCA assay (Thermo Fisher Scientific, Rockford, IL). Equal amounts of protein (5 μg) were separated by SDS/PAGE on 12% polyacrylamide gels, followed by either silver staining or electro-transfer to nitrocellulose membranes. The membranes were blocked with 5% nonfat dry milk and incubated with rhodopsin antibodies 1D4 (1:3,000), a gift from Dr. James McGinnis (Department of Ophthalmology, University of Oklahoma Health Sciences Center, Oklahoma City, OK). Membranes were stripped and reprobed with M- and S-opsin antibodies (Santa Cruz Biotechnology, Inc., Dallas, TX) at 1:1,000, and then with monoclonal antibody against β-actin (Sigma-Aldrich). Immunoreactivity of horseradish peroxidase-conjugated donkey anti-rabbit or goat anti-mouse IgG secondary antibodies (GE Healthcare Bio-Sciences, Pittsburgh, PA) was detected using Super-Signal West Dura extended duration substrate (Thermo Fisher Scientific).<ref>

**Lipid extraction and fatty acid analysis**

Total lipids were extracted from 100 μg protein of the three retinal membrane fractions, 100 μl of plasma, or whole retinas following the method of Bligh and Dyer (38) with minor modifications (39). The tubes were sealed under N2 gas and then set at 100°C overnight. The tubes were cooled on ice and fatty acid methyl esters (FAMEs) were extracted from lipids and derivatized using the Pierce BCA assay (Thermo Fisher Scientific, San Jose, CA) operating at a resolution of 100,000 (at m/z 400) using a chip-based nano-ESI source (Advion NanoMate, Ithaca, NY) operating in infusion mode. PC, PE, and PS molecular species were determined by high-resolution MS and MS/MS using accurate mass measurements in positive ion mode (40). Quantification of lipid molecular species was performed using the Lipid Mass Spectrum Analysis (LIMSA) software’s peak model fit algorithm (41–45).

**Statistical analysis**

Statistical analyses were performed using GraphPad Prism 5 software. Results are expressed as the mean ± SD. All relative mole percentages of n3 and n6 LC-PUFAs and VLC-PUFAs were determined by comparison to internal standards. The mean ± SD of the relative mole percentages of n3 and n6 LC-PUFAs and VLC-PUFAs were found to be 100 μg ± 2.0 μg of protein. For all figures, significant differences are indicated by P < 0.05, while n.s. indicates no significant difference at P > 0.05, using a multivariate ANOVA with Neuman-Keuls posthoc test or Bonferroni’s multiple comparisons test.

**RESULTS**

**Enrichment and purity of OS membrane preparations**

To determine the enrichment and purity of OS membrane preparations, equal amounts of protein of OS membranes from WT mouse (C57B6/J), Nrl−/− mouse, Nrl−/− mouse, rat, and T LGS retinas were resolved on SDS-PAGE, as described by Laemmli (44), and either stained with silver nitrate or immunoblotted with anti-opsin, anti-rod transducin, anti-M-opsin, or anti-actin antibodies (Fig. 1A, B). Band I, representing enriched OS membranes from WT and Nrl−/− retinas, showed prominent staining in the regions generally accepted for rhodopsin and rhodopsin polymers. This staining was absent in the Nrl−/− OS membranes. Similar staining patterns were found for band II, which represented broken OS and inner segment membranes, although the amounts of rhodopsin were less. There was very little staining for rhodopsin in the pellet membranes. M-opsin was present in the OS membranes from the three groups of mice, showing that all three had the visual pigment found in mouse cones. Rod transducin and rod transducin were
found only in OS preparations from WT and Nrl/− retinas, indicating that the Nrl/− OS membranes did not contain these two prominent components of ROSs. OS preparations from TLGS retinas were also examined by Western blot (Fig. 1B). We did not detect significant rhodopsin labeling from the all-cone TLGS OS membranes, which was expected due to the cone-dominant nature of the TLGS retina. However, it is possible that the 1D4 antibody does not recognize TLGS rhodopsin. Rat ROSs also contained a small amount of M-opsin, which was present along with S-opsin in OS membranes from TLGS.

