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An invited review following *the Soujinkai Award*: Cytochrome P450—Generated Metabolites of ω-3 Fatty Acids Ameliorate Choroidal Neovascularization

Ryoji Yanai,[1,2] *Koh-Hei Sonoda*[1] *and Kip M. Connor*[2]

[1] Department of Ophthalmology, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan
[2] Department of Ophthalmology, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA 02114, USA
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Correspondence to Ryoji Yanai, M.D., Ph.D. E-mail: yanai@yamaguchi-u.ac.jp

Abstract Age-related macular degeneration (AMD) is the primary cause of blindness in elderly individuals of industrialized countries, with a projected 50% increase in prevalence by 2020. There is an urgent need for new nutritional or pharmacological interventions that are safe over the long term for the treatment or prevention of AMD. Prospective clinical studies have suggested that dietary intake of ω-3 long-chain polyunsaturated fatty acids (LCPUFAs) may protect against AMD. We recently characterized a mechanism by which dietary ω-3 LCPUFAs promote regression of choroidal neovessels in a well-characterized mouse model of neovascular AMD. The concentrations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were increased in serum of mice fed a diet enriched in these ω-3 LCPUFAs. Cytochrome P450 enzymes catalyze the epoxidation of these primary ω-3 LCPUFAs to form 17,18-epoxyeicosatetraenoic acid and 19,20-epoxydocosapentaenoic acid, respectively, and intraperitoneal injection of these epoxyeicosanoids mimicked the beneficial effects of dietary ω-3 LCPUFAs on CNV resolution. Dietary intake of ω-3 LCPUFAs also suppressed leukocyte recruitment to CNV lesions by down-regulating endothelial expression of the adhesion molecules ICAM-1 and E-selectin as well as leukocyte expression of the ICAM-1 ligands CD11b and CD18. Bioactive lipid metabolites derived from ω-3 LCPUFAs thus show potential for the treatment or prevention of AMD.

Key words: ω-3 long-chain polyunsaturated fatty acids, cytochrome P450, choroidal neovascularization, immune cell recruitment

Introduction

The omega-3 long-chain polyunsaturated fatty acids (ω-3 LCPUFAs) are a class of dietary lipids that are highly enriched in the central nervous system and retina. The major dietary ω-3 LCPUFAs are docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and mammals depend on dietary intake of these fatty acids because they lack the enzymes required for their de novo synthesis. The ω-3 LCPUFAs possess anti-inflammatory properties, and they compete with ω-6 LCPUFAs as sources for the synthesis of lipid metabolites such as prostaglandins and leukotrienes as well as those generated by cytochrome P450 (CYP) isoforms. In this review, we focus on the suppressive effect of dietary supplementation with ω-3 LCPUFAs on choroidal neovascularization (CNV) in a mouse model of age-related macular degeneration (AMD), a leading cause of blindness, as well as on the mechanism of this effect.

AMD and its current treatment

Advanced AMD is characterized as "atrophic" or "neovascular," the former showing loss of outer retinal layers, and the latter the presence of CNV.[1,2] In neovascular AMD, the abnormal blood vessels grow from the choroidal vasculature, through breaks in Bruch's membrane, toward the outer retina.[1] These blood vessels are immature in nature and leak fluid below or within the retina. The two forms of AMD can occur together and share pathologies of cell death and fibroglial replacement.[3,4] The neovascular form accounts for 10% to 15% of AMD cases, develops abruptly, and rapidly leads to substantial loss of vision.[1] Although growth factors appear to play an important role in the late stage of neovascular AMD progression, they likely do not contribute to the underlying cause of the disease. The current standard of care for individuals with CNV is based on the targeting of vascular endothelial growth factor (VEGF),[5-7] which promotes angiogenesis and increases vascular permeability. However, although anti-VEGF therapy attenuates vascular permeability and angiogenesis, it does not lead to complete vascular regression.[6] Moreover, a substantial improvement in vision occurs in only about one-third of patients treated with VEGF antagonists, with one-sixth of such treated patients still progressing to legal blindness.[5,7] There is thus an urgent need for safe nutritional or pharmacological interventions for the treatment or, ideally, the prevention of AMD.

