Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Progress in Retinal and Eye Research 24 (2005) 87–138
Progress in RETINAL AND EYE RESEARCH
www.elsevier.com/locate/prer

The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina

John Paul SanGiovanni*, Emily Y. Chew

Division of Epidemiology and Clinical Research, National Eye Insitute, National Institutes of Health, 31 Center Drive, Building 31, Room 6A52, MSC 2510, Bethesda, MD 20892-2510, USA

Abstract

In this work we advance the hypothesis that omega-3 (ω-3) long-chain polyunsaturated fatty acids (LCPUFAs) exhibit cytoprotective and cytotherapeutic actions contributing to a number of anti-angiogenic and neuroprotective mechanisms within the retina. ω-3 LCPUFAs may modulate metabolic processes and attenuate effects of environmental exposures that activate molecules implicated in pathogenesis of vasoproliferative and neurodegenerative retinal diseases. These processes and exposures include ischemia, chronic light exposure, oxidative stress, inflammation, cellular signaling mechanisms, and aging. A number of bioactive molecules within the retina affect, and are effected by such conditions. These molecules operate within complex systems and include compounds classified as eicosanoids, angiogenic factors, matrix metalloproteinases, reactive oxygen species, cyclic nucleotides, neurotransmitters and neuromodulators, pro-inflammatory and immunoregulatory cytokines, and inflammatory phospholipids. We discuss the relationship of LCPUFAs with these bioactivators and bioactive compounds in the context of three blinding retinal diseases of public health significance that exhibit both vascular and neural pathology.

How is ω-3 LCPUFA status related to retinal structure and function? Docosahexaenoic acid (DHA), a major dietary ω-3 LCPUFA, is also a major structural lipid of retinal photoreceptor outer segment membranes. Biophysical and biochemical properties of DHA may affect photoreceptor membrane function by altering permeability, fluidity, thickness, and lipid phase properties. Tissue DHA status affects retinal cell signaling mechanisms involved in phototransduction. DHA may operate in signaling cascades to enhance activation of membrane-bound retinal proteins and may also be involved in rhodopsin regeneration. Tissue DHA insufficiency is associated with alterations in retinal function. Visual processing deficits have been ameliorated with DHA supplementation in some cases.

What evidence exists to suggest that LCPUFAs modulate factors and processes implicated in diseases of the vascular and neural retina? Tissue status of LCPUFAs is modifiable by and dependent upon dietary intake. Certain LCPUFAs are selectively accreted and efficiently conserved within the neural retina. On the most basic level, ω-3 LCPUFAs influence retinal cell gene expression, cellular differentiation, and cellular survival. DHA activates a number of nuclear hormone receptors that operate as transcription factors for molecules that modulate reduction-oxidation-sensitive and proinflammatory genes; these include the peroxisome proliferator-activated receptor-α (PPAR-α) and the retinoid X receptor. In the case of PPAR-α, this action is thought to prevent endothelial cell dysfunction and vascular remodeling through inhibition of: vascular smooth muscle cell proliferation, inducible

Abbreviations: A2E, *N*-retinylidene-*N*-retinylethanolamine; AA, arachidonic acid (20:4ω-6); AMD, age-related macular degeneration; ARM, age-related maculopathy; COX, cyclooxygenase; DHA, docosahexaenoic acid (22:6ω-3); DPA, docosapentaenoic acid; DR, diabetic retinopathy; EFA, essential fatty acid; EPA, eicosapentaenoic acid (20:5ω-3); GA, geographic atrophy; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; HUVEC, human umbilical vein endothelial cell; ICAM, intracellular cell adhesion molecule; IFN, interferon; Ig, immunoglobulin; IL, interleukin; IPM, interphotoreceptor matrix; LA, linoleic acid (18:2ω-6); LCPUFA, long-chain polyunsaturated fatty acid; LOX, lipoxygenase; LT, leukotriene; NFκB, nuclear-factor kappa B; NPDR, non-proliferative diabetic retinopathy; NV, neovascular; PAF, platelet-activating factor; PC, phosphatidylcholine; PDR, proliferative diabetic retinopathy; PEA, phosphatidylethanolamine; PG, prostaglandin; PI, phosphatidylinositol; PKC, protein kinase C; PLA_2, phospholipase A_2; PPAR, peroxisome proliferator-activated receptor; PS, phosphatidylserine; redox, oxidation-reduction; ROP, retinopathy of prematurity; RPE, retinal pigment epithelium; RXR, retinoid X receptor; TNF, tumor necrosis factor; TX, thromboxane; uPA, urokinase-type plasminogen activator; VEGF, vascular endothelial growth factor; α-LLNA, α-linolenic acid (18:3ω-3)

*Corresponding author. Tel.: +1-301-496-1331; fax: +1-301-496-2297.
E-mail address: jpsangio@nei.nih.gov (J.P. SanGiovanni).

1350-9462/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.preteyeres.2004.06.002

nitric oxide synthase production, interleukin-1 induced cyclooxygenase (COX)-2 production, and thrombin-induced endothelin 1 production.

Research on model systems demonstrates that ω-3 LCPUFAs also have the capacity to affect production and activation of angiogenic growth factors, arachidonic acid (AA)-based vasoregulatory eicosanoids, and MMPs. Eicosapentaenoic acid (EPA), a substrate for DHA, is the parent fatty acid for a family of eicosanoids that have the potential to affect AA-derived eicosanoids implicated in abnormal retinal neovascularization, vascular permeability, and inflammation. EPA depresses vascular endothelial growth factor (VEGF)—specific tyrosine kinase receptor activation and expression. VEGF plays an essential role in induction of: endothelial cell migration and proliferation, microvascular permeability, endothelial cell release of metalloproteinases and interstitial collagenases, and endothelial cell tube formation. The mechanism of VEGF receptor down-regulation is believed to occur at the tyrosine kinase nuclear factor-kappa B (NFκB). NFκB is a nuclear transcription factor that up-regulates COX-2 expression, intracellular adhesion molecule, thrombin, and nitric oxide synthase. All four factors are associated with vascular instability. COX-2 drives conversion of AA to a number angiogenic and proinflammatory eicosanoids. Our general conclusion is that there is consistent evidence to suggest that ω-3 LCPUFAs may act in a protective role against ischemia-, light-, oxygen-, inflammatory-, and age-associated pathology of the vascular and neural retina.
Published by Elsevier Ltd.

Contents

1. Introduction . . . 89

2. LCPUFAs: general descriptions, functions, actions, and associations . . . 90
   2.1. DHA, EPA, and AA are LCPUFAs . . . 90
   2.2. DHA, EPA, and AA are fatty acids of physiological significance . . . 91

3. Metabolism, transport, accretion, and intake of EFAs and LCPUFAs . . . 92
   3.1. LCPUFAs are obtained through diet or biosynthesized from EFAs . . . 92
   3.2. Transport and accretion of LCPUFAs . . . 92
      3.2.1. LCPUFAs in RPE and photoreceptors . . . 94
      3.2.2. LCPUFAs in the vascular retina . . . 95
   3.3. EFA and LCPUFA intake . . . 95

4. Role of LCPUFAs in structure and function of sensory retina . . . 96
   4.1. DHA is an essential structural component of retinal membranes . . . 96
   4.2. DHA tissue status is associated with alterations in retinal and visual function . . . 96
      4.2.1. Inherited retinal degenerations . . . 97
      4.2.2. Metabolic insufficiency . . . 97
      4.2.3. Dietary insufficiency . . . 97
   4.3. DHA affects retinal cell signaling mechanisms in phototransduction . . . 97
   4.4. LCPUFAs influence retinal cell gene expression, differentiation, and survival . . . 98
      4.4.1. Gene expression . . . 98
      4.4.2. Cellular differentiation . . . 98
      4.4.3. Survival . . . 98

5. Metabolic and environmental bioactivators . . . 98
   5.1. Role of PLA_2 in LCPUFA hydrolysis . . . 99
   5.2. Role of COX in eicosanoid biosynthesis . . . 99
   5.3. Role of LOX in eicosanoid biosynthesis . . . 99
   5.4. Retinal ischemia . . . 99
      5.4.1. Vascular networks in the retina . . . 100
      5.4.2. LCPUFAs affect factors and processes implicated retinal ischemia: vasoregulatory eicosanoids and vascular response . . . 100
      5.4.3. Lipoprotein metabolism . . . 101
      5.4.4. LCPUFAs affect energy production, regulation, and metabolism . . . 101
   5.5. Light exposure . . . 102
      5.5.1. LCPUFAs affect factors and processes implicated in retinal light damage . . . 102
   5.6. Oxidation-reduction balance . . . 102
      5.6.1. Reactive oxygen species and free radicals . . . 102
      5.6.2. Metabolic and environmental bioactivators affect redox balance . . . 103

5.6.3. LCPUFAs affect factors and processes implicated in maintaining redox balance . . . 104
5.7. Inflammation . . . 104
5.7.1. Eicosanoid metabolism . . . 105
5.7.2. LCPUFAs affect factors and processes implicated in ocular inflammation . . . 105
5.7.3. LCPUFAs are ligands for nuclear hormone receptors involved in signaling pathways . . . 108
5.8. Neuroactive compounds and cell signaling pathways . . . 108
5.8.1. Cyclic nucleotides . . . 109
5.8.2. Endocannabinoids . . . 109
5.8.3. Glutamate and PAF . . . 109
5.8.4. Dopamine . . . 110
5.8.5. Ionic transport and channel dynamics . . . 110
5.9. Developmental processes . . . 111
5.9.1. Aging and the chororiocapillaris–Bruch's membrane–RPE—photoreceptor complex . . . 111
5.9.2. Aging and oxidative stress . . . 112
5.9.3. Lipofuscin . . . 113
5.9.4. Astrocytes . . . 113
5.9.5. LCPUFAs affect factors and processes implicated in aging . . . 113

6. Role of LCPUFAs in structure and function of vascular retina . . . 114
6.1. LCPUFAs affect pathogenic processes implicated in neovasculaization . . . 114
6.1.1. Production and release of angiogenic factors . . . 114
6.1.2. Growth factors bind to receptors on endothelial cells . . . 118
6.1.3. Endothelial cells are activated and send signals to the nucleus for production of signaling molecules and enzymes . . . 118
6.1.4. Enzymes digest the basement membrane . . . 118
6.1.5. Endothelial cells divide and migrate through basement membrane toward growth factors . . . 118
6.1.6. Adhesion molecules, or integrins ($\alpha_v\beta_3$, $\alpha_v\beta_5$) help pull the blood vessel sprout forward . . . 118
6.1.7. MMP are produced to dissolve the tissue in front of the sprouting vessel tip in order to accommodate it. As the vessel extends, the tissue is remolded around the vessel . . . 119
6.1.8. Sprouting endothelial cells form tubes . . . 119

7. Retinal diseases of public health significance . . . 119
7.1. Diabetic retinopathy . . . 119
7.1.1. DR and the vascular retina . . . 120
7.1.2. DR and the neural retina . . . 120
7.1.3. Putative role of ω-3 LCPUFAs in modulating factors and processes implicated in pathogenesis of DR . . 120
7.2. Age-related macular degeneration . . . 121
7.2.1. Advanced AMD and CNV . . . 121
7.2.2. Advanced AMD and degeneration of RPE and neural retina . . . 122
7.2.3. Putative role of ω-3 LCPUFAs in modulating factors and processes implicated in pathogenesis of AMD 122
7.3. Retinopathy of prematurity . . . 123
7.3.1. ROP and the vascular retina . . . 123
7.3.2. ROP and the neural retina . . . 125
7.3.3. Putative role of ω-3 LCPUFAs in modulating factors and processes implicated in pathogenesis of ROP . 125

8. Summary and future directions . . . 125

Acknowledgements . . . 126

References . . . 126

1. Introduction

Long-chain polyunsaturated fatty acids (LCPUFAs) demonstrate anti-angiogenic, anti-vasoproliferative, and neuroprotective actions on factors and processes implicated in the pathogenesis of proliferative and degenerative retinal diseases. Many retinal diseases of public health significance manifest tissue and cellular dysfunction in the forms of abnormal angiogenesis, proliferative neovascularization, excessive vascular permeability, immunoregulatory dysfunction, alterations in physiologic reduction-oxidation (redox) balance, or neuronal/retinal pigment epithelial (RPE) cell degeneration. A number of bioactive molecules within the eye

affect, and are effected by, such conditions. These molecules are activated in response to ischemia, light exposure, oxygen/energy metabolism and oxidative stress, apoptosis, cell signaling pathways, inflammation, and developmental processes associated with aging. They operate within complex systems and include eicosanoids, angiogenic factors, matrix metalloproteinases (MMPs), reactive oxygen species, cyclic nucleotides, neurotransmitters and neuromodulators, proinflammatory and immunoregulatory cytokines, and inflammatory phospholipids. Effects and actions of metabolic and environmental bioactivators and bioactive molecules include activation of phospholipase A_2 (PLA_2), cyclooxgenase (COX), and lipoxygenase (LOX). Activation of this enzyme system yields a pool of LCPUFAs and bioactive eicosanoids.