Characterization and quantification of fatty acids in cone-dominant versus rod-dominant retinas

We determined the fatty acid composition of whole retina lipids from rod-dominant WT and Nrl/− mice, relative to cone-dominant retinas of Nrl/− mice and TLGSs. To our surprise, whole retina fatty acids from Nrl/− mice and TLGS yielded 23.6 and 15.2 relative mole percent of DHA (22:6n3), respectively, which is significantly lower than the 34% 22:6n3 content of WT and Nrl/− retinas (Fig. 2). The reduced 22:6n3 in cone-dominant retinas was accompanied by a relative increase in stearic acid (18:0), oleic acid (18:1), and AA (20:4n6) (Fig. 2). ROS membranes are known to be enriched in 22:6n3, which has been shown to modulate rod phototransduction processes. Hence, we analyzed FAMEs prepared from equal amounts of retinal OS membranes from WT, Nrl/−, Nrl/+ and TLGSs (Fig. 3). Consistent with the whole-retina results (Fig. 2), 22:6n3 was the most abundant fatty acid in WT and Nrl/+ OS membranes (Fig. 3), which showed no statistically significant differences in their fatty acid composition. However, OS membranes from the Nrl/− retinas had significantly lower levels of 22:6n3, with compensatory increases in 18:0, 18:1, and 20:4n6. The OS membranes from the cone-dominant TLGS retinas had even lower 22:6n3 levels than did the Nrl/− OS membranes (Fig. 3). Compared with the rod-dominant retinas, the cone-dominant retinas also had slightly more adrenic acid (22:4n6) without any significant enrichment in n6 docosapentaenoic acid (22:5n6), which is usually increased in pathological n3 fatty acid deficiency (45–49). This further confirmed our findings that cone photoreceptors indeed have a different lipid profile from...
rod photoreceptors, and this difference is not an artifact of captivity.

Composition of retinal VLC-PUFAs in rod-dominant versus cone-dominant retinas

Having identified differences in LC-PUFAs, we next compared the relative composition of VLC-PUFAs in rod-dominant retinas to that of cone-dominant retinas. In total, the sum of VLC-PUFAs was significantly less in Nrl−/− retinas when compared with whole retinas from WT and Nrl+/− mice (Fig. 4). The major VLC-PUFAs present in the mouse retina membranes included 32:6n3, 34:5n3, 34:6n3, and 36:6n3, all of which were similar in WT and Nrl+/− retinas, but significantly lower in Nrl−/− retinas, with the exception of 36:5n3 and 36:6n3 (Fig. 4). Because VLC-PUFAs are elongated products of C20–C26 n3 and n6 PUFAs, reduced levels of these PUFAs in the retina are thus consistent with lower levels of VLC-PUFAs. To determine whether reduced levels of 22:6n3 in the TLGSs are intrinsic to cone photoreceptors, or whether they are due to influence from laboratory diets, we also analyzed the fatty acid composition of total retinas and OS membrane preparations collected directly from wild TLGSs caught near the University of Wisconsin, Oshkosh. We found that the relative mole percent of 22:6n3 in the TLGS retinas, whether captive-bred or wild-caught, was 12–14% compared with 33–35% of 22:6n3 in Nrl−/− and WT mouse retinas. This suggests that reduced levels of retinal 22:6n3 and VLC-PUFAs in cone-dominant retinas are a function of cone retinal physiology and metabolism, rather than reduced amounts of plasma VLC-PUFA precursors.

Glycerophospholipid analysis and fatty acid composition of cone-dominant retinas

We next determined the molecular species composition of the three major phospholipids, PC, PE, and PS, in rod- and cone-dominant retinas. We compared nocturnal rod-dominant mouse and rat retinas with those of diurnal cone-dominant TLGSs, tree squirrels, and tree shrews using MS/MS. Within the mouse strains (WT, Nrl−/−, and Nrl+/−), there was a significant reduction between WT and Nrl−/− mice in PC, PE, and PS species containing two molecules of 22:6n3 (PC-, PE-, PS-22:6/22:6), while there was no difference between the WT and Nrl+/− retinas (Figs. 5, 6A, B; 7A, B). These findings support the fatty acid data that indicate reduced levels of 22:6n3 in cone-dominant Nrl−/− mice. Within retinal dipoly-22:6n3-PC and VLC-PUFA-PC...
species, we observed a significant reduction in PC-44:12 (22:6/22:6), PC-54:12 (32:6/22:6), PC-56:12 (34:6/22:6), and PC-56:11 (34:5/22:6) in Nrl<sup>−/−</sup> retinas compared with WT and Nrl<sup>+/−</sup> retinas (Fig. 5). PC-56:12 (34:6/22:6) is the most abundant VLC-PUFA-PC in the mouse retina, followed by PC-54:12 (32:6/22:6), PC-58:12 (36:6/22:6), and PC-56:11 (34:5/22:6). We further confirmed these results in OS preparations in which we found a significant reduction in 32:6/22:6, 34:5/22:6, and 34:6/22:6 PC in Nrl<sup>−/−</sup> versus WT and Nrl<sup>+/−</sup> retinas. Interestingly, there was no difference in PC-58:12 (36:6/22:6) between the three genotypes. Collectively, however, there was a significant reduction in the sum of retinal VLC-PUFA-PC in Nrl<sup>−/−</sup> mice compared with WT and Nrl<sup>+/−</sup> mice (Fig. 5). To determine whether the decreased 22:6n3/22:6n3 in PC could be accounted for in the other glycerophospholipid species, we analyzed the composition of retinal PE (Fig. 6A, B) and PS (Fig. 7A, B). Similar to the PC data, both PE and PS species had decreased levels of 22:6/22:6 in the cone-dominant retina compared with the WT and Nrl<sup>−/−</sup> retinas (Figs. 6A, B; 7A, B). The PE and PS species did not contain any VLC-PUFAs.