ω-3 LCPUFAs and AMD

The ω-3 and ω-6 LCPUFAs are two classes of dietary lipids that are highly enriched in the retina and have opposing physiological effects. The ω-3 LCPUFAs have antithrombotic and anti-inflammatory properties, and they compete with ω-6 LCPUFAs as sources for eicosanoid synthesis at the CYP, cyclooxygenase, and lipoxygenase levels. Mammals lack the enzymes required for de novo synthesis of LCPUFAs, however. ω-6 LCPUFAs are the primary polyunsaturated fatty acids present in Western diets. Dietary enrichment with ω-3 LCPUFAs has been shown to be protective against pathological angiogenesis, which occurs in cancer and retinopathies.[8-12] Prospective clinical studies have suggested that dietary ω-3 LCPUFAs may also protect against AMD.[13,14] In a prospective cohort study of 1837 participants at moderate-to-high risk of developing advanced AMD, those who reported the highest intake of ω-3 LCPUFAs (median of 0.11% of total energy intake) were 30% less likely than their peers to develop the condition over a 12-year period.[15] In a follow-up study to the Age-Related Eye Disease Study (AREDS),[13,14] the U.S. National Institutes of Health is currently testing the effects of ω-3 LCPUFA supplements in >4000 subjects aged 50 to 85 years at increased risk of developing advanced neovascular AMD. However, two recent randomized, prospective, placebo-controlled clinical trials found no beneficial effect of ω-3 LCPUFA supplementation on late-stage AMD development.[16,17] The findings to date suggest that the bioavailability of ω-3 LCPUFAs provided as dietary supplements may differ among individuals, and that for those individuals who are unable to achieve elevated physiological levels of these fatty acids by taking such supplements may benefit from treatment with downstream bioactive lipid metabolites with regard to protection against neovascular AMD. Identification of the bioactive lipid metabolites with antiangiogenic and anti-inflammatory activities is therefore of substantial importance for such dietary nonresponders. We have pursued this goal with an animal model subject to strict dietary control.

Effects of dietary intake of ω-3 LCPUFAs in an AMD model

To investigate how dietary ω-3 LCPUFAs might prevent AMD, we characterized a pathway by which dietary intake of these fatty acids promotes resolution of choroidal neovessels in mice with laser-induced CNV, an animal model of neovascular AMD. To study the effects of ω-3 LCPUFAs on CNV, we fed mice one of two diets: a diet containing ω-3 LCPUFAs (1% DHA and 1% EPA, with no arachidonic acid [AA]), or a diet devoid of ω-3 LCPUFAs but containing an ω-6 LCPUFA (2% AA).[8] These diets were started 2 weeks before CNV induction. Given that the typical intake of ω-3 LCPUFAs in the United States (100 to 200 mg/day) provides ~0.05 to 0.1% of

total calories, the amount of these fatty acids in the experimental diet is physiological and attainable by patients. To evaluate the effects of LCPUFAs on the development of CNV, we first examined choroidal flat-mount preparations after lectin staining for blood vessels.[18] Lesion size at 5 days after photocoagulation (previously shown to be the time of maximal lesion size and severity)[19] did not differ substantially between mice fed ω-3 or ω-6 LCPUFAs, whereas that at 7 days was significantly smaller in mice fed the ω-3 LCPUFA diet, indicating that ω-3 LCPUFAs promote disease resolution in this laser-induced AMD model.[20]