Omega-3 (ω-3) LCPUFAs demonstrate the capacity to modulate production, activation, and potency of bioactive molecules. In some cases these LCPUFAs operate as lipid–protein complexes via signaling cascades in nuclear and cytosolic compartments. In others, they affect substrate pools or availability of biosynthetic enzymes. They influence gene expression as ligands to a number of transcription factors and act as endocannabinoid autocoids. Docosahexaenoic acid (DHA, C22:6ω-3), a major dietary ω-3 LCPUFA, is also a major structural lipid in sensory and vascular retina. Metabolic and dietary DHA insufficiency is associated with alterations in visual system structure and function. DHA and its substrate, eicosapentaenoic acid (EPA, C20:5ω-3), influence eicosanoid metabolism by reducing ω-6 LCPUFA levels (mainly arachidonic acid (C20:4ω-6, AA)) and competing for enzymes (COX and LOX) used to produce AA-based angiogenic and proinflammatory series 2- and 4-eicosanoids.

In this work we present the body evidence implicating LCPUFAs as key modulators of processes influencing retinal health and disease. Section 2 contains a general overview of properties, functions, and actions of LCPUFAs; a more detailed treatment of the issue appears in Chow (2000). Section 3 contains an overview of LCPUFA metabolism, intake, transport, and accretion to the retina; additional information exists in Neuringer (1993), Salem et al. (2001), and Bazan et al. (1993). In Section 4 we consider actions of LCPUFAs on biochemical and biophysical processes that define properties of retinal membranes and signaling systems. Section 5 contains information on metabolic and environmental factors and processes that activate molecules driving retinal neovascularization and neural cell death. These bioactivating factors include ischemia, chronic light exposure, cellular redox balance, cell death, inflammation, neuroactive signaling molecules, and the aging process. In Section 6 we consider the role of LCPUFAs in the structure and function of the vascular retina. In Section 7 we consider the means by which ω-3 LCPUFAs may operate as protective factors in retinal diseases that manifest vascular and neural pathology; we present three examples: diabetic retinopathy (DR), age-related macular degeneration (AMD), and retinopathy of prematurity (ROP). These diseases were selected on the basis of life-span risk, the burden they exert on society, and the coexistence of vascular and neural degenerative pathologies. Our general conclusion is that there is consistent evidence to suggest that ω-3 LCPUFAs may act in a protective role against ischemia-, light-, oxygen-, inflammatory-, or age-associated retinal diseases. Section conclusions are displayed in Table 1.

2. LCPUFAs: general descriptions, functions, actions, and associations

2.1. DHA, EPA, and AA are LCPUFAs

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. ω-3 and ω-6 LCPUFAs contain a carboxyl head group and an even numbered carbon chain ($\geqslant$18 carbons) with two-or-more methylene-interrupted double (unsaturated) bonds. EFAs and LCPUFAs 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. Fatty acids of the ω-3 family contain a double bond at the third carbon; those of the ω-6 family contain a double bond at the sixth carbon. The chemical structure of fatty acids is commonly abbreviated by a listing of the number of carbons, the number of double bonds, and the location of the first double bond from the methyl terminal. For example, DHA is represented as C22:6ω-3, indicating carbon chain length of 22 with 6 double bonds; the first unsaturated bond is inserted at carbon 3. Body stores of LCPUFAs exist mainly as esterified complexes in the *sn-2* position of gylcerophosphates (also known as glycerophospholipids or phospholipids) or trihydric glycerols (also known as triacylglycerols or triglycerides). Within the neural retina, phospholipids represent the predominant LCPUFA-rich lipid class; these compounds are stored mainly as structural elements of membranes. Phosphatidylcholine (PC) composes 40–50% of retinal phospholipids and is localized mainly in the outer leaflet of the membrane. Phosphatidylethanolamine (PEA) and phosphatidylserine (PS) represent 30–35% and 5–10% of retinal phospholipids, respectively; both species tend to orient within the cytoplasmic leaflet. Phosphatidylinositol (PI) composes 3–6% of retinal phospholipids and may be a constituent of membrane domains acting in signaling cascades (Gordon and Bazan, 1997).

Table 1
Section conclusions of this report

DESCRIPTIONS, FUNCTIONS, ACTIONS, AND ASSOCIATIONS OF LCPUFAs

- DHA is a ω-3 LCPUFA. It has 22 carbons and 6 methylene-interupted double bonds.
- EPA is a ω-3 LCPUFA. It has 20 carbons and 5 methylene-interupted double bonds.
- AA is a ω-6 LCPUFA. It has 20 carbons and 4 methylene-interupted double bonds.
- DHA, EPA, and AA are LCPUFAs of physiologic significance, as they act as constituents of lipid–protein complexes, substrates for bioactive eicosanoids or endocannabinoids, and natural ligands to nuclear transcription factors.

LCPUFA METABOLISM, INTAKE, TRANSPORT, AND ACCRETION

- LCPUFAs may be of dietary or cellular origin. The body does not have the enzymatic capacity to meet tissue needs for LCPUFA through biosynthesis. Tissue status is modifiable and dependent on intake.
- DHA is selectively accreted and efficiently retained in photoreceptors.
- The hepatocyte is the major site of LCPUFA biosynthesis.
- LCPUFAs are esterified into triglycerides and phospholipids, integrated with chylomicrons or very low-density lipoproteins before transport to the choriocapillaris.
- LCPUFA-rich phospholipids are hydrolyzed and taken up by a high affinity, receptor-mediated process at the choroid-RPE. They are then transported through the interphotoreceptor matrix to the photoreceptor inner segment. Esterified DHA-phospholipid compounds are then hydrolyzed, actively transferred to the cytosol of the inner segment and re-esterified into phospholipids. These moieties are then incorporated into photoreceptor disk membranes and transferred to the outer segment. Disks migrate to the apical tip of the photoreceptor with time, they are shed and phagocytized by RPE cells. DHA is then stored within oil droplets in the RPE and efficiently recycled to the inner segment via a receptor mediated process.
- LCPUFAs of cellular origin may also be biosynthesized on neural (astrocytes, photoreceptor) and vascular retinal endoplasmic reticulum and peroxisomes.
- ω-3 LCPUFA-rich foods are limited and less frequently consumed than other foods in Western diets

LCPUFAs IN RETINAL STRUCTURE AND FUNCTION OF THE SENSORY RETINA

- DHA is a major structural component of retinal membranes
- DHA tissue status insufficiency is associated with reduced visual processing capacity.
- DHA affects retinal cell signaling mechanisms involved in phototransduction.
- LCPUFAs influence retinal cell gene expression, differentiation, and survival.

METABOLIC AND ENVIRONMENTAL ACTIVATORS

- PLA_2 hydrolyzes LCPUFAs from their esterified form within membranes and lipoproteins to a free form capable of acting as a substrate for eicosanoid synthesis. PLA_2 is activated by ischemia, light exposure, oxidative stress, apoptosis, inflammation, cell signaling molecules, and aging. Retinal diseases of public health significance are associated both with PLA_2 activity and with these metabolic and environmental factors.
- COX and LOX catalyze conversion of LCPUFAs from free forms to eicosanoids. COX and LOX are activated by ischemia, light exposure, oxidative stress, apoptosis, inflammation, cell signaling molecules, and aging. Retinal diseases of public health significance are associated both with COX/LOX activity and with these metabolic and environmental factors.

LCPUFAs IN RETINAL STRUCTURE AND FUNCTION OF THE VASCULAR RETINA

- Long-chain polyunsaturated fatty acids (LCPUFAs) demonstrate anti-angiogenic, anti-vasoproliferative, and neuroprotective actions on factors and processes implicated in the pathogenesis of proliferative and degenerative retinal diseases.
- These actions affect eicosanoids, angiogenic factors, matrix metalloproteinases, reactive oxygen species, cyclic nucleotides, neurotransmitters and neuromodulators, pro-inflammatory and immunoregulatory cytokines, and inflammatory phospholipids.

LCPUFAs AND RETINAL DISEASES OF PUBLIC HEALTH SIGNIFICANCE

- LCPUFAs have the capacity to affect pathogenic factors and processes implicated in retinal neovascularization
- LCPUFAs have the capacity to affect pathogenic factors and processes implicated in retinal neural degeneration

2.2. DHA, EPA, and AA are fatty acids of physiological significance

DHA (Δ4,7,10,13,16,19-DHA; $C_{22}H_{32}O_2$) is an ω-3 LCPUFA with a molecular weight of 328.488. Highest body concentrations of DHA per unit weight are found in phospholipids of retinal photoreceptor outer segments; DHA is also found in substantial amounts within retinal vascular tissue and glia. PEA and PS are the dominant retinal DHA-containing phospholipid species. EPA (Δ5,8,11,14,17-EPA; $C_{20}H_{30}O_2$) is the other major dietary ω-3 LCPUFA. This compound contains 5 double bonds and has a molecular weight of 302.451. EPA is present in blood components, but is not accreted to tissue in great amounts as it is quickly used in DHA or eicosanoid biosynthesis (reviewed in Nelson, 2000). AA (Δ5,8,11,14-eicosatetraenoic acid; $C_{20}H_{32}O_2$) is an ω-6 LCPUFA with 4 double bonds and a molecular weight of 304.467. AA is a major fatty acid of neural and vascular tissue of the retina and brain. Highest concentrations of AA in human retina are found in PC, and then PEA.

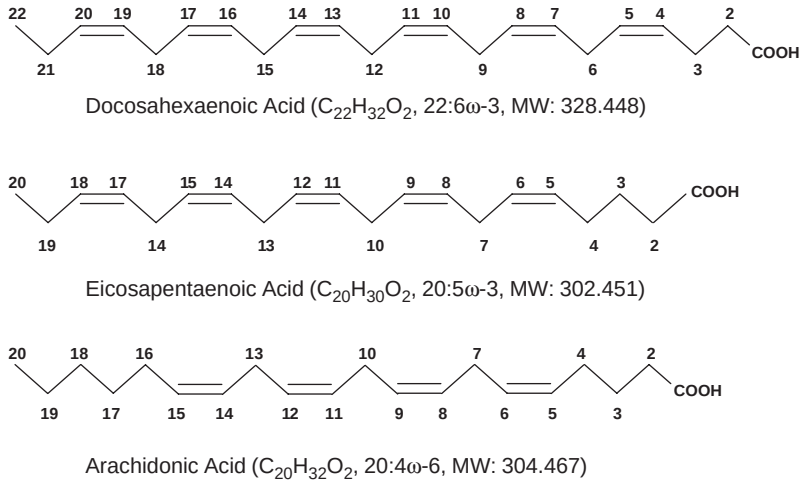


Fig. 1. Chemical structures of DHA (Δ4,7,10,13,16,19-docosahexaenoic acid), EPA (Δ5,8,11,14,17-eicosapentaenoic acid), and AA (Δ5,8,11,14-eicosatetraenoic acid). Molecules are oriented with methyl (omega) terminal on the reader's left.