Fig. 5. Comparison of di-poly-DHA-PC and VLC-PUFA-PC in Nrl<sup>−/−</sup>, Nrl<sup>+/−</sup>, and WT retina. Consistent with decreased retinal DHA and VLC-PUFAs, di-poly-DHA-PC and VLC-PUFA-PC and the sum of retinal VLC-PUFA-PC are significantly decreased in Nrl<sup>−/−</sup> retina relative to Nrl<sup>+/−</sup> and WT retinal VLC-PUFA-PC. The most abundant VLC-PUFA-PC species in the Nrl<sup>−/−</sup> and WT retina include 32:6/22:6, 34:5/22:6, 34:6/22:6, and 36:6/22:6. Statistically significant differences are indicated as: AWT versus Nrl<sup>+/−</sup>; BWT versus Nrl<sup>−/−</sup>; and DNrl<sup>+/−</sup> versus Nrl<sup>−/−</sup>; P < 0.05 to P < 0.001; mean ± SD.

Fig. 6. Retinal LC-PUFA-PE levels in rod-dominant retinas compared with cone-dominant retinas. The most abundant LC-PUFA-PE in both rod- and cone-rich retinas was 18:0/22:6 (A, B). The 22:6/22:6-PUFA-PE is significantly decreased in cone-dominant retinas compared with rod-dominant WT and Nrl<sup>−/−</sup> retinas (A). However, among the cone-rich retinas, there was no significant difference in 22:6/22:6-PUFA-PE levels. No VLC-PUFA-PEs were detected. Statistically significant differences are indicated as: AWT versus Nrl<sup>−/−</sup>; BWT versus Nrl<sup>−/−</sup>; DWT versus Nrl<sup>−/−</sup>; ENrl<sup>+/−</sup> versus Nrl<sup>−/−</sup>; F Nrl<sup>−/−</sup> versus TLGS; GNrl<sup>−/−</sup> versus tree squirrel; HNrl<sup>−/−</sup> versus tree shrew; 1tree squirrel versus TLGS; 1tree squirrel versus tree shrew; and 6TLGS versus tree shrew; P < 0.05 to P < 0.001; mean ± SD.
Statistically significant differences are indicated as: BWT versus nas, without any differences detected in the cone-rich retina, except compared with WT and 44:12, PS-46:12 (Figs. 5, 6, 7), and VLC-PUFA-PC (Fig. 5) had significantly lower levels of PC-44:12, PE-44:12, PS-46:12, and PC-46:12 (P < 0.05) than did rod-dominant retinas. The 18:0/22:6-PS presents the major PS in both rod- and cone-rich retinas (A, B). Consistent with PC and PE data, 22:6/22:6-PS is significantly decreased in the cone-dominant retinas compared with rod-dominant WT and Nrl−/− retinas, without any differences detected in the cone-rich retina, except for Nrl−/− and TLGS that were significantly different at P < 0.05. Statistically significant differences are indicated as: WT versus Nrl−/−; 1WT versus Nrl−/−; 2Nrl−/− versus WT; 3Nrl−/− versus TLGS; 4Nrl−/− versus tree squirrel; 5Nrl−/− versus tree shrew; 6tree squirrel versus TLGS; 7tree squirrel versus tree shrew; and 8TLGS versus tree shrew; P < 0.05 to P < 0.001; mean ± SD.