Spectral domain-optical coherence tomography (SD-OCT) allows detailed and noninvasive evaluation of the retinal architecture in vivo and has been found to reflect retinal morphological changes during AMD.[21] We therefore applied SD-OCT to determine the cross-sectional area of CNV lesions in mice fed either ω-3 or ω-6 LCPUFAs. Again, we found that lesion size did not differ between mice fed the two experimental diets at 5 days after CNV induction, but that it was significantly smaller in mice fed ω-3 LCPUFAs than in those fed the ω-6 LCPUFA diet at 7 days. Moreover, fluorescein angiography revealed that the extent of leakage from the new choroidal vessels at both 5 and 7 days after CNV induction was less pronounced in mice fed ω-3 LCPUFAs than in those on the ω-6 LCPUFA diet. The incidence of clinically significant (grade 2B) CNV lesions at 7 days after photocoagulation was thus only 30.0% in mice receiving ω-3 LCPUFAs versus 58.3% for those fed the ω-6 LCPUFA diet.

Lipid profiles for serum and the retina of mice fed ω-3 LCPUFAs

To gain mechanistic insight into the effect of dietary ω-3 LCPUFAs on CNV lesion size, we analyzed the lipid profiles of both serum and the retina for mice fed the two diets by liquid chromatography and tandem mass spectrometry (LC-MS/MS). At 7 days after CNV induction, the concentrations of the principal ω-3 LCPUFAs (EPA and DHA) and of total ω-3 LCPUFAs as well as the DHA/ω-6 docosapentaenoic acid (DPA) ratio were significantly increased, whereas the ω-6/ω-3 LCPUFA ratio was significantly decreased, in serum of mice fed ω-3 LCPUFAs compared with their counterparts fed the ω-6 LCPUFA diet.[20] The amount of EPA as well as the DHA/ω-6 DPA ratio were also significantly increased, whereas the levels of AA and ω-6 DPA were reduced, in the retina of mice fed the ω-3 LCPUFA diet.[20] Both EPA and DHA are efficient alternative substrates of the AA-metabolizing CYP isoforms and are predominantly epoxidized at their ω-3 double bonds to yield 17,18-epoxyeicosatetraenoic acid (17,18-EEQ) and 19,20-epoxydocosapentaenoic acid (19,20-EDP), respectively, as the main products.[22-24] We again applied LC-MS/MS analysis to measure the amounts of endogenous CYP-generated epoxyeicosanoids in serum and the retina at 7 days after CNV induction in mice fed ω-3 or ω-6 LCPUFAs. The serum levels of AA-derived 14,15-dihydroxyeicosatrienoic acid (DHET) and epoxyeicosatrienoic acid (EET) were significantly reduced, whereas those of EPA-derived 11,12- and 17,18-dihydroxyeicosaquatraenoic acid (DHEQ) and EEQ and of DHA-derived 7,8- and 19,20-dihydroxydocosapentaenoic acid (DHDP) and EDP were significantly increased, in the ω-3 LCPUFA-fed mice. Intraperitoneal injection of EPA-derived 17,18-EEQ immediately before laser irradiation conferred significant and dose-dependent protection from CNV and from vascular leakage evaluated at 7 days after CNV induction.[20] Similar effects were also apparent in mice injected with DHA-derived 19,20-EDP.[20] These data thus suggested that eicosanoids generated from ω-3 LCPUFAs by CYP mediate the resolution of laser-induced CNV by dietary ω-3 LCPUFAs.

Role of PPARγ in the amelioration of CNV by dietary ω-3 LCPUFAs

Given that ω-3 LCPUFAs had previously been shown to attenuate retinal neovascularization via activation of peroxisome proliferator-activated receptor γ (PPARγ), an endogenous receptor for these fatty acids,[10,25] we investigated the effects of the ω-3 and ω-6 LCPUFA diets on PPARγ expression in mice with laser-induced CNV. Reverse transcription and real-time polymerase chain reaction analysis revealed that the amount of PPARγ mRNA in the choroid at 7 days after CNV induction was significantly increased in

mice fed ω-3 LCPUFAs compared with those fed the ω-6 LCPUFA diet, whereas immunoblot analysis showed that the abundance of PPARγ protein was significantly increased in both the retina and choroid of the ω-3 LCPUFA-fed mice at this time.[20] The transactivation activity of PPARγ in retinal nuclear extracts was also significantly higher in mice receiving ω-3 LCPUFAs.[20]

