• Review •

The omega-3 and retinopathy of prematurity relationship

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Abstract

• The aim of this article is to examine the effect of omega-3 (ω-3) long-chain polyunsaturated fatty acids (LCPUFAs) intake on retinopathy of prematurity (ROP) by reviewing the experimental and clinical trials conducted on animal models and infants. LCPUFAs demonstrate cytoprotective and cytotherapeutic actions contributing to a number of anti-angiogenic and neuroprotective mechanisms within the retina. Their intake appears to have a beneficial effect on ischemia, oxidative stress, inflammation and cellular signaling mechanisms, influencing retinal cell gene expression and cellular differentiation. ω-3 LCPUFAs may modulate metabolic processes that activate molecules implicated in the pathogenesis of vasoproliferative and neurodegenerative retinal diseases such as ROP.

• KEYWORDS: retinopathy of prematurity; omega-3
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INTRODUCTION

Retinopathy of prematurity (ROP) represents a major cause of childhood blindness[1-6]. It is a condition in which a variety of factors take part at different stages of the disease leading to microvascular degeneration followed by neovascularisation. Manipulation of oxygen administration has been the golden standard of prevention. This single intervention approach for a multifactorial disease may be one of the reasons why it continues to present with significant morbidity in premature infants. Reports of *in vivo* animal and also a few clinical trials in the recent years that present evidence suggesting long-chain polyunsaturated fatty acids (LCPUFAs) are key modulators of processes affecting retinal health and disease, while the use of preventive dietary supplementation with ω-3 polyunsaturated fatty acids (PUFAs) may be promising. In this report, we review such evidence, indicating that LCPUFAs may act as protective factors in retinal diseases involving vascular and neural pathology, and more specifically ROP.

BIOCHEMISTRY

Fatty acids are compounds synthesized through condensation of malonyl coenzyme A units by a fatty acid synthase complex. Two families of essential fatty acids (EFAs) exist in nature, ω-3 and ω-6. They contain a carboxyl head group and an even numbered carbon chain (×18 carbons) with two or more methylene-interrupted double (unsaturated) bonds. They are structurally classified by the number of carbons, double bonds, and proximity of the first double bond to the methyl (omega) terminal of the fatty acid acyl chain. The ω-3 family of fatty acids contains a double bond at the third carbon; those of the ω-6 family contain a double bond at the sixth carbon.

Docosahexaenoic acid (DHA; C22: 6ω-3) is a major structural lipid in the sensory and vascular retina. Along with its substrate, eicosapentaenoic acid (EPA; C20: 5ω-3), they affect eicosanoid metabolism by reducing ω-6 LCPUFA levels [mainly arachidonic acid (AA; C20: 4ω-6)] and by competing for enzymes [cyclooxygenase (COX) and lipoxygenase (LOX)] used to produce AA-based angiogenic and proinflammatory series 2-a and 4-eicosanoids.

EFAs may be of dietary or cellular source. The human organism does not have the enzymatic capability to meet tissue needs for them through biosynthesis. They are esterified into triglycerides and phospholipids, unified with chylomicrons or very low-density lipoproteins before transport to the choriocapillaris, acting as key structural constituents of phospholipid membranes. DHA and AA are major fatty acids of neural and vascular retinal tissue[7]. In addition, they are ligands to transcription factors for genes, influencing cellular differentiation, growth and lipid, protein, and carbohydrate metabolism. Together with AA, they affect gene expression

Table 1 Major LCPUFAs present in the retina

LCPUFAs	Foods containing this molecule[18-20]	Highest concentration of ω-3 fatty acids in the body
ω-3		
DHA	Fish, fish oils, specialty egg/dairy products	Non-myelin part of the central nervous system, as exemplified by the grey matter of the brain and the rods and cones of the retina[21-22].
EPA	Fish, fish oils, marine sources	
ALA	Flaxseed, canola oil, English walnuts, specialty eggs	
ω-6		
AA	Animal sources only (meat, eggs)	
LA	Vegetable oils (corn, sunflower, safflower, soybean), animal meats	