Based on the finding that cone-dominant Nrl−/− retinas had significantly lower levels of PC-44:12, PE-44:12, PS-44:12, and VLC-PUFA-PC (Fig. 5) compared with WT and Nrl−/− retinas, we determined the levels of these molecular species in retinas from Sprague-Dawley rats, TLGSs, tree squirrels, and tree shrews. Between these four species, as well as Nrl−/−, there were no significant differences in levels of PC-32:0 (16:0/16:0), PC-34:01 (16:0/18:1), PC-34:04 (16:0/20:4), PC-36:01 (18:0/18:1), and PC-38:04 (18:0/20:4) (data not shown). This finding suggests that, biochemically, cone-dominant retinas have reduced levels of 22:6n3 diacyl-glycerophospholipids. Similarly, there was a significant decrease in PE-44:12 and PS-44:12 in the TLGS, tree squirrel, and tree shrew retinas (Figs. 6B, 7B) when compared with rod-dominant rat retinas (Figs. 6A, 7A). Furthermore, within the cone-dominant retinas, VLC-PUFA-PC classes were significantly reduced compared with rat retina (Fig. 8). While PC-54:12 and PC-56:12 were enriched in the rat retinas, they were significantly reduced in the squirrel retinas, with the tree shrew retinas having higher levels than the squirrel retinas (Fig. 8). Similarly, PC-54:11, PC-54:10, and PC-56:11 were enriched in rat retinas compared with cone-dominant retinas (Fig. 8). Consistent with the mouse retina data, PC-58:12 was enriched in the cone-dominant retinas (Fig. 8).

**DISCUSSION**

Increasing life expectancy has brought in its wake increasing numbers of people with various aging diseases, including vision loss due to AMD, diabetic retinopathy, glaucoma, and cataracts. Multiple experimental, epidemiological, and clinical trial results have indicated that dietary n3 PUFAs might attenuate AMD progression (50-56). Reports from experimental studies have shown that n3 PUFAs reduce the risk of diabetic retinopathy through inhibition of retinal vascular damage, enhancing the reparative function of endothelial progenitor cells and reducing the expression of inflammatory markers (57-61). The Blue Mountains Eye Study reported an association between reduced incidence of nuclear cataracts with higher dietary intake of n3 PUFAs (62). However, the recent AREDS2 and the Nutritional AMD Treatments-2 studies surprisingly reported that dietary n3 PUFAs had no beneficial effect on the outcome of AMD (63). To gain a better understanding of a possible biochemical basis for these confounding results, we compared the fatty acid and molecular species compositions of lipids from whole retinas and OS membranes from rod- and cone-rich retinas.

The most significant findings of the present study are that cone-dominant retinas and OS membranes have about 4-fold less 22:6n3 than do rod-dominant retinas and that PC molecular species containing VLC-PUFAs are significantly less abundant in cone-rich retinas and their OS membranes. Our data thus provide support for previous studies that reported less 22:6n3 and VLC-PUFAs in human macula than in the peripheral retina (26, 28). Interestingly, our results contrast with those reported in Nile rats (Arvicanthis ansorgei, 33% cones), which had 14% higher levels of 22:6n3 compared with Norwegian rats (Rattus norvegicus, 2-3% cones) (64). Our data, however, are consistent with the reported average 15.9 relative mole percent of 22:6n3 found in human macula (26), as well as low 22:6n3 levels in cone-rich chicken retinas (65). Our findings are also different from a previous report that found high percentages of DHA in PC and PS (55% and 63%, respectively) in the P521 cone-rich retinas of Tokay geckos (Gekko gecko) (66). Nevertheless, for mammals, it seems safe to conclude that COS membranes contain lower levels of DHA than do ROS membranes. We also report significantly
lower levels of retinal and OS VLC-PUFAs in cone-dominant retinas than in rod-dominant retinas, which is consistent with recent reports by Liu et al. (28) that the total VLC-PUFAs and the n3/n6 ratio of VLC-PUFAs in human retinas were significantly higher in rod-enriched peripheral retina, compared with the cone-rich macula. While Liu et al. (28) attributed these differences to the possibility of lipids within the relatively thick inner retina overwhelming detection of VLC-PUFAs within the macula, our studies suggest that cone photoreceptors intrinsically have less 22:6n3 and VLC-PUFAs than do rods.

DHA plays essential roles in phototransduction and protection of photoreceptor cells against cellular insults, such that DHA depletion affects retinal function. Within the retina, G protein-coupled signal transduction is essential for sensory processing and visual perception, which are compromised by 22:6n3 deficiency, which occurs in rats raised on an n3-deficient diet (45, 48). Previous studies demonstrate that rats raised on an n3-deficient diet have an almost stoichiometric replacement of 22:6n3 by 22:5n6 in the retina (45, 48, 67). However, the replacement of 22:6n3 with 22:5n6 in ROS membranes of rats results in functional deficiencies in the photoreceptor signal transduction pathway (68). In contrast, DHA-rich phospholipids optimize photoreceptor signaling, which underscores the importance of DHA in neural G protein-coupled receptor signaling. The cone-dominant retinas do not have increased 22:5n6, which demonstrates that reduction of DHA in COSs is not the result of a limited supply of 22:6n3 to these cells. Rather, the reduced amounts of DHA and VLC-PUFAs suggest that the biophysical lipid requirement for these fatty acids in visual function in cone photoreceptor cells is intrinsically different from rod photoreceptors.