The chemical structures for DHA, EPA, and AA are represented in Fig. 1. These compounds act as:

- *Key structural constituents of phospholipid membranes.* DHA and AA are major fatty acids of neural and vascular retinal tissue.
- *Ligands to transcription factors* for genes influencing: (a) cellular differentiation and growth; (b) lipid, protein, and carbohydrate metabolism. DHA, EPA, and AA affect gene expression through regulation of transcription factor activity and concentration within the nucleus. Transcription factors containing an LCPUFA binding domain include peroxisome proliferator-activated receptor (PPAR), retinoid X receptor (RXR), nuclear-factor kappa B (NFκB), and sterol regulatory element binding proteins (SREBPs). In some cases, metabolites of the LCPUFAs also act as ligands.
- *Effectors of signal transduction pathways regulating gene transcription*. These pathways include enzyme-based LOX, COX, protein kinase C (PKC), and sphingomyelinase. LCPUFAs may also regulate pathways affecting tyrosine kinase-linked- and G-protein receptors.
- *Substrates for eicosanoid or endocannabinoid autocoids* involved in inter- and intracellular signaling cascades that influence vascular, neural, and immune function.

3. Metabolism, transport, accretion, and intake of EFAs and LCPUFAs

3.1. LCPUFAs are obtained through diet or biosynthesized from EFAs

Humans do not have capacity for de novo biosynthesis of EFAs (α-linolenic and linoleic acid, LA), due to a natural absence of Δ-15 and-12 desaturase enzymes. We are thus dependent on dietary sources of these compounds. LCPUFAs may be obtained directly through the diet or formed from 18-carbon EFAs. Enzymatic reactions yielding LCPUFAs do not satisfy the body's requirements.

After EFAs are obtained through the diet they are desaturated (by insertion of double bonds) and elongated (by addition of 2-carbon units) to LCPUFAs on the hepatic or retinal endoplasmic reticulum (ER). α-linolenic acid (α-LLNA, C18:3ω-3) is the dietary precursor to EPA and DHA. Linolenic acid (LA, C18:2ω-6) is the dietary precursor to AA. Conversion from 24 to 22 carbon LCPUFAs requires β-oxidation in the peroxisome. Fig. 2 displays biosynthetic pathways for the ω-3 and ω-6 families. Because both EFA families compete for the same biosynthetic enzymes, dietary lipid balance and composition will affect production and tissue accretion of these nutrients. Although biosynthesis of LCPUFAs from EFAs is possible, the efficiency of tissue accretion is highest when they are ingested in the preformed state (Su et al., 1999).

3.2. Transport and accretion of LCPUFAs

Gordon and Bazan (1997) and Rodriguez de Turco et al. (1999) discuss pathways by which LCPUFAs may be accreted to the retina (see Fig. 3). EFAs and LCPUFAs exist mainly in esterified forms as triacylglycerols (TG, triacylglycerol) within foods. During early phases of dietary lipid absorption, free fatty acids are cleaved (hydrolyzed) from the *sn-1* and *sn-3* positions of *triglycerides* by pancreatic lipase within the intestine. DHA appears to predominantly occupy the *sn-2* position of the resulting 2-monoglyceride. EPA may occupy the *sn-3*, and to a lesser extent, the *sn-1* position (Nettleton, 1995). Free LCPUFA and

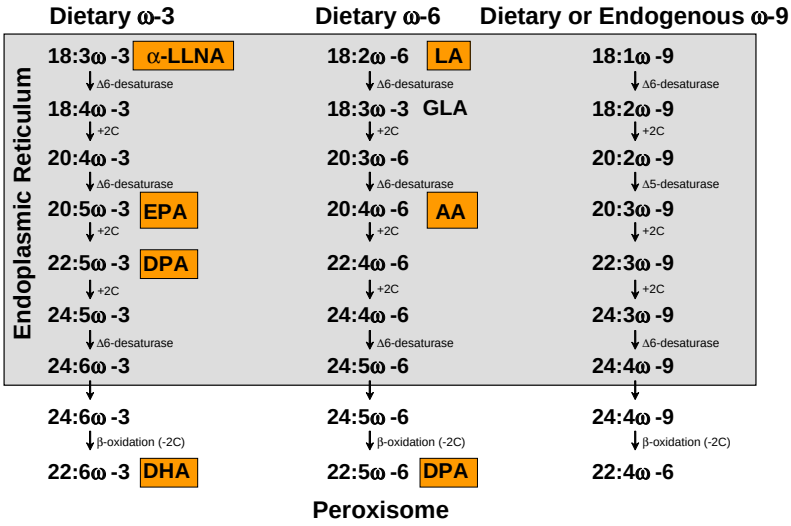


Fig. 2. Biosynthetic pathways of ω-3, -6, and -9 fatty acids. Fatty acid notation represents total number of carbons: number of double bonds, position of the first double bond relative to the methyl terminal of the hydrocarbon chain. For example, 22:6ω-3 indicates that the fatty acid chain is 22 carbons long with the first of 6 double bonds inserted between the third and fourth carbons from the methyl terminal. α-LLNA = α-linolenic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, LA = linoleic acid, AA = arachidonic acid, DPA = docosapentaenoic acid. A detailed representation of this pathway with chemical structures is presented in Bazan (1990).

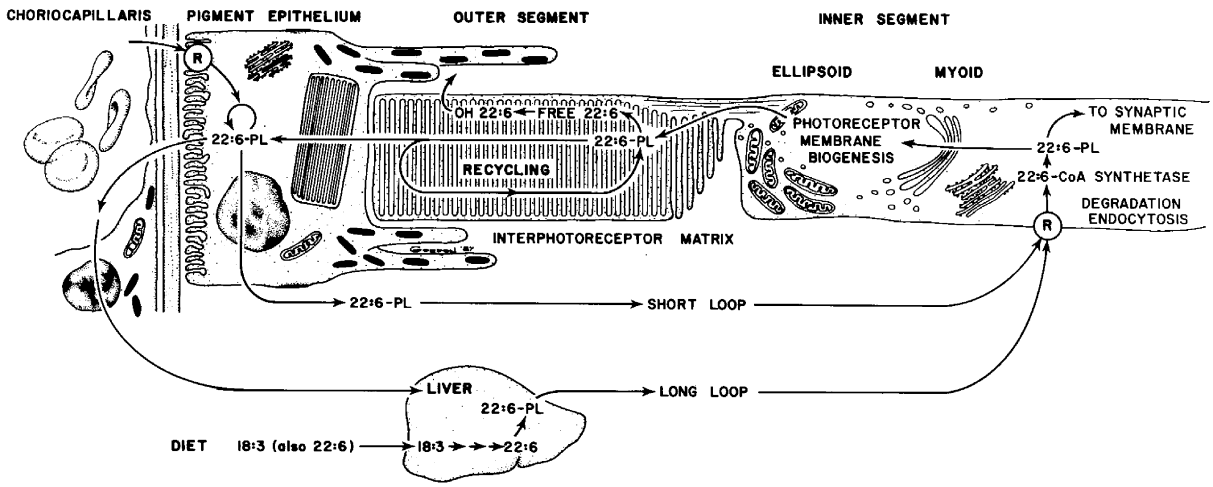


Fig. 3. Pathway of DHA transport from the liver to the choriocapillaris to the RPE to the photoreceptor. DHA is carried from the liver on phospholipids and triacylglycerols synthesized in the liver and packaged into lipoproteins. At the basal surface of the RPE DHA is taken up via a receptor-mediated process® (e.g. highly specific fatty acid binding protein + lipoprotein lipase). This process may occur simultaneously or subsequently via receptor mediated uptake at the myoid region of the inner segment. Transport through the IPM may be mediated by interphotoreceptor binding protein. DHA is then packaged into disk membranes and transported to the outer segment. As the apical membranes of the outer segment are shed and phagocytized by RPE cells, DHA-rich phospholipids from degraded phagosomes are immediately reintroduced to the pathway. This leads to a very efficient local conservation of DHA (from Bazan, NG. In: LaVail MM, Anderson RE, Hollyfield JG, eds. Inherited and environmentally induced retinal degenerations; Copyright © 1989, Alan Liss, New York. This material is used by permission of John Wiley & Sons, Inc.).

LCPUFA-monoacylglycerol complexes are then re-esterified to triglycerides and phospholipids within enterocytes of the intestinal epithelium. Triglycerides and phospholipids are then integrated to chylomicrons and very low density lipoproteins (VLDLs), secreted into the lymphatic system, and circulated from the thoracic duct via blood to the liver. DHA of cellular origin is synthesized mainly from α-LLNA within the liver (Scott and Bazan, 1989). α-LLNA enters the hepatocyte through a receptor-mediated process and is activated by coenzyme A (CoA). The complex then enters the smooth ER where it is elongated and desaturated to DHA–CoA. DHA–CoA enters the rough ER where it is esterified to phospholipids and forms a

complex with apoproteins. These complexes are transported in vesicles to the Golgi bodies where they are assembled into lipoproteins and secreted (Bazan, 1990). DHA of cellular origin is then transported with dietary DHA via VLDL lipoproteins to the choriocapillaris. Lipoprotein lipase hydrolyzes chylomicrons remnants and VLDL within the choriocapillaris.

The bulk of lymph-borne esterified LCPUFAs are carried in the triglyceride class of the chylomicron and VLDL fractions; they exist to a lesser extent as free fatty acids and within other lipid pools (PC, cholesterol ester, monoglycerides, and diglycerides). DHA is accreted mainly to phospholipids species composing membranes (PEA, PC, PS) in the retina. Within the circulation LCPUFAs on chylomicron-bound triglycerides are hydrolyzed to their free forms by capillary-endothelial-cell-derived lipoprotein lipase. These free fatty acids may subsequently form loose (non-covalent) bonds with albumin in blood plasma for delivery to tissues.

The capacity of photoreceptors to synthesize DHA is limited (Wang and Anderson, 1993; Wetzel et al., 1991). RPE (Wang and Anderson, 1993), retinal endothelium (Delton-Vandenbroucke et al., 1997), and brain astrocytes (Moore, 2001) are able to synthesize DHA. Retinal biosynthesis of DHA is slow (Wetzel et al., 1991) and may be insufficient to support the needs of photoreceptors. Gordon and Bazan (1989) and Li et al. (2001) have demonstrated that the liver is a key site for LCPUFA biosynthesis. Availability and distribution of LCPUFAs in plasma lipids and lipoproteins are driven by liver biosynthesis, lipoprotein assembly, and tissue uptake (Gordon and Bazan, 1997). Transport via the choriocapillaris to the RPE and inner segments appears to be mediated by a high affinity receptor mediated uptake.

Hepatectomized rats demonstrate rapid accretion of LCPUFAs in neural tissue (Anderson and Connor, 1988), suggesting that transport mechanisms and specific binding proteins for these compounds operate effectively within the nervous system. DHA is transported from the choriocapillaris via the RPE cells and interphotoreceptor matrix (IPM, an extracellular region between the RPE and outer limiting membrane). The hydrophobic nature of fatty acids requires specialized cytoplasmic transport systems, specific binding proteins, and receptors to transfer LCPUFAs to the photoreceptors. As there is no direct contact between photoreceptors and the choroidal circulation, adjacent cell types (RPE cells, astrocytes, and Müller cells) must aid in the process.

3.2.1. LCPUFAs in RPE and photoreceptors

Gordon and Bazan (1989) have traced the fate of radiolabeled α-LLNA (injected intraperitoneally) from the liver to the retina in the rat. Li et al. (2001) have used similar techniques to trace orally ingested compounds. Study results were concordant on a number of factors. Both reports identify the liver as the key site for LCPUFA metabolism, observe that retinal accretion of radiolabeled α-LLNA-derived DHA was negligible, and suggest that delivery of LCPUFAs is in part regulated at the choroid-RPE interface. Li et al. (2001) state that dietary LCPUFAs are first esterified to triglycerides within enterocytes of the intestinal epithelium. Triglycerides are then integrated with chylomicrons, secreted to the lymph, and enter the circulation via the thoracic duct. At 4 h post-ingestion, and thereafter, labeled compounds appeared predominantly in the phospholipid classes of the liver. These authors suggest such a profile may indicate lipolytic actions on the chylomicron triglycerides as a first step in biosynthesis of phospholipids. Specific activity of DHA peaked in liver at 4 h post-ingestion and had dropped by more than half by 24 h. Radiolabeled DHA was detected in rod outer segments at 1 h post-ingestion, increased rapidly from 2 to 4 h (approximately eight-fold), peaked at 24 h, and remained at levels at least seven-fold higher than those measured at the 2-hour sample for the duration of the study (96 h).