LCPUFAs: Long-chain polyunsaturated fatty acids; ω-3: Omega-3; DHA: Docosahexaenoic acid; EPA: Eicosapentaenoic acid; ALA: A-linolenic acid; AA: Arachidonic acid; LA: Linoleic acid.

through adjustment of transcription factor activity and concentration within the nucleus. They are effectors of signal transduction pathways regulating gene transcription and may also adjust pathways affecting tyrosine kinase-linked- and G-protein receptors.

DHA is an substantial structural component of the retina, as it is the major fatty acid in structural lipids of retinal photoreceptor outer segment disc membranes[8]. Outer segment discs contain rhodopsin, the photopigment necessary for initiating visual sensation. It is efficiently incorporated and selectively preserved in the disc. Highest body concentrations of DHA per unit area are found in the disc membranes and the overall percentage of DHA (30% of total retinal fatty acids) is 50 mol% greater than in the next most concentrated tissue[9]. Composition of retinal photoreceptor outer segments is unique in that 80%-90% of structural lipids are glycerophospholipids and 8%-10% are neutral lipids[10]. Retinal phospholipids are unique because many are polyenoic in nature.

Biochemical characteristics of DHA may interpret why it is concentrated in the metabolically active retinal photoreceptor outer segment. Fatty acids in membrane phospholipids are a primary source of signaling molecules that modulate intercellular communication and autocrine signaling from the plasma membrane. These processes influence nuclear control of gene expression[11-15]. Although AA is more efficiently released from membrane stores than DHA[16] retinal astrocytes probably supply a readily mobilized source of the latter for such purposes[17] (Table 1).

PHYSIOLOGY (ANGIOGENESIS-ANIMAL MODELS)

Several experimental models have been used to demonstrate the benefits of ω-3 in the retina. One study used six-week-old mice fed with laboratory chow with 5% EPA or linoleic acid (LA) for 4wk. Laser photocoagulation was performed to induce choroidal neovascularisation (CNV), and the volume of CNV tissue was evaluated by volumetric measurements. The CNV volume in the former group of animals was significantly suppressed compared with that in control mice, whereas the latter group did not influence the neovascular tissue. The mRNA expression and protein levels of intercellular adhesion molecule 1 (ICAM-1), monocyte chemotactic protein 1 (MCP-1), vascular endothelial growth factor (VEGF), and interleukin (IL)-6 after CNV induction were significantly reduced in EPA-supplemented mice. EPA application *in vitro* led to significant inhibition of mRNA and protein levels of ICAM-1 and MCP-1 in endothelial cells and VEGF and IL-6 in macrophages. They demonstrated significantly higher levels of EPA and lower levels of AA in the serum and the retinal pigment epithelium (RPE)-choroid than control animals. Moreover, it led to considerable reduction of serum levels of IL-6 and CRP after neovascular induction[23].

In another study mice were treated either with vehicle control or with neuroprotectin D1 (NDP1), a stereospecific derivative of DHA, and CNV was then induced by laser abruption of Bruch's membrane. Treatment was given throughout the first week of recovery. At the seventh day after pathological neovascularisation, NPD1-treated mice had 60% less clinically relevant lesions than controls, dropping to 80% fewer by 14d. They demonstrated 25% smaller leakage area than controls at 7d and 44% smaller extent at 14d. Volumetric immunofluorescence revealed 46% less vascular endothelial cell volume in 7d NPD1-treated mice than in 7d controls, and by 14d NPD1 treatment was 68% lower than controls. Moreover, comparison of 7 and 14d vascular endothelial cell volumes of NPD1-treated mice showed a 50% reduction at 14d. There are at least two possible mechanisms that could interpret this neuroprotective effect. Nuclear factor-kappaB could be inhibited with a reduction in cyclooxygenase-2 (COX-2) to reduce VEGF expression, and/or activation of the resolution phase of the inflammatory response/survival pathways could be upregulated. Furthermore, NPD1 continues to be effective after treatment is completed, indicating sustained protection and highlighting the potential applicability of this lipid mediator in preventing or altering endothelial cell growth in pathoangiogenesis[24].