The daily shedding of DHA- and VLC-PUFA-enriched OS membranes and subsequent phagocytic activity of retinal pigment epithelium (RPE) cells result in recycling and conservation of 22:6n3 between photoreceptor cells and the RPE cells (69–73). Phospholipase activity on membrane phospholipids of photoreceptor and RPE cells leads to release of free fatty acids, including DHA, AA, and VLC-PUFAs, some of which are converted to bioactive lipids that mediate further intracellular signal transduction and cytoprotection of photoreceptors from cellular stress (71, 74). Bazan’s laboratory has reported that 22:6n3 can be converted by the RPE into the bioactive neuroprotective docosanoid, neuroprotecin D1 (NPD1; 10R,17S-dihydroxy-docosapentaenoic acid) in response to oxidative and other stress (74). NPD1 and other bioactive docosanoids synthesized in RPE and photoreceptor cells on demand act as potent anti-apoptotic and anti-inflammatory molecules that preserve retinal integrity in response to cellular damage (75, 76). Apart from acting as signaling molecules, DHA-derived docosanoids, such as the d-series resolvins (RvD1 and RvD2) and maresins, as well as NPD1, play essential homeostasis roles by promoting cellular repair and phagocytosis, which are essential for maintaining the structure and integrity of postmitotic cells such as retinal neurons. More recently, the Bazan laboratory also showed that ELOVL4-derived VLC-PUFAs 32:5n3 and 34:6n3 (17, 18, 77) can be converted by the RPE into neuroprotective molecules coined “elovanoids” (78). Because these precursors of neuroprotective lipids are less abundant in the OSs of cone-dominant animals and in the macula of the human retina (27, 28, 79), and because their reduction in the human macula is age-dependent (26, 27), we suggest that with age there may be a compromised supply of docosanoids and elovanoids in the central retina, which makes the RPE and, subsequently, the underlying photoreceptors more susceptible to stress-induced cell death. Our earlier studies (80) demonstrated an age-dependent increase in the susceptibility of the human macula to oxidative stress and an increase in lipid peroxidation compared with peripheral retina.

The higher n6/n3 ratios in cone photoreceptor cells could, with age, potentially tilt the balance toward pro-inflammatory AA-derived eicosanoids within macular RPE cells, thereby increasing inflammation-induced AMD progression. This reasoning is supported by studies by Liu et al.
(27) that showed that DHA and VLC-PUFA abundance in the retina and RPE/choroid peaks in middle age and decreases with age and that, when compared with normal age-matched human donors, there is a significant decrease in DHA and VLC-PUFAs in the AMD retina and RPE/choroid accompanied by increased n6/n3 PUFA ratios. Thus, it is possible that aging and epigenetic and inherited genetic factors that affect DHA and VLC-PUFA biosynthesis, such as in STGD3 (12, 13, 81, 82), contribute to the exacerbation of macular degeneration.

CONCLUSIONS

Compared with well-characterized rod-dominant retinas, cone-dominant retinas have significantly less 22:6n3 and VLC-PUFAs in whole retinas and OS membranes. Because 22:6n3 is necessary for optimal G protein-coupled receptor signaling in rods, these findings suggest that cones and cone signaling may not have the same lipid requirements as rods. Moreover, our studies confirm the differences in LC-PUFAs found in human macula relative to the peripheral retina. While this study was done on rod data, the support decreased DHA and VLC-PUFAs found in human macula. AREDS2 results showed that DHA supplementation is not able to attenuate progression of macular degeneration. Our data show that cone photoreceptors intrinsically have low levels of DHA, which suggests that perhaps increasing cone DHA levels after onset of retinal degeneration will not provide therapeutic benefits. On the other hand, it might also suggest that cone photoreceptors do not readily take up and retain DHA as do rod photoreceptors, so dietary DHA is not able to influence cone photoreceptor DHA levels. We propose that genetic and epigenetic modification coupled with age-related reduction in macular 22:6n3 and VLC-PUFAs (which are precursors of neuroprotective bioactive lipids that are synthesized in the RPE) increase the susceptibility of the macula to age-related RPE and photoreceptor cell degeneration. Hence, an increase in the dietary intake of LC-PUFAs and VLC-PUFAs early in life would maintain retinal PUFA and PUFA-derived neuroprotective bioactive lipid levels that are beneficial for visual function.

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