In vivo tracer studies on amphibians have demonstrated a higher degree of LCPUFA labeling in RPE than in photoreceptors. As in mammalian species, RPE labeling preceded that of photoreceptors and the time course of transport from initial exposure to the tracer was substantially shorter. Authors of these studies speculate that RPE plays an important role in regulation and release of DHA from plasma to the IPM (reviewed in Gordon and Bazan, 1997). In amphibians there was a selective accretion of LCPUFAs with carbon-chain lengths greater than 20 to the neural retina (Chen and Anderson, 1993). The RPE contained 20- and 22-carbon LCPUFAs; the bulk of 20-carbon species were AA. In a tracer study on rats, level of AA in plasma was equivalent to that in RPE after 14 weeks of feeding (Wang and Anderson, 1992). Relative to RPE, the specific activity of AA in rod outer segments was 4–16 times lower. The amount of 22-carbon LCPUFAs (DHA and docosapentaenoic acid (DPA) (22:5 ω-6)) in photoreceptors was 3–5 times greater than that in RPE. These studies support the notion of a selective accretion of 22-carbon LCPUFAs in the photoreceptors.

How is DHA delivered to subcellular membrane systems in the photoreceptor? LCPUFA-containing phospholipids enter the RPE or photoreceptor inner segment via a receptor mediated transport process. Gordon and Bazan (1997) suggest operation of a high affinity fatty acid binding protein with a lipoprotein lipase. LCPUFAs enter the inner segment in a smooth ER-dense area adjacent to the base of the outer segment (the myoid) that exhibits a preferential uptake of DHA. After enzymatic degradation of the DHA-triglyceride in the inner segment, activation of fatty acid co-enzyme A

leads to re-esterification of DHA to phosphatidic acid. A process of de novo phospholipid, di- and triglyceride biosynthesis then occurs. Tracer studies indicate DHA-containing phospholipids are then integrated as structural constituents of photoreceptor disk membranes and are retained in proximity to rhodopsin molecules across the life-span of these organelles. There are effective and efficient mechanisms of repair to oxidized DHA that allow it to remain within disks. These properties of repair and selective retention are unique among photoreceptor lipids. As disks migrate to the outer segment-RPE interface, are shed, and phagocytized the photoreceptor DHA content remains unchanged. Phagosomes are degraded in the RPE to form large oil droplets containing DHA-rich triglycerides. Triglycerides are then and transported back to the myoid for re-uptake. It is interesting to note that RPE cytosol remains virtually free of DHA-containing lipid and lipoprotein species. This condition may have important consequences for disease prevention, as discussed in later sections.

3.2.2. LCPUFAs in the vascular retina

Lecomte et al. (1996) determined the fatty acid composition of isolated bovine retinal microvessels and confluent endothelial cell/pericyte monolayers. DHA and AA each represented approximately 10% of total fatty acids in purified intact vessels. In primary cultures the value for DHA was reduced by approximately 2% and the value for AA did not change. DHA levels in cultured endothelial cells and pericytes were restored with 10 μM supplementation of unesterified DHA. In the case of endothelial cells, supplementation did not alter AA concentration; in the case of pericytes, AA concentration was reduced. Levels of EPA in all preparations were more than 10 times less than those of DHA and AA. Although substantial variation existed across tissue types, the mol% of EPA in retinal microvessels was 5 times higher than that in non-vascular retina (0.5% vs. 0.1%). In human serum, retroconversion of DHA to EPA is estimated at 9–11% (Conquer and Holub, 1996, 1997). Likewise, endothelial cultures from bovine macrovascular networks (aorta) exhibit considerable retroconversion. In the Lecomte et al. (1996) report, retroconversion was negligible, indicating a specificity of fatty acid metabolism, based upon the origin of vascular tissue.

Delton-Vandenbroucke et al. (1997) examined capacity of cultured bovine retinal endothelial cells to produce DHA and concluded that, in this model system it is possible via desaturation of DPA (C22:5) of the ω-3 family. While EPA was the major metabolite of DPA ω-3 desaturation, DPA has been shown to constitute 2 mol% of isolated and purified bovine microvessels (Lecomte et al., 1996).

3.3. EFA and LCPUFA intake

Typical intake of total ω-3 fatty acids is 1.6 g/d in the US (approximately 0.7% of total energy intake) (Kris-Etherton et al., 2000). Most is in the form of α-LLNA. EPA and DHA usually compose 6–12% of this value (0.1–0.2 g/d). The main sources of α-LLNA are vegetable oils; of common types, linseed, canola, and soybean oils have highest levels. EPA and DHA are concentrated in fatty fish and marine mammals and these are the main sources in the Western diet. Approximately 10% of DHA is typically derived from eggs. ω-3 LCPUFAs are also commercially available as dietary supplements in the form of oil and capsules. Capsules typically contain 120 mg DHA and 180 mg EPA (Kris-Etherton et al., 2002). The main source of EPA for these products is fish oil. DHA may be derived from fish oil or single-celled organisms. A list of commercially available supplements containing DHA and/or EPA, the nutrient composition of these supplements, and the supplement manufacturers exists at The Natural Medicines Comprehensive Database (http://www.naturaldatabase.com). As this chapter went to press, this database listed 121 products. The main dietary sources of LA are sunflower, safflower, corn and soybean oils. Intake of ω-6 fatty acids is 12–16 g/d in the US (approximately 6.0% of total energy intake). AA consumption is approximately 0.1 g/d. Major dietary sources of AA are terrestrial animal meats, organ meats, and egg yolk.

Because DHA and EPA are concentrated only in a small number of less-frequently consumed marine-based foods, these nutrients may show merit as modifiable factors in diet- or nutrient-based interventions designed to reduce the risk of vascular and degenerative retinal diseases. A scientific statement issued by the American Heart Association (AHA) on ω-3 fatty acids and cardiovascular disease reviews safety of ω-3 fatty acids and fish (Kris-Etherton et al., 2002). The AHA statement cites formal population-based dietary intake recommendations of 0.3–0.5 g/d of EPA + DHA from The World Health Organization, North Atlantic Treaty Organization, and National Health ministries of Australia, Canada, Japan, Sweden, and the United Kingdom. The US Food and Drug Administration (FDA, 1997) has formally stated that consumption of up to 3 g/d of marine-based ω-3 fatty acids is generally regarded as safe (GRAS). The FDA (2002) has also approved a health claim for DHA and EPA in supplement form.

Governmental regulatory bodies have issued statements concerning the potential for hemorrhagic risk with intake of ω-3 LCPUFAs > 3 g/d (2002) (discussed in Kris-Etherton et al., 2002). The anti-thrombotic and anti-haemostatic effects of ω-3 LCPUFAs operate within physiologic limits at intakes between 1.0 and 3.0 g/d (Dyerberg and Bang, 1979; Levine et al., 1989;

Li and Steiner, 1991; Vericel et al., 1999; von Schacky et al., 1985); at these levels hemorrhagic risk is not considered a major issue.

4. Role of LCPUFAs in structure and function of sensory retina

The contents of this section describe the role of LCPUFAs in the structure and function of the sensory retina. Neuringer et al. (forthcoming publication in Progress in Retinal and Eye Research) review these issues in detail.

4.1. DHA is an essential structural component of retinal membranes

DHA is the major fatty acid in structural lipids of retinal photoreceptor outer segment disc membranes (Fliesler and Anderson, 1983). Outer segment discs contain rhodopsin, the photopigment necessary for initiating visual sensation; DHA is efficiently incorporated and selectively retained in disc membranes (Bazan et al., 1993). Highest body concentrations of DHA per unit area are found in the disc membranes and the overall percent of DHA (30% of total retinal fatty acids) is 50 mol% greater than in the next most concentrated tissue (Neuringer, 1993).

Composition of retinal photoreceptor outer segments is unique in that 80–90% of structural lipids are glycerophospholipids and 8–10% are neutral lipids (Daemen, 1973; Fliesler and Anderson, 1983). Neutral lipid species are mainly cholesterol, with a lower concentration of free fatty acids. A phospholipid is a polar molecule with a hydrophilic phosphate head group and two hydrophobic fatty acid tails on a glycerol backbone. Retinal phospholipids are unique because many are polyenoic in nature. Polyenoic phospholipids contain polyunsaturated fatty acids (PUFAs) in the C_1 (*sn*-1) or C_2 (*sn*-2) positions of the molecule's glycerol backbone. The majority of phospholipid species in the outer segments are dipolyenoic (Aveldano and Sprecher, 1987; Choe and Anderson, 1990; Wiegand et al., 1991). Dipolyenoic species are known to increase the rate of rhodopsin activation (metarhodopsin II formation) in model membrane systems (Litman and Mitchell, 1996); this is an essential event in the process of phototransduction.

Fliesler and Anderson (1983) provide a detailed review on chemistry and metabolism of lipids in the vertebrate retina. Retinal phospholipid species include PEA as ~40% of outer segment lipids, PS as ~12%, and PC as ~10% of total outer segment lipids. PC, PE, PS, PI represent ~48%, 32%, 9%, and 4% of retinal phospholipids, respectively. DHA composes approximately 20% of the fatty acids for outer segment PC, and ~30% for each of PEA and PS (Anderson, 1970; Fliesler and Anderson, 1983). Half of all PC fatty acids are saturated (~30% palmitic acid and ~20% stearic acid); in PE these values are ~10% and 36%, respectively. Thirty percent of PS fatty acids are saturated, with the greatest proportion being stearic acid (~28%).

What is the functional significance of the unique fatty acid composition in retinal outer segment photoreceptor disc membranes? The biophysical and biochemical properties of DHA affect membrane function by altering permeability, fluidity, thickness, lipid phase properties, and the activation of membrane-bound proteins (Clandinin et al., 1994; Jumpsen and Clandinin, 1997). DHA-rich membranes impart properties to outer segment discs that influence the dynamic of the inter- and intracellular communication (Litman and Mitchell, 1996; Litman et al., 2001; Mitchell et al., 2001; Niu et al., 2001, 2002; Treen et al., 1992). The stereochemical structure of DHA with its 22 carbons and 6 double bonds allows an efficient conformational change of the transmembrane protein rhodopsin, in response to light absorption (photon capture). Membranes highly concentrated with PUFAs exhibit less rigid global properties than membranes concentrated in sterol esters or saturated fatty acids, because the multiple unsaturated bonds in PUFAs do not allow dense packing of the hydrophobic fatty acid components. LCPUFAs, with their long-chain nature, also contribute to a less-dense structure. A more fluid membrane allows a faster response to stimulation. For DHA, the position of the first unsaturated bond at the ω-3 (between Δ-20 and Δ-19) carbon provides an advantage in efficiency of membrane dynamics over that observed in an otherwise structurally identical fatty acid with the first double bond at the ω-6 carbon (Mitchell et al., 2003).

Biochemical characteristics of DHA may also explain why it is concentrated in the metabolically active retinal 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 (de Urquiza et al., 2000; Doucet et al., 1990; Lin et al., 1999a; Miles and Calder, 1998; Yaqoob, 1998). Although esterified AA is more efficiently released from membrane stores than DHA (Salem et al., 2001), retinal astrocytes probably provide a readily mobilized source of DHA for such purposes (Kim and Edsall, 1999).

4.2. DHA tissue status is associated with alterations in retinal and visual function

DHA deficiency is associated with structural and functional abnormalities in the visual system (Uauy et al., 2000, 2001). Evidence to support this concept

exists for genetic, metabolic, and behavioral factors that influence DHA tissue status and dietary intake. Three examples are provided below.