Dietary intake of ω-3 LCPUFAs has also been shown to down-regulate the expression of adhesion molecules in retinal neovessels in a PPARγ-dependent manner.[10] Similarly, we found that the levels of mRNAs for the adhesion molecules ICAM-1 (intercellular adhesion molecule-1) and E-selectin in the retinal and choroidal vasculature at 7 days after the induction of CNV were significantly reduced in mice fed ω-3 LCPUFAs compared with their counterparts fed the ω-6 LCPUFA diet. In contrast, the amounts of VCAM-1 (vascular cell adhesion molecule-1) and P-selectin mRNAs in the retina and choroid did not differ between the two groups or mice. Laser-capture microdissection confirmed that the levels of ICAM-1 and E-selectin mRNAs were reduced specifically in the CNV lesions of ω-3 LCPUFA-fed mice. Furthermore, the protein levels of these adhesion molecules in the choroid were also significantly reduced between 2 and 7 days after CNV induction in mice fed ω-3 LCPUFAs.[20] These observations thus suggested that the recruitment of systemic leukocytes to CNV lesions by endothelial adhesion molecules might be down-regulated by dietary ω-3 LCPUFAs.

Effects of dietary ω-3 LCPUFAs on leukocyte recruitment and macrophage infiltration in CNV lesions

Recruitment of systemic leukocytes to CNV lesions has been thought to worsen disease.[26] To assess the impact of ω-3 LCPUFAs on systemic leukocyte recruitment during CNV, we measured the rolling velocity of peripheral blood leukocytes (PBLs) at 3 days after CNV induction (the peak of immune cell infiltration in this model)[27] with the use of an autoperfused microflow chamber coated with P-selectin or with the combination of P-selectin and ICAM-1. The rolling velocity of PBLs in the chamber coated with P-selectin and ICAM-1 was significantly higher for mice fed ω-3 LCPUFAs (1.87 ± 0.18 μm/s) than for those fed the ω-6 LCPUFA diet (1.24 ± 0.07 μm/s). PBLs from both groups of mice rolled at similar velocities on immobilized P-selectin. The average shear stress values and the number of interacting leukocytes per field of view also did not differ between PBLs of the two groups of mice in these experiments.[20] Together, our data were thus suggestive of functional down-regulation of both ICAM-1 on endothelial cells and ICAM-1 ligand on the surface of leukocytes in mice fed the ω-3 LCPUFA diet.

To investigate further the increase in the ICAM-1-dependent rolling velocity of PBLs from mice fed ω-3 LCPUFAs, we measured the expression of the ICAM-1 ligands CD11b and CD18 on the cell surface. Flow cytometry revealed that the surface expression levels of both CD11b and CD18 on PBLs were significantly lower for mice fed ω-3 LCPUFAs than for those fed the ω-6 LCPUFA diet, consistent with the notion that the increased rolling velocity of leukocytes from the former mice is attributable, at least in part, to down-regulation of CD11b and CD18.[20]

Inflammatory cell invasion has been detected in surgically excised CNV lesions from individuals with AMD as well as in postmortem eyes with CNV.[28,29] To investigate whether down-regulation of leukocyte adhesion molecules by ω-3 LCPUFA intervention affects leukocyte infiltration in the eye, we quantified the number of leukocytes in the retina and choroid after CNV induction. Mice fed ω-3 LCPUFAs manifested significantly fewer macrophages in the retina and choroid at 7 days after CNV induction compared with those fed the ω-6 LCPUFA diet.[20]