Other researches use neuronal cultures from albino Wistar rats. Rotstein *et al*[25] demonstrated that DHA prevents retinal

photoreceptor apoptosis during their early development *in vitro*, and upon oxidative stress. Connor *et al*[26] studied the influence of EFAs on vascular loss, vascular regrowth after injury, and pathological neovascularisation induced by hypoxia in a mouse model of oxygen-induced retinopathy. They show that increasing ω-3 tissue levels by dietary or genetic means decreased the avascular area of the retina by increasing vessel regrowth after injury, thus reducing the hypoxic stimulus for neovascularization. The ω-3-PUFA-derived mediators NPD1, resolvin D1 and resolvin E1 also potently protected against neovascularisation. Their protective result and their bioactive metabolites were partially mediated through suppression of tumor necrosis factor-alpha. This inflammation-related cytokine was found in a subset of microglia that was closely connected with retinal vessels. These findings show that increasing the sources of ω-3-fatty acids or their bioactive products reduces pathological angiogenesis.

CLINICAL TRIALS

Long-chain Polyunsaturated Fatty Acids in Human Infants

Randomized controlled trials involving preterm infants that tested EFAs-supplemented formulas have provided evidence that ω-3 fatty acids are needed for normal retinal development and appear to play a protective role against retinal neovascularization. Preterm neonates fed LCPUFA-enriched formulas have enhanced development of the visual system, including improved retinal sensitivity and visual acuity, in comparison with those fed unsupplemented formulas.

In a trial conducted by Birch *et al*[27], very-low-birth-weight (VLBW) neonates with different ω-3 and ω-6 intake were assessed to define whether retinal function was influenced. Neonates born at an average of 30.4wk gestation age were randomized to receive either mother's milk (naturally containing both ω-6 and ω-3) or one of three infant formulas: formula A containing mainly LA and low in all ω-3 fatty acids, formula B containing adequate a-linolenic acid but no long-chain ω-3, or formula C, supplemented with both ALA and marine oils, thus comparable to human milk in long-chain ω-3. Full-field electroretinograms (ERGs) were obtained several weeks after. Ten healthy preterm infants born at 35wk gestation age, who had had intrauterine retinal development, served as controls. Infants fed formula A had significantly higher rod ERG thresholds than infants receiving long-chain ω-3 (human milk, formula C, and intrauterine). Neonates receiving formula B had intermediate thresholds that were considerably higher than those of infants taking intrauterine nutrition. Analysis of the leading edge of the a-wave showed that b-wave differences come from the photoreceptor level. Oscillatory potentials had significantly longer implicit times in infants fed formula A than in infants receiving human milk. These findings point out that retinal function varies with the dietary supply of ω-3 fatty acids in VLBW infants[27].

Another similar clinical trial tested the influence of dietary ω-3 supply on visual acuity development in VLBW infants using visual-evoked potential (VEP) and forced-choice preferential-looking (FPL) procedures at 36 and 57wk postconception. The VLBW neonates born at 27-33wk postconception were randomized to one of three diet groups: corn oil (solely LA), soy oil (linoleic and a-linolenic acids), or soy/marine oil (linoleic, a-linolenic acids, and preformed long chain ω-3 fatty acids). The neonates in the soy/marine oil group had better VEP and FPL acuities than infants in the corn oil group. The soy oil group had significantly poorer VEP acuity at 57wk compared with the soy/marine oil group. The soy/marine oil group had acuities similar to the "gold standards" of VLBW infants fed human milk and preterm infants who were born and tested at 35-36wk post conception. Moreover, VEP and FPL acuity were poorer in a nonrandomized group of formula-fed full-term newborns than in breast-fed full-term infants. The results indicate that dietary ω-3 fatty acid intake may play an essential role in early human visual development[28].