4.2.1. Inherited retinal degenerations

Retinitis pigmentosa (RP) is the term used to describe a family inherited retinal diseases defined by photoreceptor atrophy, progressive night blindness, and loss of peripheral visual fields (Hoffman et al., 2001). Photoreceptor outer segment lipid DHA concentration was reduced in canine progressive rod-cone degeneration (pcrd) (Aguirre et al., 1997) and rodent transgenic models of RP (Anderson et al., 2001). A study of subjects with X-linked RP demonstrated a 30–40% lower concentration of erythrocyte (RBC) ω-3 LCPUFAs than that observed in a normally sighted comparison group (Hoffman and Birch, 1995; Schaefer et al., 1995). When people with non-X-linked RP were compared with a normally sighted group, their plasma and RBC DHA were lower. Similar results for circulating DHA were observed in canine models of pcrd (Anderson et al., 1999, 1991). Hoffman et al. (2001) have suggested that these differences may be due to decreased biosynthesis of DHA in people with RP. ELOVL4 is a photoreceptor-specific gene responsible for two dominant forms of macular dystrophy (Stargardt-like macular dystrophy and autosomal dominant macular dystrophy). This gene has a homology to a family of yeast proteins (ELO) that operate within the ER to elongate fatty acids. Zhang et al. (2001) suggest that ELOVL4 may be an essential factor in the biosythesis of DHA.

4.2.2. Metabolic insufficiency

Human populations with certain peroxisomal disorders (Zellweger syndrome, neonatal adrenal leukodystrophy, and infantile Refsum disease) exhibit abnormalities in metabolism of LCPUFAs. In these disorders, tissue deficiency of DHA is present in retina, brain, liver, kidney, and blood (Martinez, 1989, 1990, 1992). People with these diseases demonstrate gross visual processing deficits that can be ameliorated with ω-3 LCPUFA supplementation (Martinez et al., 2000).

4.2.3. Dietary insufficiency

Feeding studies in mice, rats, rabbits, and non-human primates have demonstrated electroretinogram (ERG) wave form differences that vary on the basis of dietary ω-3 EFA/LCPUFA intake (reviewed in (Jeffrey et al., 2001). Studies on primates demonstrate alterations in visual resolution acuity is associated with dietary LCPUFA composition and tissue markers of retinal LCPUFAs. Clinical trials in preterm infants have demonstrated transient differences in visual resolution acuity at 2- and 4-months of age, favoring DHA supplementation over DHA-free formulas (reviewed in (SanGiovanni et al., 2000b). Studies on full-term infants demonstrate differences at 2-months-of-age (reviewed in (SanGiovanni et al., 2000a).

4.3. DHA affects retinal cell signaling mechanisms in phototransduction

Photoreceptor outer segment phospholipid fatty acid composition affects the efficiency of intercellular signaling in the visual transduction pathway. The leading portion of the ERG a-wave is associated with the phototransduction pathway; ω-3 deficient animals produce wave forms that are both delayed and of reduced amplitude when compared to those of ω-3 replete animals (Connor and Neuringer, 1988; Pawlosky et al., 1997; Weisinger et al., 1996).

Litman et al. have investigated mechanisms by which membrane composition may affect aspects of phototransduction (Litman and Mitchell, 1996; Litman et al., 2001; Niu et al., 2001). Interpreting this body of evidence requires a basic understanding of the phototransduction process. Phototransduction is the process through which the retina processes light energy and converts it to a pattern of neuronal activity. In a dark-adapted state, retinal photoreceptors generate a depolarizing 'dark current' that is mediated by the effect of high cytosolic concentrations of 3′,5′-cyclic guanosine monophosphate (cGMP) that open Na^+/Ca^{2+} channels. Phototransduction is initiated with the capture of a photon by rhodopsin. Rhodopsin is then transformed to metarhodpsin II (M(II)). M(II) binds to and activates the α subunit of the trimeric G-protein transducin. The M(II)–transducin complex binds to and activates tetrameric cGMP phosphodiesterase (PDE) through removal of one of its inhibitory γ subunits. Activated PDE hydrolyzes cGMP to GMP, which results in membrane hyperpolarization due to dissociation of cGMP from Na^+/Ca^{2+} ion channels. The hyperpolarized state of the photoreceptor leads to a graded decrease of in release of the neurotransmitter glutamate into the photoreceptor synapses on horizontal and bipolar cells. Bipolar cells form synapses with retinal ganglion cells; axons of the retinal ganglion cells constitute the optic nerve. The process is deactivated when rhodopsin is phosphorylated by rhodopsin kinase and then bound with visual arrestin; this process inhibits formation of the M(II)–transducin complex.

How does DHA tissue status alter dynamics of the phototransduction cascade?: M(II) formation to an activated membrane-bound receptor state is higher in DHA-containing model membrane systems than in those containing the AA and cholesterol (Litman and Mitchell, 1996) DHA also enhances production of M(II). Activation of the M(II)–transducin complex is more than two times greater in DHA containing systems than it is in those concentrated with saturated and

monounsaturated fatty acid species (Salem et al., 2001). This indicates that efficiency of interaction in M(II)–transducin coupling is enhanced for DHA-rich membranes. Similar relationships have been observed for PDE activity (Litman et al., 2001).

How may these findings relate to alterations in the timing and magnitude of the ERG a-wave? Salem et al. (2001) suggest that displacement of the leading edge of the ERG a-wave may be constrained by formation of the M(II)–transducin complex and that the reduced a-wave amplitude may be related to a reduced activation of the formed complex (Salem et al., 2001). Others have suggested that a rate limiting step may also exist downstream from activation of the M(II)–transducin complex, in the process of maintaining an adequate supply of photopigment. To this end, DHA may have a role in the regeneration of rhodopsin, as it affects the transport of 11-*cis*-retinal (a component of rhodopsin) by interphotoreceptor retinal binding protein (IRBP) from the RPE (Chen et al., 1996).

4.4. LCPUFAs influence retinal cell gene expression, differentiation, and survival

4.4.1. Gene expression

DHA is a ligand to the nuclear hormone receptors peroxisome PPAR (Lin et al., 1999) and RXR (de Urquiza et al., 2000). DHA binds to specific DNA motifs present on *cis*-regulatory elements in promoter regions of target genes. This event modulates activation of the PPAR and RXR receptors that subsequently operate as transcription factors (Gottlicher et al., 1993). Isoforms of PPAR receptors affected by DHA include α, β, and γ (Dreyer et al., 1993; Gottlicher et al., 1993; Yu et al., 1995). DHA also may act directly in transcription, as it is highly concentrated in PS, a negatively charged aminophospholipid known to activate protein kinases involved in gene expression (Salem et al., 2001). DHA may operate at the posttranscriptional level by acting as a ligand to induce changes of phosphorylation events in native mRNA processing, mRNA transport and stabilization, and mRNA degradation rates (Uauy et al., 2001).

4.4.2. Cellular differentiation

DHA operates as a trophic molecule in photoreceptor development, differentiation, and growth. It has been shown to increase opsin expression and apical process differentiation in developing rat photoreceptors in vitro (Politi et al., 2001; Rotstein et al., 1997, 1998). The protein opsin combines with the 11-*cis*-retinal to form rhodopsin; the relevance of this issue for retinal health is that expression of the opsin gene may be required for assembly of photoreceptor disc membranes (Uauy et al., 2001).

4.4.3. Survival

DHA prolongs survival of rat photoreceptors in vitro (Politi et al., 2001; Rotstein et al., 1996, 1997, 1998). The number photoreceptors supplemented with DHA that survived for 11 days in vitro was approximately twice of that observed within a culture existing on DHA-free media. The proportion DHA-fed cells expressing opsin was significantly higher than in those from a DHA-free culture. Measures of apoptosis (fragmented photoreceptor nuclei and dysfunctional mitochondria) suggested a protective effect of DHA at post-plating days 7 and 11. Serum-starved PC-12 and Neuro-2A cells preincubated in DHA and vitamin E for at least 24 h had lower amounts of genomic DNA fragmentation than cultures fed DHA-free media (Kim et al., 2001). Caspase-3 is an enzyme that mediates mammalian apoptosis. In serum-starved, DHA-enriched cultures, the activity of caspase-3 was maintained at the levels of adequately fed control cultures. DHA-feeding also reduced the expression of caspase-3 mRNA. This was not the case of serum-starved cultures incubated with DHA-free media (Kim et al., 2001).

5. Metabolic and environmental bioactivators

A number of metabolic and environmental factors and processes serve as bioactivators of molecules associated with abnormal angiogenesis, proliferative neovascularization, excessive vascular permeability, immunoregulatory dysfunction, alterations in physiologic redox balance, and neuronal/RPE cell degeneration. Key factors and processes affecting the retina include ischemia, light exposure, oxidative stress, apoptosis, inflammation, neuroactive cell signaling molecules, and developmental processes associated with aging. In addition to affecting molecules associated with the pathogenesis of retinal disease, such factors and processes also modulate: (1) release of unesterified LCPUFAs from phospholipid membranes by PLA_2; and (2) activation of COXs and LOXs that catalyze eicosanoid synthesis. It is important at this point to acknowledge the dominant role of diet in affecting the LCPUFA substrate pool. Metabolic and environmental factors and processes affect fatty acid cleavage- and biosynthetic enzymes. As the concentration and composition of ω-3 LCPUFAs stored in phospholipid membranes is modifiable by and dependent upon dietary intake, the balance of free LCPUFAs and their metabolites is thus affected after activation of PLA_2, COX, and LOX.

In this section we discuss seven major metabolic and environmental factors and processes associated with activation or generation of eicosanoids, angiogenic growth factors, MMPs, reactive oxygen species, cyclic nucleotides, neurotransmitters and neuromodulators,

pro-inflammatory and immunoregulatory cytokines, and inflammatory phospholipids operating in vascular and degenerative retinal diseases. The metabolic and environmental bioactivators presented here work in an interrelated and recursive system as a complex of aetiologic agents. Considering the role of factors capable of altering the concentrations of free LCPUFAs and the activity of key fatty acid cleaveage and biosynthetic enzymes is essential when investigating potential actions of ω-3 LCPUFAs in the retina. As such, we first review basic concepts related to PLA_2, COX, and LOX in the context of the retinal metabolic and environmental exposures.

5.1. Role of PLA_2 in LCPUFA hydrolysis

PLA_2s catalyze hydrolysis of fatty acids from the ester bond at the *sn-2* position of phospholipids to yield free LCPUFAs and lysophospholipids. At least 19 groups of PLA_2s have been identified and they are generally classified into cytosolic ($cPLA_2$), secretory ($sPLA_2$), and calcium-independent ($iPLA_2$) isoforms (Phillis and O'Regan, 2003). $cPLA_2$s are high molecular weight and preferentially cleave AA; there are Ca^{2+}-dependent and independent forms. Intracellular $sPLA_2$s are low molecular weight; while these enzymes do not show specificity for particular fatty acids, Han et al. (2003) demonstrated that group IIa and V $sPLA_2$s can regulate $cPLA_2\alpha$ activity to affect AA release. $cPLA_2\alpha$ has an N-terminal calcium-dependent phospholipid domain that may allow post-translational regulation by calcium or phosphorylation via mitogen-activated protein kinase (MAPK) and PKC (Geijsen et al., 2000; Kramer et al., 1996; Lin et al., 1993; Nemenoff et al., 1993; Qiu and Leslie, 1994). Extracellular signal-regulated kinases (ERKs) are implicated in communication between $cPLA_2\alpha$ and $sPLA_2$s (Balsinde and Dennis, 1996; Hernandez et al., 1998). DHA has been shown to decrease PLA_2 activity in nerve growth cones of nerve growth factor-differentiated PC12 cells, with a predominant effect on $sPLA_2$ in calcium-independent pathways (Martin, 1998). PLA_2 is activated in response to ischemia (Kolko et al., 2002; Phillis and O'Regan, 2003), light exposure (Jung and Reme, 1994), oxidative stress (Martinez and Moreno, 2001; Goldman et al., 1997), apoptosis (Goldman et al., 1997), inflammation (Bazan et al., 2002), cell signaling molecules (Hayakawa et al., 1996; Schalkwijk et al., 1996), and developmental processes associated with aging (Balazy and Nigam, 2003).