Infiltrated macrophages and other leukocytes are a source of inflammatory cytokines[30] and proangiogenic molecules such as VEGF that contribute to CNV pathogenesis.[25] The amount of VEGF-A mRNA in the retina or choroid at 7 days after CNV induction did not differ between mice fed the ω-3 or ω-6 LCPUFA diets. However, the abundance of VEGF protein in both the retina and choroid at 7 days (but not at 5 days) after CNV induction was significantly reduced in the ω-3 LCPUFA-fed mice compared with mice on the

ω-6 LCPUFA diet.[20] This temporal pattern of VEGF expression correlated closely with that of CNV size and vessel leakage, suggesting that the modulation of ocular angiogenesis and inflammation by ω-3 LCPUFAs contributes to their protective effect against CNV.

Conclusion

Diets rich in ω-3 LCPUFAs have been found to protect against the development of various conditions, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases,[31,32] although the mechanisms underlying such protection have remained unknown. Our recent results suggest that ω-3 LCPUFAs inhibit pathological angiogenesis via a pathway dependent on CYP-generated eicosanoids (Fig. 1)[20] and PPARγ-mediated down-regulation of adhesion molecule expression and leukocyte invasion. Our observations may thus provide new insight into the complex mechanisms that link essential dietary fatty acids to the development of AMD. Further studies are therefore warranted to determine the benefit of dietary intake of ω-3 LCPUFAs, either as an adjunct or possible alternative to current VEGF-targeted treatment, for prevention or amelioration of AMD.