In a randomized, masked, controlled trial of supplemented premature infant formulas, multiple indices of visual development were evaluated. Formulas were supplemented with oils containing AA and DHA, either from fish/fungal oil or from egg-derived triglyceride/fish oil (egg-TG)/fish oil. Premature infants (birth weights 750 to 1800 g) were assigned to: 1) AA+DHA from fish/fungal oil; 2) AA+DHA from egg-derived triglyceride (egg-TG)/fish oil; 3) unsupplemented formula; or 4) exclusively human milk (EHM). Visual acuity measured by swept-parameter VEP was better in both the fish/fungal and egg-TG/fish and closer to that of the EHM group (16.0±0.2) at 6mo to term corrected age. These results showed a benefit of supplementing formulas for premature infants with AA and DHA[29].

A double-masked, randomized trial was conducted to estimate the effect of four amounts of DHA supplementation of formula-fed infants on their visual acuity at 12 months of age. Three hundred and forty-three healthy, term, newborns were randomly assigned at 1-9 days of age to be fed one of the following four infant formulas containing equivalent nutrient amounts, but a different amount of DHA: control (0 DHA), 0.32% DHA, 0.64% DHA, or 0.96% DHA; DHA-supplemented formulas also provided 0.64% AA. Visual acuity was measured by VEP in 244 infants who completed the 12-month primary outcome examination. Infants who were fed with control formula had significantly poorer VEP visual acuity than did infants who received any of the DHA-supplemented formulas (P<0.001). There were no notable differences in VEP visual acuity between the 3 amounts of DHA supplementation[30].

Table 2 ω-3 trials conducted

Authors	Year	Type	Study
Koto *et al*[23]	2007	Animal model	Eicosapentaenoic acid is anti-inflammatory in preventing choroidal neovascularization in mice
Sheets *et al*[24]	2010	Animal model	Neuroprotectin D1 attenuates laser-induced choroidal neovascularization in mouse
Rotstein *et al*[25]	2003	Animal model	Protective effect of docosahexaenoic acid on oxidative stress-induced apoptosis of retina photoreceptors
Connor *et al*[26]	2007	Animal model	Increased dietary intake of omega-3-polyunsaturated fatty acids reduces pathological retinal angiogenesis.
Birch *et al*[27]	1992	Clinical trial	Retinal development in very-low-birth-weight infants fed diets differing in omega-3 fatty acids
Birch *et al*[28]	1992	Clinical trial	Dietary essential fatty acid supply and visual acuity development
O'Connor *et al*[29]	2001	Clinical trial	Growth and development in preterm infants fed long-chain polyunsaturated fatty acids: a prospective, randomized controlled trial
Birch *et al*[30]	2010	Clinical trial	The DIAMOND (DHA intake and measurement of neural development) Study: a double-masked, randomized controlled clinical trial of the maturation of infant visual acuity as a function of the dietary level of docosahexaenoic acid
Carlson *et al*[31]	1993	Clinical trial	Visual-acuity development in healthy preterm infants: effect of marine-oil supplementation
Pawlik *et al*[32]	2011	Clinical trial	Fish-oil fat emulsion supplementation may reduce the risk of severe retinopathy in VLBW infants
Fu *et al*[33]	2015	Clinical trial	Dietary ω-3 polyunsaturated fatty acids decrease retinal neovascularization by adipose-endoplasmic reticulum stress reduction to increase adiponectin

Carlson *et al*[31] hypothesized that preterm infants fed with formulas with marine oil as a source of DHA would have better visual acuities than infantsfed with formulas without marine oil, as measured by the Teller Acuity Card Test. Marine-oil-supplemented infants had better visual acuity at 2 and 4 months of age than those fed with standard formulas. The authors conclude that the formula improved visual acuity of preterm infants through 4 months of age by improving DHA status.