5.2. Role of COX in eicosanoid biosynthesis

COX (prostaglandin endoperoxide synthase) is a protein complex that first converts 20-carbon LCPUFA substrates from ω-6 (AA) and ω-3 (EPA) families to G-PG endoperoxides via hydrogen subtraction (at carbon 11) and subsequent addition of 2 molecules of oxygen. A hydroperoxidase (HOX) then uses glutathione to convert the G-PGs to H-PGs. FitzGerald (2003) reviews basic aspects of COX production, structure, and metabolite actions. The constitutive form (COX-1) is found in most cell types (mainly in the gastric mucosa, kidney, and platelets) and operates primarily in the role of haemostatic regulation. The inducible form (COX-2) is found constitutively in the central nervous system, is activated by cytokines and mitogens, and acts in formation of PGs in inflammatory response. Ringbom et al. (2001) have demonstrated that DHA and EPA are effective in inhibiting COX-1 and COX-2 catalyzed PG biosynthesis an in vitro assay. There was a higher potency of inhibition for COX-2. Corey et al. (1983) discuss potential for DHA to operate as a competitive inhibitor of COX. COXs are activated in response to PLA_2 activation and free LCPUFA concentration. As such, COX-2 activation is associated with ischemia (Ju et al., 2003; Candelario-Jalil, 2003), light exposure (Hendrickx et al., 2003), oxidative stress (Kiritoshi et al., 2003; Feng et al., 1995), cell death (Bizik et al., 2004), inflammation (Sennlaub et al., 2003; Bazan et al., 1997; Dubois et al., 1998), neuroactive cell signaling molecules (Nakamichi et al., 2003; Hurst and Bazan, 1995), and developmental processes associated with aging (reviewed in Han et al., 2004).

5.3. Role of LOX in eicosanoid biosynthesis

5-Lipoxygenase (5-LOX) converts AA or EPA to hydroperoxides (hydroperoxyeicosatetraenoic acids, HPETE) via removal of hydrogen at carbon 7 and insertion of moleular oxygen at carbon 5. HPETE is used in leukotriene (LT) biosynthesis; it may also be reduced to hydroxyeicosatetraenoic acid (HETE). Activation of 5-LOX is modulated by calcium, adenosine triphosphate (ATP), and 5-LOX activating protein (FLAP). Upon activation 5-LOX is translocated to the nuclear membrane. 5-LOX metabolites operate in immunoregulation within the inflammatory response (reviewed in Romano and Claria, 2003). LOXs are activated in response to PLA_2 activation and free LCPUFA concentration. As such, LOXs activation is associated with ischemia (Phillis and O'Regan, 2003), light exposure (Naveh et al., 2000), oxidative stress (Werz et al., 2000) and inflammation (Flamand et al., 2002). 12- and 15-LOX are other LOX enzymes that catalyze bioconversion of 20-carbon chain LCPUFAs to compounds of physiological significance.

5.4. Retinal ischemia

Retinal ischemia activates PLA_2 and influences processes implicated the pathogenesis of DR (Frank,

2004), AMD (Ambati et al., 2003), and ROP (Kohner and Chibber, 2001). Retinal ischemia is a state wherein the blood supply is insufficient to meet the metabolic needs of the retina; it is associated with alterations in oxygen delivery and regulation. Osborne et al. (2004) provide a comprehensive review on the subject. DHA and AA are major fatty acids within intraretinal (Lecomte et al., 1996) and choroidal (Kulkarni et al., 2002) capillary networks of vertebrate species.

5.4.1. Vascular networks in the retina

The metabolic demand for oxygen in retina exceeds that of most other tissues (reviewed in Beatty et al., 2000; Wangsa-Wirawan and Linsenmeier, 2003). Since storage of oxygen within retina tissue is not possible, it must be provided continuously by the vascular system (Vanderkooi et al., 1991). The central retinal (ophthalmic) artery and choriocapillaris (from the posterior ciliary arteries) supply blood to the mammalian retina. Two-thirds to 85% of retinal blood flow travels to the choroid. The majority of oxygen carried via this pathway supports photoreceptor metabolism (Wangsa-Wirawan and Linsenmeier, 2003). There are three vascular networks within the choroidal system: (1) the inner layer is adjacent to Bruch's membrane and the RPE; (2) the outer layer is adjacent to the sclera; (3) the medial layer is positioned between these two. Vascular endothelium of the choriocapillaris is thin and fenestrated in the area adjacent to the RPE. It is interesting to note that VEGF is secreted constitutively from the basal surface of RPE cells. Vascular endothelium is thick and non-fenestrated in the area adjacent to the choroid (Mancini et al., 1986).

Twenty to 33% of retinal blood supply enters at the optic nerve head from the ophthalmic artery to form an endartery (often called intraretinal) network supporting the inner retina. There are three major intraretinal capillary networks: (1) radial peripapillary capillaries (RPCs); (2) the inner capillary layer; and (3) the outer capillary layer. The RPCs occupy the inner area of the nerve fiber layer. In the healthy retina, astrocytes are present exclusively in the nerve fiber layer, were their processes cover the vascular tissue (Gardner et al., 2002). The inner capillary layer occupies the ganglion cell layer. The outer layer projects from the inner- to outer-plexiform layers. Vascular endothelium in the intraretinal vascular network is characterized by tight junctions and is impermeable to macromolecules (Gardner et al., 2002; Antonetti et al., 1999).

The intraretinal microvascular system does not receive autonomic input. In order to permit transmission of light to the photoreceptors, intraretinal blood vessels are sparsely distributed at intercapillary distances more of than 50 μm. At the same time, this vascular network must deliver energy substrates (oxygen, glucose, and lipids), enzymatic substrates (nutrients), and remove waste products from this tissue of high metabolic activity. Because retinal neurons are not able to sustain prolonged ischemic insult and the nature of the tightly sealed capillaries of the blood-retina barrier, the system must operate efficiently in controlling perfusion. One suggested pathway has been through modulation of pericyte activity. Pericytes encase vascular endothelial cells and demonstrate contractile properties in vitro (Kawamura et al., 2003; Kelley et al., 1987; Matsugi et al., 1997; Wu et al., 2003). Pericyte loss is a prominent feature in vascular forms of DR.

The unique characteristics of systems modulating retinal oxygenation and their effects on pathogenesis and treatment of ischemia-induced retinopathies are the subject of a recent review (Wangsa-Wirawan and Linsenmeier, 2003). These characteristics include the dual circulatory systems discussed above—with absence of metabolic oxygen regulation in one (choroid) and autoregulation in the other (intraretinal), and dense concentrations of mitochondria within photoreceptor inner segments.

In retinal or choroidal vascular disease, blood flow may be affected by capillary occlusion or increases in platelet activity and aggregation. Occlusion may lead to infarction and concomitant structural alterations, necrosis, and functional loss in vascular tissue. Diseases affecting blood or vessel walls often manifest neural comorbidity. Modulators of retinal ischemia operate within blood and on vascular membranes. Eicosanoids, reactive oxygen species, and cytokines are potent modulators. Actions of these compounds are discussed in detail in following sections. As such ω-3-derived compounds may show some advantage in modulating vasoregulatory processes.

5.4.2. LCPUFAs affect factors and processes implicated retinal ischemia: vasoregulatory eicosanoids and vascular response

How may retinal blood flow and oxygen regulation be affected by LCPUFA intake and status? One means is by providing a substrate for eicosanoids that act as auto- or paracrine effectors of vascular membrane response and alter properties of blood constituents. Another is by altering lipoprotein metabolism. Membrane composition and concentration of LCPUFAs will determine the nature of the resultant free fatty acid pool that serves as substrates for eicosanoid biosynthesis. Vasoregulatory eicosanoids vary in bioactivity and structure on the basis of their LCPUFA substrate. EPA serves as the substrate for series-3 compounds and AA is the precursor to series-2 compounds. Eicosanoids are produced in diverse cell types. Thromboxanes (TX) and PG are the main eicosanoids produced within platelets and vascular endothelial cells that are associated with haemostasis and vasomotility. TXA_2 is a potent vasoconstrictor and induces platelet aggregation;

the ω-3-based analog, TXA_3, is approximately ten-fold less prothrombotic. Prostacyclins (PGI) of the 2- and 3-series have equivalent potency in vasodilatation and platelet anti-aggregation.

5.4.2.1. Blood pressure. Results of two meta-analysis suggest reduction in blood pressure among persons with hypertension who consumed ω-3 LCPUFAs (Appel et al., 1993; Morris et al., 1993). Results have been observed for untreated hypertensive subjects consuming >3 g/d of ω-3 LCPUFAs (Appel et al., 1993). The effect of the anti-hypertensive beta-blocker propranolol is enhanced with fish oil intake (Singer et al., 1990). Bazan and Rodriquez de Turco comment that interventions with amphiphilic cationic drugs (such as propranolol) demonstrate active esterification of DHA into membrane lipids through endogenous biosynthesis (Bazan and Rodriguez de Turco, 1994).

5.4.2.2. Blood flow, vasomotility, thrombosis, platelet activity and aggregation. Knapp (1997) has reviewed the role of fatty acids in modulating blood flow, haemostasis, and thrombosis. A number of studies demonstrate anti-aggregant effects of ω-3 LCPUFAs (Dyerberg and Bang, 1979; Levine et al., 1989; Vericel et al., 1999; von Schacky et al., 1985). Bayon et al. (1995) have observed that since DHA is less likely to be hydrolyzed to a free fatty acid form by PLA_2 than either EPA or AA, that it may prevent platelet aggregation, while in a membrane-bound esterified form (as opposed to serving as a mobilizeable source of EPA). This group has demonstrated that when DHA was esterified in into PC of platelet membranes, affinity of eicosanoids to platelet TXA_2/PGH_2 receptor was reduced. Platelet aggregation is reduced with increased consumption of ω-3 LCPUFAs (Agren et al., 1997; Knapp, 1997; Mori et al., 1997). Effects of LCPUFAs on thrombosis are equivocal, as measured by alteration in the coagulation factors fibrinogen (Barcelli et al., 1985; Marckmann et al., 1997; Shahar et al., 1993), Factor VIII (Archer et al., 1998; Marckmann et al., 1997; Shahar et al., 1993), von Willebrand factor (Archer et al., 1998; Marckmann et al., 1997; Shahar et al., 1993), and thrombomodulin (Johansen et al., 1999).

5.4.3. Lipoprotein metabolism

Lipoprotein metabolism and composition affects ischemia and oxygen regulation. Harris (1997) has reviewed data on the relationship of dietary ω-3 LCPUFAs with serum lipoprotein response. The relationship of ω-3 LCPUFA intake with triglyceride lowering follows a dose–response pattern. Reductions were observed with consumption of <2 g/d (Roche and Gibney, 1996). Consumption of approximately 4 g/d of ω-3 LCPUFAs from fish oil was associated with a 25–30% reduction in serum triglycerides. LCPUFAs exert this effect mainly by reducing triglyceride synthesis in the liver and release of VLDL into the circulation (Bordin et al., 1998; Nenseter et al., 1992; Nestel, 2000; Vasandani et al., 2002). The mechanism driving these events is believed to occur via ω-3 LCPUFA binding to nuclear transcription factors involved in fatty acid and triglyceride regulation. ω-3 LCPUFAs act as ligands to PPAR genes; PPARs form a heterodimer with RXR in binding with DNA in promoter regions of genes involved in fatty acid transport, fatty acid binding, and PUFA desatuarion (Jump, 2004). LCPUFAs operate in a inhibitory feedback mechanism to reduce the nuclear abundance of SREBPs; this process occurs via modulation of proteolytic SREBP processing or SREBP-1c transcription (Jump, 2002). SREBPs are involved in fatty acid biosynthesis and triglyceride metabolism (Horton et al., 2002). SREBP-1a increases transcription of all SREBP-responsive genes and is thus involved in cholesterol, triglyceride, and fatty acid biosynthesis. SREBP-1c activates transcription of genes for acetyl CoA carboxylase, fatty acid synthase, stearoyl CoA desaturase-1; it is associated with de novo fatty acid synthesis and desaturation. SREBP-2 activates genes involved in the inhibition of triglyceride biosynthesis (HMG-CoA reductase, HMG-CoA synthase, farneysyl diphosphate synthase, squaline synthase) (Price et al., 2000). The reasoning is that increase in fatty acid catabolism and decrease in fatty acid biosythesis reduces the pool of materials essential for triglyceride synthesis. This subsequently influences triglyceride release into the circulation as well as the rate of VLDL production (Harris et al., 1990; Roche and Gibney, 2000). Harris (1997) also reports ω-3 LCPUFA-intake-related increases in serum high-density lipoprotein (HDL) of 1–3% and in LDL of 5–10%.