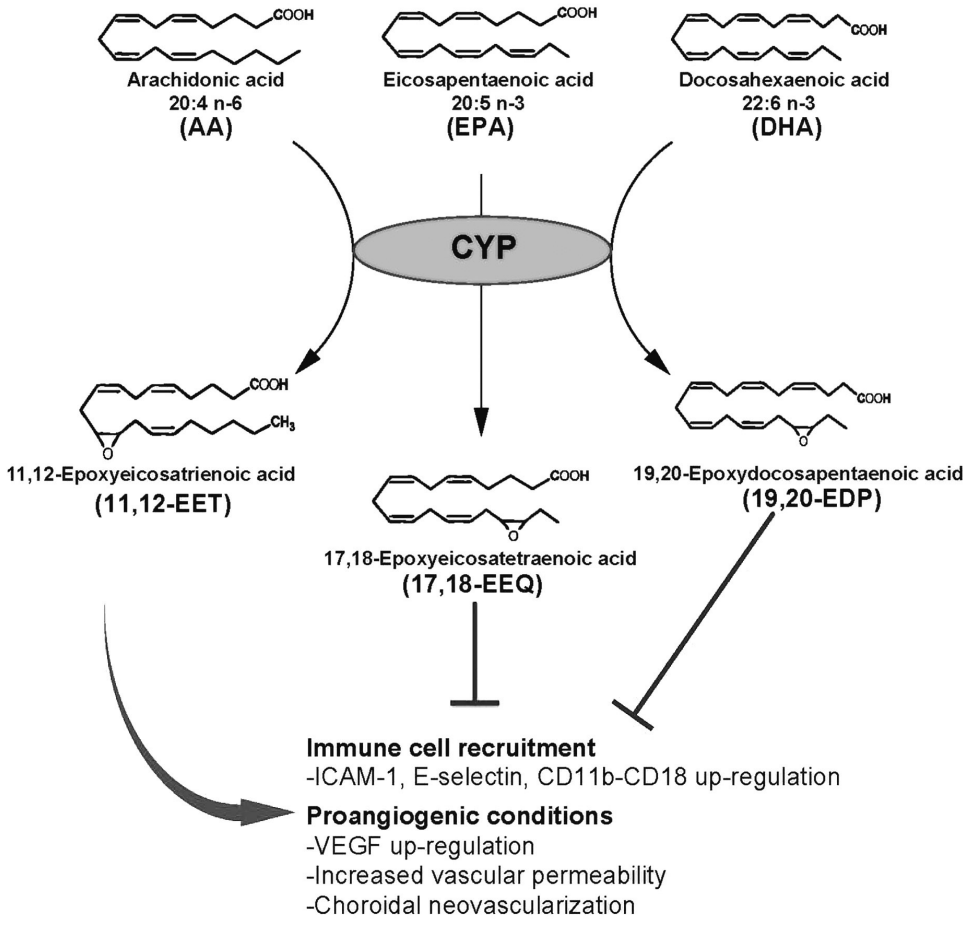

Fig. 1 Proposed roles of CYP-generated lipid metabolites in CNV.

EPA and DHA are efficient alternative substrates for CYP isoforms that metabolize AA, with their metabolism resulting in reduced levels of the AA-derived metabolite 11,12-EET and increased levels of the EPA-derived metabolite 17,18-EEQ and the DHA-derived metabolite 19,20-EDP. These CYP-generated metabolites of ω-3 LCPUFAs promote resolution of CNV by down-regulating inflammatory and proangiogenic conditions.

Conflict of Interest

The authors state no conflict of interest.

References

1. Lim, L. S., Mitchell, P., Seddon, J. M., Holz, F. G. and Wong, T.Y.: Age-related macular degeneration. *Lancet*, **379**: 1728-1738, 2012.
2. Zhang, K., Zhang, L. and Weinreb, R. N.: Ophthalmic drug discovery: novel targets and mechanisms for retinal diseases and glaucoma. *Nat. Rev. Drug Discov.*, **11**: 541-559, 2012.
3. Wang, L., Clark, M. E., Crossman, D. K., Kojima, K., Messinger, J. D., Mobley, J. A. and Curcio, C. A.: Abundant lipid and protein components of drusen. *PLoS One*, **5**: e10329, 2010.
4. Curcio, C. A., Rudolf, M. and Wang, L.: Histochemistry and lipid profiling combine for insights into aging and age-related maculopathy. *Methods Mol. Biol.*, **580**: 267-281, 2009.
5. Gragoudas, E. S., Adamis, A. P., Cunningham, E. T., Jr., Feinsod, M. and Guyer, D. R.: Pegaptanib for neovascular age-related macular degeneration. *N. Engl. J. Med.*, **351**: 2805-2816, 2004.
6. Ng, E. W. and Adamis, A. P.: Anti-VEGF aptamer (pegaptanib) therapy for ocular vascular diseases. *Ann. N.Y. Acad. Sci.*, **1082**: 151-171, 2006.
7. Rosenfeld, P. J. Brown, D. M., Heier, J. S., Boyer, D. S., Kaiser, P. K., Chung, C. Y. and Kim, R. Y.; MARINA Study Group: Ranibizumab for neovascular age-related macular degeneration. *N. Engl. J. Med.*, **355**: 1419-1431, 2006.
8. Connor, K. M., SanGiovanni, J. P., Lofqvist, C., Aderman, C. M., Chen, J., Higuchi, A., Hong, S., Pravda, E. A., Majchrzak, S., Carper, D., Hellstrom, A., Kang, J. X., Chew, E. Y., Salem, N., Jr., Serhan, C. N. and Smith, L. E.: Increased dietary intake of omega-3-polyunsaturated fatty acids reduces pathological retinal angiogenesis. *Nat. Med.*, **13**: 868-873, 2007.