Pawlik *et al*[32] designed a study to compare the safety and efficacy outcomes of an intravenous fat emulsion that consists of fish-oil emulsion (which contains DHA) with soybean and olive oil, administered from the first day of life to 40 neonates with birth weight <1250 g. The authors found a considerably lower risk of laser therapy for ROP in infants who received an emulsion of soybean, olive oil, and fish oil.

Recently, Fu *et al*[33] studied the influence of adiponectin in ROP development and whether circulating adiponectin levels are increased by dietary intake of ω-3 LCPUFAs to mediate the protective effect in ROP in a mouse model. They found that in preterm infants, low serum adiponectin concentrations positively associate with ROP, and serum adiponectin concentrations positively associate with serum ω-3 concentrations. Their findings imply that increasing adiponectin by ω-3 supplementation in parental diet for preterm infants may suppress ROP (Table 2).

Polyunsaturated Fatty Acid and Retinal Disease The role of fatty acids in ocular neovascularization has also been investigated in diseases other than ROP. Several *in vivo* and *in vitro* studies have demonstrated the benefit of DHA for retinal function, photoreceptor survival and rhodopsin regeneration[25,34-36]. In contrast, tissue insufficiency can negatively affect retinal signaling and is associated with alterations in retinal function[37]. *In vivo* studies point to the effects of ω-3-PUFAs on the vascular retina.

Sheets *et al*[24] examined the effects of NPD1, a stereospecific derivative of DHA, on pathological neovascilarisation in a laser-induced mouse model. They demonstrated that intraperitoneal injections of the NPD1 can attenuate laser-induced CNV in mice.

Koto *et al*[23] investigated the role of EPA in the development of CNV in mice. They reported that an EPA-rich diet results in significant suppression of CNV and CNV-related inflammatory molecules in mice and in cultured macrophages and endothelial cells.

Another study by Stahl *et al*[38] aimed to identify a major mechanism by which ω-3 attenuate retinal neovascularization. Administering ω-3-PUFAs exclusively during the neovascular stage of the mouse model of oxygen-induced retinopathy induces a direct angiogenesis reduction of more than 40% without altering vaso-obliteration or the regrowth of normal vessels.

Finally, epidemiological studies have revealed an important inverse relationship between dietary intake of the ω-3-PUFA and risk of developing advanced age-related macular degeneration (AMD)[39-43].

CONCLUSION

EFAs serve as key structural and signalling molecules and contemporary research focuses on their interrelationship with

the neural and vascular structure and function of the retina. Dietary lipids appear to demonstrate potent actions in cellular communication that extend beyond the traditional perception of these molecules as energy substrates. Recent studies indicate the role of ω-3-PUFAs as inhibitors of angiogenesis and give them therapeutic potential as protectors against angiogenic diseases. As lipid-dependent signalling mechanisms are clarified, researchers may be guided in the discovery of key pathways driving retinal response to developmental, environmental, and metabolic factors.

Since retinal tissue status of LCPUFAs is dependent upon and modifiable by diet, we may finally arrive at some reasonable understanding of whether alterations in dietary lipid composition may be effectively used as a preventive intervention against ROP. These studies demonstrate that administration of ω-3 to preterm infants from the first day of life showed a significantly lower incidence and severity of ROP. Treatments with ω-3 fatty acids appear to be a promising therapy for prevention and treatment of ROP, but there is a lack of multicenter randomized clinical trials. New treatment modalities are underway. Currently the most encouraging are therapies that aim at the VEGF-insulin-like growth factor 1 (IGF1) pathway, along with a diet enriched with ω-3. The ideal treatment would be prevention through providing an environment as similar as that of the maternal uterus, to allow for normal retinal vascular maturation to occur[44].

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