5.4.4. LCPUFAs affect energy production, regulation, and metabolism

The metabolically active neural retina supports its energy requirements in the form of ATP that is produced from oxygen and nutrient-based substrates (fatty acids and glucose, pyruvate, and lactate) within mitochondria of the photoreceptor inner segments. Mitochondria consume 90% of oxygen used by the body. ω-3 LCPUFAs may influence efficiency of energy production within the retina. After ischemic challenge, recovery of mitochondrial function in cardiac tissue of rats fed a fish oil diet was better than that observed in a group consuming an ω-3 LCPUFA-free diet (Demaison et al., 1994). Increased efficiency of ATP production and energy use within mitochondrial membranes in cardiac tissue of animals with higher levels of phospholipid ω-3 LCPUFAs has also been observed (Grynberg and Demaison, 1996). These results suggest that ω-3 LCPUFAs enhance processes of energy metabolism with minimal cost of energy substrate expenditure. The

mitochondria are a major site of reactive oxygen species generation and gains in energy processing efficiency are believed to lower production rates and volume of these compounds.

5.5. Light exposure

Light exposure induces PLA_2 activation and liberation of LCPUFAs bound to the *sn-2* position of membrane phospholipids (Jung and Reme, 1994; Reinboth et al., 1996). Photic damage influences processes implicated the pathogenesis of AMD. Under normal physiological conditions, retinal photic damage is unlikely to occur, despite the high density of photosensitive compounds (chromophores) and the chronic nature of light exposure. Boulton et al. (2001) discuss 3 processes by which light damage may occur. Light-induced ionization is a mechanical process initiated by high irradiance, high frequency exposures; the retina is less susceptible to this form of damage than others. The destructive physical effect of ionization on ocular tissue is caused by periodic 'shock waves.' Light-induced thermal photocoagulation is the result of energy capture, retention, and non-radioactive decay within photothermal chromophores. Thermal damage is associated with cellular temperature increases that occur as chromophores are reduced from activated to ground states. Light-induced photochemical retinal damage is a process initiated after an activated chromophore shifts from a singlet to a more stable triplet energy state. In this triplet form, the activated chromophore may exist long enough to transfer energy to substrates of reactive oxygen species; the result is production of singlet oxygen and free radicals. Photochemical damage is assumed to occur under exposure to ambient light and, depending on spectral sensitivity of chromophores, may affect rod outer segments or rod outer segments and RPE cells (rhodopsin-based, green action spectrum peak), or RPE cells (non-rhodopsin-based, blue action spectrum peak).

5.5.1. LCPUFAs affect factors and processes implicated in retinal light damage

How may ω-3 LCPUFA intake and status facilitate cytoprotective mechanisms in response to light damage? Cellular response to chronic light exposure involves regulation of rhodopsin and membrane lipid concentration. The purpose of this process is to allow the sensory retina to maintain a stable capacity for photon capture that is independent of stimulus intensity. DHA may contribute to this process as it binds to transport proteins implicated in regulation of photopigment regeneration. Boulton et al. (2001) discuss the potential for photosensitized chromophore-oxidation products to form cytotoxic compounds. All-*trans*-retinaldehyde (vitamin A aldehyde) is hydrolyzed from opsin during the isomerization of 11-*cis*-retinaldehyde. All-*trans*-retinaldehyde is subsequently reduced to all-*trans*-retinol by all-*trans*-retinol dehydrogenase. All-*trans*-retinol is carried to the RPE where it undergoes oxidation and isomerization to 11-*cis*-retinaldehyde. Because all-*trans*-retinaldehyde exhibits a peak absorption spectrum in the range of high-energy short-wavelength light, increased concentrations of this compound may increase the potential for photic damage. All-*trans*-retinol exhibits membranolytic characteristics (reviewed in Boulton et al., 2001); the fact that this compound is concentrated both in photoreceptor outer segments and in RPE cells indicates that accumulation may have pervasive effects on retinal structure and function.

Accumulation of all-*trans*-retinol, and some other retinoids of the visual cycle, is modulated by retinoid binding proteins that travel across the IPM. IRBP is a 140-kDa glycoprotein that constitutes the major soluble protein fraction of the IPM (Chen et al., 1996). IRBP contains 2 retinoid binding sites and exhibits highest affinity for 11-*cis*-retinaldehyde and all-*trans*-retinol. IRBP also demonstrates affinity for LCPUFAs with highest specificity for DHA. In bovine retina DHA rapidly and specifically displaced 11-*cis*-retinaldehyde from IRBP (Chen et al., 1996). On the basis of these findings, and information suggesting a steep gradient of DHA between RPE (3.5% of total lipids as DHA) and photoreceptor cells (20% of total lipids as DHA), Chen et al. proposed a model by which lipids and retinoids may interact with IRBP in the regeneration of visual photopigment. The model posits that when IRBP is in the proximity of the RPE, the hydrophilic retinoid-binding site is occupied by 11-*cis*-retinal (this compound has a higher specificity to the receptor than RPE-associated lipids). As the protein comes in contact with the DHA-rich photoreceptor, the 11-*cis* retinoid is released, and the site is occupied by DHA. All-*trans*-retinol also exhibits a high affinity to the receptor and may thus bind to the complex as it approaches the outer segments in transit to the RPE.

Light adaptation is linked to reduction of oxidative stress, as it associated with a decrease in photoreceptor oxygen consumption (Wangsa-Wirawan and Linsenmeier, 2003). Evidence to suggest that light damage and photopigment concentration affect oxidative processes is based on the observation that heme oxygenase, an oxygen sensitive stress protein, is upregulated by retinal photic injury and rhodopsin loss (Organisciak et al., 1998).

5.6. Oxidation-reduction balance

5.6.1. Reactive oxygen species and free radicals

Reactive oxygen species activate PLA_2. Alterations in cellular redox balance are implicated in the pathogenesis of AMD (Beatty et al., 2000), DR (Cai and Boulton, 2002) and ROP (Hutcheson, 2003). As part of a

comprehensive review on the role of oxidative stress in AMD, Beatty et al. (2000) discuss the basic biochemistry of oxidative processes, as well as the generation and specific actions of reactive oxygen species in the retina. Oxidation involves the removal of electrons from an atom or ion and results in an electropositive state. Reactive oxygen species include free radicals (e.g. lipid peroxyl ($ROO^{\bullet}$), hydroperoxyl ($HO_2^{\bullet}$), hydroxyl ($HO^{\bullet}$), nitric oxide ($^{\bullet}NO$) and superoxide anion ($O_2^{-\bullet}$)), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2). Free radicals are unstable molecules characterized by one or more unpaired electrons in their outer bond orbitals; they accept electrons (hydrogen) from other molecules to attain a balanced electric state. The molecule operating as the electron donor in this electron transfer reaction then becomes unstable and, in the process, acts to extract electrons from an adjacent molecule. This may result in an oxidative cascade. Reactivity and half-life influence the effects of these compounds. Most free radicals have a half life of a few seconds to $\sim 10^{-6}$ s. Free radicals react with bases in nucleic acids, amino acid side chains in proteins, and unsaturated bonds in fatty acids.

Singlet oxygen is formed when molecular oxygen is energized to redistribute both electrons in the outer-shell octet (that exist in separate pi*2p orbitals) to a single pi*2 orbital. The outer bond orbit of singlet oxygen is complete, but the molecule exhibits a higher energy state than molecular oxygen since the 2 electrons in its outer shell orbit in opposite trajectories. As singlet oxgyen degrades to molecular oxygen, energy is released—this can damage adjacent molecules.

Mitochondria are the main site for superoxide generation; since this molecule is highly reactive, it is unlikely to exist far from the cytosolic regions containing mitochondria. The superoxide anion is yielded via addition of an electron to molecular oxygen. Superoxide reacts with nitric oxide to form peroxynitrate ($ONOO^-$). The hydroxyl radical is the product of peroxynitrate degradation. Superoxide may also be enzymatically converted to to hydrogen peroxide. Hydrogen peroxide has a relatively long half-life and that allows it to travel to the nuclear domain; hydrogen peroxide oxidizes–SH groups of resident proteins. Hydrogen peroxide also reacts with divalent metal catalysts (released from injury or haemolysis), and via single electron transfer, yields highly reactive hydroxyl radicals. The hydroxyl radical is the most reactive oxygen species present in the body. While the half-life of of the hydroxyl radical is relatively short ($\sim 10^{-8}$ s), it has a relatively high oxidation potential. This radical may be formed in the nucleus and lead to covalent cross-linking of nucleic acid bases. The hydroxyl radical also reacts with membrane-bound lipids to yield lipid radicals. Lipid radicals combine with oxygen to yield highly reactive lipid peroxyl and hydroperoxyl radicals. Lipid peroxyl and hydroperoxyl radicals exist mainly in biological membranes rich in PUFA.

Phospholipid bilayers that constitute cell membranes are rich sources of electrons in the case that the acyl chains of their fatty acids contain unsaturated double bonds. Balazy and Nigam (2003) review the multiple aspects of lipid peroxidation. Free radicals extract hydrogen from these unsaturated bonds, yielding lipid peroxyl radicals and lipid peroxides. Adjacent fatty acids are subsequently oxidized in the attempt to reduce the peroxyl radical to a stable compound.

Beatty et al. (2000) discuss retinal characteristics that facilitate imbalance of cellular redox balance to favor oxidation. These are: the high volume of oxygen consumption necessary to support the metabolic needs of the photoreceptors, the high concentration of photosensitizing compounds in the photoreceptors and RPE, the high concentration of unsaturated fatty acids in photoreceptors, and active phagocytosis of photoreceptor outer segments by the RPE. These characteristics are discussed in the following section within the context of metabolic, environmental, and developmental bioactivators.

5.6.2. Metabolic and environmental bioactivators affect redox balance

Redox balance may be altered by natural and pathologic metabolic processes. Cellular concentrations of reactive oxygen intermediates may be associated with energy metabolism; as nutrient-based energy substrates (carbohydrates, lipids, and proteins) are oxidized to CO_2 and H_2O they yield hydrogen atoms that are stored within reduced nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD(2H)). These coenzymes donate electrons to oxygen within the electron transport chain to yield energy that drives the oxidative phosphorylation of adenosine 5′-diphosphate (ADP) to adenosine 5′-triphosphate (ATP) by ATP synthase within the mitochondria. Most free radicals are by-products of mitochondrial respiration. As a means of supporting the metabolic needs of the cell, photoreceptor inner segments are densely packed with these with these organelles.

Retinal oxygen delivery occurs mainly via the choriocapillaris and factors affecting blood flow (blood or vascular tissue) and oxygen saturation will alter rates of energy production; these conditions affect rates of reactive oxygen intermediate generation. As partial pressure of oxygen increases there is a concomitant rise in reactive oxygen intermediates. It is important to note that oxygen regulation does not occur within choriocapillaris. Likewise, the effects of hyperoxia on vasoobliteration seen in the intraretinal capillary beds are not observed in the choroid. The inflammatory response is associated with increases in reactive oxygen species production and thus affects redox balance.

Cellular redox balance may be altered by environmental exposures. Chronic and intense acute retinal

irradiation increase production of free radicals and H_2O_2. Cigarette smoke, O_3, and NO_2 are other environmentally based factors that increase production of reactive oxygen species. Cellular redox balance may be altered in response to the aging process (Balazy and Nigam, 2003) under conditions of chronic exposure. The cellular structure and metabolic efficiency of the retina changes across developmental periods (see Section 5.9.1). Mitochondrial structure and function are affected with age; this may favor increased production of reactive oxygen species. In addition to the potential effects of age-related cumulative oxygen load, there is a concomitant alteration in tissue status of aqueous and lipid soluble vitamins with anti-oxidant properties (reviewed in Boulton et al., 2001).