9. Bazan, N. G.: Homeostatic regulation of photoreceptor cell integrity: significance of the potent mediator neuroprotectin D1 biosynthesized from docosahexaenoic acid: the Proctor Lecture. *Invest. Ophthalmol. Vis. Sci.*, **48**: 4866-4881, 2007.
10. Stahl, A., Sapieha, P., Connor, K. M., SanGiovanni, J. P., Chen, J., Aderman, C. M., Willett, K. L., Krah, N. M., Dennison, R. J., Seaward, M. R., Guerin, K. I., Hua, J. and Smith, L. E.: PPAR gamma mediates a direct antiangiogenic effect of omega 3-PUFAs in proliferative retinopathy. *Circ. Res.*, **107**: 495-500, 2010.
11. Kang, J. X. and Liu, A.: The role of the tissure omega-6/omega-3 fatty acid ratio in regulating tumor angiogenesis. *Cancer Metast. Rev.*, **32**: 201-210, 2013.
12. Sheets, K. G., Zhou, Y., Ertel, M. K., Knott, E. J., Regan, C.E. Jr., Elison, J. R., Gordon, W. C., Gjorstrup, P. and Bazan, N. G.: Neuroprotectin D1 attenuates laser-induced choroidal neovascularization in mouse. *Mol. Vis.*, **16**: 320-329, 2010.
13. SanGiovanni, J. P., Chew, E. Y., Clemons, T. E., Davis, M. D., Ferris, F. L., 3rd, Gensler, G. R., Kurinij, N., Lindblad, A. S., Milton, R. C., Seddon, J. M. and Sperduto, R. D.:Age-Related Eye Disease Study Research Group: The relationship of dietary lipid intake and age-related macular degeneration in a case-control study: AREDS report no. 20. *Arch. Ophthalmol.*, **125**: 671-679, 2007.
14. SanGiovanni, J. P., Chew, E. Y., Agrón, E., Clemons, T. E., Ferris, F. L., 3rd, Gensler, G., Lindblad, A. S., Milton, R. C., Seddon, J. M., Klein, R. and Sperduto, R. D.: Age-Related Eye Disease Study Research Group: The relationship of dietary omega-3 long-chain polyunsaturated fatty acid intake with incident age-related macular degeneration: AREDS report no. 23. *Arch. Ophthalmol.*, **126**: 1274-1279, 2008.
15. SanGiovanni, J. P., Agrón, E., Meleth, A. D., Reed, G. F., Sperduto, R. D., Clemons, T. E. and Chew, E. Y.: Age-Related Eye Disease Study Research Group: ω-3 Long-chain polyunsaturated fatty acid intake and 12-y incidence of neovascular age-related macular degeneration and central geographic atrophy: AREDS report 30, a prospective cohort study from the Age-Related Eye Disease Study. *Am.*

J. Clin. Nutr., **90**: 1601-1607, 2009.
16. Age-Related Eye Disease Study 2 Research Group: Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA*, 309: 2005-2015, 2013. Erratum: JAMA, **310**: 208, 2013.
17. Souied, E. H., Delcourt, C., Querques, G., Bassols, A., Merle, B., Zourdani, A., Smith, T. and Benlian, P.: Nutritional AMD Treatment 2 Study Group: Oral docosahexaenoic acid in the prevention of exudative age-related macular degeneration: the Nutritional AMD Treatment 2 Study. *Ophthalmology*, **120**: 1619-1631, 2013.
18. Afzal, A., Caballero, S., Palii, S. S., Jurczyk, S., Pardue, M., Geroski, D., Edelhauser, H., Hochhaus, G., Kim, M., Franklin, A., Shapiro, G. and Grant, M. B.: Targeting retinal and choroid neovascularization using the small molecule inhibitor carboxyamidotriazole. *Brain Res. Bull.*, **81**: 320-326, 2010.
19. Giani, A., Luiselli, C., Esmaili, D. D., Salvetti, P., Cigada, M., Miller, J. W. and Staurenghi, G.: Spectral-domain optical coherence tomography as an indicator of fluorescein angiography leakage from choroidal neovascularization. *Invest. Ophthalmol. Vis. Sci.*, **52**: 5579-5586, 2011.
20. Yanai, R., Mulki, L., Hasegawa, E., Takeuchi, K., Sweigard, H., Suzuki, J., Gaissert, P., Vavvas, D.G., Sonoda, K.H., Rothe, M., Schunck, W.H., Miller, J.W. and Connor, K.M.: Cytochrome P450-generated metabolites derived from ω-3 fatty acids attenuate neovascularization. *Proc. Natl. Acad. Sci. U. S. A.*, **111**: 9603-9608, 2014.
21. Sulzbacher, F., Kiss, C., Kaider, A., Eisenkoelbl, S., Munk, M., Roberts, P., Sacu, S. and Schmidt-Erfurth, U.: Correlation of SD-OCT features and retinal sensitivity in neovascular age-related macular degeneration. Invest. *Ophthalmol. Vis. Sci.*, **53**: 6448-6455, 2012.
22. Arnold, C., Konkel, A., Fischer, R. and Schunck, W. H.: Cytochrome P450-dependent metabolism of omega-6 and omega-3 long-chain polyunsaturated fatty acids. *Pharmacol. Rep.*, **62**: 536-547, 2010.
23. Arnold, C., Markovic, M., Blossey, K., Wallukat, G., Fischer, R., Dechend, R., Konkel, A., von Schacky, C., Luft, F. C., Muller, D. N., Rothe, M. and Schunck, W. H.: Arachidonic acid-metabolizing cytochrome P450 enzymes are targets of ω-3 fatty acids. *J. Biol. Chem.*, **285**: 32720-32733, 2010.
24. Konkel, A. and Schunck, W. H.: Role of cytochrome P450 enzymes in the bioactivation of polyunsaturated fatty acids. *Biochim. Biophys. Acta*, **1814**: 210-222, 2011.
25. Serhan, C. N. and Devchand, P. R.: Novel antiinflammatory targets for asthma. A role for PPARgamma? *Am. J. Respir. Cell Mol. Biol.*, **24**: 658-661, 2001.
26. Ambati, J. and Fowler, B. J.: Mechanisms of age-related macular degeneration. *Neuron*, **75**, 26-39, 2012.
27. Noda, K., She, H., Nakazawa, T., Hisatomi, T., Nakao, S., Almulki, L., Zandi, S., Miyahara, S., Ito, Y., Thomas, K. L., Garland, R. C., Miller, J. W., Gragoudas, E. S., Mashima, Y. and Hafezi-Moghadam, A.: Vascular adhesion protein-1 blockade suppresses choroidal neovascularization. *FASEB J.*, **22**: 2928-2935, 2008.
28. Cherepanoff, S., McMenamin, P., Gillies, M. C., Kettle, E. and Sarks, S. H.: Bruch's membrane and choroidal macrophages in early and advanced age-related macular degeneration. *Br. J. Ophthalmol.*, **94**: 918-925, 2010.
29. Zarbin, M. A.: Current concepts in the pathogenesis of age-related macular degeneration. *Arch. Ophthalmol.*, **122**: 598-614, 2004.
30. Ishida, S., Yamashiro, K., Usui, T., Kaji, Y., Ogura, Y., Hida, T., Honda, Y., Oguchi, Y. and Adamis, A. P.: Leukocytes mediate retinal vascular remodeling during development and vaso-obliteration in disease. *Nat. Med.*, **9**: 781-788, 2003.
31. Daviglus, M. L., Stamler, J., Orencia, A. J., Dyer, A. R., Liu, K., Greenland, P., Walsh, M. K., Morris, D. and Shekelle, R. B.: Fish consumption and the 30-year risk of fatal myocardial infarction. *N. Engl. J. Med.*, **336**: 1046-1053, 1997.
32. Miles, E. A. and Calder, P. C.: Influence of marine n-3 polyunsaturated fatty acids on immune function and a systematic

review of their effects on clinical outcomes in rheumatoid arthritis. *Br. J. Nutr.*, **107** (suppl. 2): S171-S184, 2012.