5.6.3. *LCPUFAs affect factors and processes implicated in maintaining redox balance*

The biochemical nature of DHA and AA and the accretion of these compounds to metabolically active neural retinal tissue would appear to facilitate formation of lipid radicals, lipid peroxyl radicals, and lipid peroxides. Considering the high concentration of DHA in retinal photoreceptor outer segments (areas chronically exposed to high levels of irradiation) the selective tissue distribution of these compounds is perplexing. The importance of LCPUFAs in the retina is indicated by the efficient conservation and use of these 'easily oxidized' lipids in areas highly susceptible to oxidative stress and under conditions that facilitate production of reactive oxygen species (Gordon and Bazan, 1997). There is evidence to suggest that foveal regions exposed to highest intensity light have lower concentration of LCPUFAs (van Kuijk and Buck, 1992); in age-related (chronic) retinal disease the fovea is often spared until late stages of disease.

In vitro studies on model membranes and liposomes have generally reported reactive LCPUFA peroxidation in response to energy or oxygen exposure. This has not been the case for most in vivo studies. Muggli (2003)) reviews studies examining the relationship of ω-3 LCPUFA or fish intake with reactive oxygen species-mediated events, effects on reactive oxygen species biomarkers, and effects on anti-oxidant defense systems. Free radical-induced haemolysis (Mabile et al., 2001) and in vitro LDL oxidation (Wander et al., 1998) were both reduced in samples from people consuming fish oil. Urinary F2-isoprostanes are in vivo markers of lipid peroxidation and oxidant stress. Non-smoking, treated-hypertensive, type 2 diabetic subjects consuming 4 g/d of purified EPA and DHA had lower levels of this biomarker than subjects sharing these characteristics, but consuming an olive oil supplement (Mori et al., 2000). In human tissue, fish oil exposure is also associated with reduction in superoxide anion generation (Chen et al., 1994; Luostarinen and Saldeen, 1996). In some cases, in vivo oxidation of LDL was not altered as a function of LCPUFA intake (Higgins et al., 2001; Brude et al., 1997; Bonanome et al., 1996; Higdon et al., 2000; Frankel et al., 1994); in others it was decreased (Ando et al., 1999). In aged subjects ω-3 LCPUFA intake at low doses (180 mg/d) was associated with decreases in oxidative stress within platelets (Vericel et al., 1999). At higher doses (50 μmol/L) DHA operated as a pro-oxidant (Vericel et al., 2003).

An in vitro study on human retina reported an age- and area-related susceptibility to peroxidation, with the posterior pole oxidation increased among tissue from the oldest subjects (De La Paz and Anderson, 1992). The oxidative damage of peripheral retina did not vary with age. Rotstein et al. (2003) applied and in vitro model of oxidative stress on pure rat retina neurons to elucidate a mechanism by which DHA may operate as a neuroprotective factor. After cells were exposed to an environmental oxidant (paraquat) that generates the superoxide anion, they were observed to die by apoptosis; loss of mitochondrial membrane integrity was seen a key factor in this event. Addition of DHA to the cultures protected photoreceptors from oxidative stress induced apoptosis. Authors speculate that DHA operates to preserve mitochondrial membrane structure and function by reducing Bax and increasing bcl-2 expression. In rats, lower DHA tissue status is associated with lower susceptibility to light damage from acute exposure of 700–800 lux followed by 90 min of darkness (Bush et al., 1991). After exposure to intense green light using intermittent or hyperthermic light treatments rats fed a depleted ω-3 diet exhibited better structural outcomes than rats fed an linolenic acid-enriched diet from flaxseed (Organisciak et al., 1996).

5.7. *Inflammation*

Inflammation activates PLA_2 and influences processes implicated the pathogenesis of AMD (Penfold et al., 2001) and DR (Gardner et al., 2000; Frank, 2004). Inflammation is an immediate biologic response to injury or infection; it is the result of increased capillary permeability and blood flow. Increased capillary permeability allows regulatory proteins (antibodies, complement, and cytokines) and leukocytes (monocytes, macrophages, natural killer lymphocytes, and granulocytes) to pass from the bloodstream across the vascular endothelial wall. Integration of this innate immune response with an acquired one then occurs as activated macrophages and monocytes present antigen to cytotoxic (CD8+) and helper (CD4+) T lymphocytes. Helper T-lymphocytes express CD4+ receptors that recognize cell surface peptide fragments bound in class II major histocompatibility complex (MHCII). These peptides are derived from extracellular pathogens that have either been phagocytosed by macrophages or

endocytosed by antigen-presenting cells (macrophages, dendritic cells, B lymphocytes).

With the cell-mediated response to the antigen, T lymphocytes secrete cytokines that: modulate B and T lymphocyte proliferation; induce B lymphocyte antibody production; and regulate monocyte, macrophage, and natural killer lymphocyte activity. Activity within innate immune system monocytes and macrophages leads to production of proinflammatory cytokines (tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6) that modulate many aspects of innate and acquired immune response.

Helper T lymphocytes are key factors in the production of immunoregulatory cytokines; they originate from a common progenitor (Th0) and are functionally classified by the effects of the cytokines they produce. Th1 cells differentiate from Th0 after exposure to IL-12 or interferon (IFN)-γ. These cells produce IL-2 and IFN-γ to activate cytotoxic T lymphocytes, natural killer lymphocytes, macrophages, and monocytes. Th2 cells differentiate after exposure to IL-4. These cells produce IL-4 (to induce IgE production by B lymphocytes and suppress the Th1 response), IL-5 (to activate eosinophils), and IL-10 (to suppress the Th1 response).

The eye is highly susceptible to attack by systemic autoimmune diseases since it contains cells originating from each of the three embryonic layers. In their diverse origin, these cells express cell surface and intracellular proteins that exist within many systems of the body. Under pathologic conditions, these proteins may be target sites for the immune system. Eicosanoids affect the activities of such factors. Eicosanoids are derived from tissue stores and circulating 20 carbon LCPUFAs; most 20 carbon LCPUFA species in the human body are of dietary origin. We discuss LCPUFA–eicosanoid–cytokine relationships in Section 5.7.1.

5.7.1. Eicosanoid metabolism

Eicosanoids are lipid-based molecules that operate as mediators of inflammation and immunity. As discussed in Section 5.4.2, EPA is the precursor for series-5 LTs and series-3 PGs and TXs. AA is the substrate for series-4 LTs and series-2 PGs and TXs. Section 6.1.1.1 presents details on eicosanoids as they relate to vascular pathology. AA-derived eicosanoids have the common effect of increasing vascular permeability and activating cells that produce proinflammatory cytokines (key processes in the inflammatory response). Autoimmune uveitis is a chronic inflammatory disease ocular structures in the uvea; certain types of uveitis manifest forms of retinal vasculitis. Autoimmune diseases commonly exhibit a dysregulated Th-1 type response (alterations in IL-1, TNF-α production) and enhanced production of AA-derived eicosanoids (particularly PGE_2 and LTB_4). The role of inflammatory mediators in DR, AMD, and ROP is discussed in Section 5.7.2. Fig. 4 represents the relationship of ω-3 LCPUFAs with AA-derived eicosanoid metabolism and neovascularization, vascular permeability, and inflammation.

The potential for ω-3 LCPUFAs to modulate production of AA-derived eicosanoids is important for a number of reasons. First LTB_4 is associated with TNF-α production (Wallace et al., 2000). TNF-α mediates production of a number of potent proinflammatory and immunoregulatory cytokines (Calder, 2001, see Fig. 5). Also, eicosanoids may operate directly on factors in the immune system or via a number soluble mediators, the inflammatory phospholipids platelet-activating factor (PAF), nitric oxide (NO), and tyrosine and serine/threonine kinases. PGE_2 decreases T-cell proliferation, lymphocyte migration, and secretion of IL-1 and IL-2. PGI_2 blocks leukocyte aggregation, T-cell proliferation, and lymphocyte migration and secretion of IL-1 and IL-2. TXA_2 increases lymphocyte proliferation. LTB_4 increases leukocyte chemotaxis and aggregation, T-cell proliferation, and the release of TNF-α, IFN-γ, IL-1, and IL-2. While AA-derived eicosanoids play different roles the inflammatory process, they are all associated with vascular leakage.

5.7.2. LCPUFAs affect factors and processes implicated in ocular inflammation

What evidence exists to implicate ω-3 LCPUFAs in alteration of the inflammatory response? Table 2

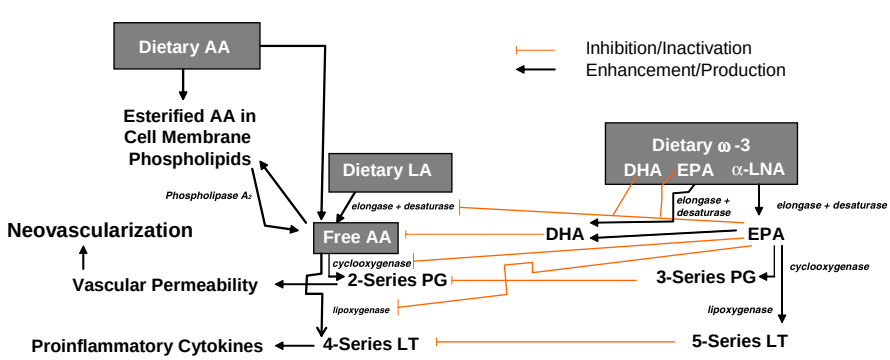


Fig. 4. The relationship of ω-3 LCPUFAs with AA-derived eicosanoids, neovascularization, vascular permeability, and inflammation. α-LNA = α-linolenic acid; AA = arachidonic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acis; LA = linoleic acid.

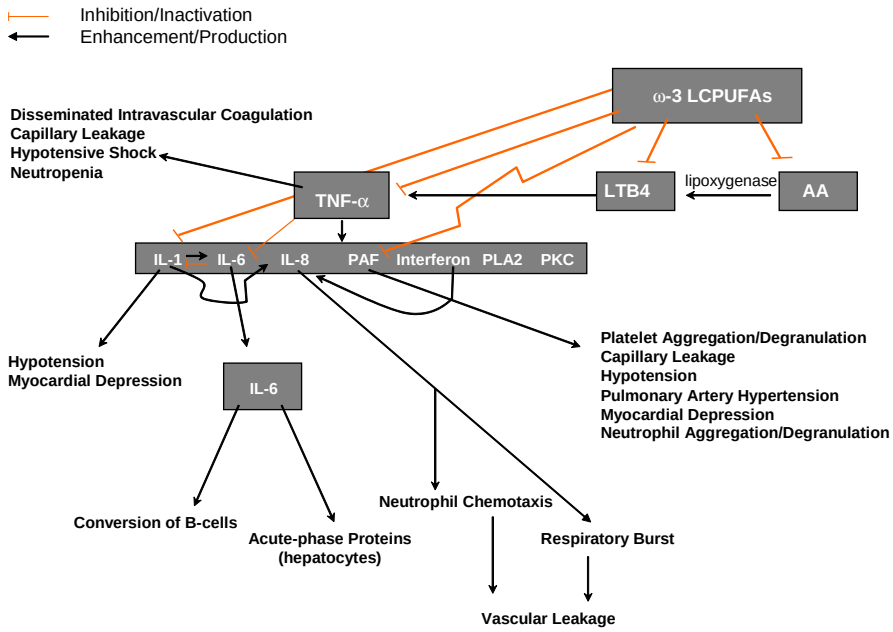


Fig. 5. The relationship of ω-3 LCPUFAs with leukotriene B_4, cytokines, and actions of cytokines. AA = arachidonic acid; IL = interleukin; PAF = platelet-activating factor. PKC = protein kinase C; PLA_2 = phospholipase A_2; TNF-α = tumor necrosis factor-α.

presents three lines of evidence to support the modulatory role of ω-3 LCPUFAs in immune and inflammatory processes. Information was extracted from a comprehensive review by Calder (2001).

LCPUFAs affect both innate and acquired immune systems. In vitro studies on human cell lines incubated with ω-3 LCPUFAs have demonstrated decreased: (1) monocyte cell surface antigen presentation (Hughes et al., 1996), TNF-α and IL-1β expression (Baldie et al., 1993); (2) neutrophil superoxide presentation (Chen et al., 1994); (3) natural killer lymphocyte activation (Purasiri et al., 1997; Yamashita et al., 1986); (4) lymphocyte proliferation (Brouard and Pascaud, 1993; Calder et al., 1992; Calder and Newsholme, 1992; Khalfoun et al., 1996; Purasiri et al., 1997; Santoli et al., 1990; Virella et al., 1991), antigen expression (Tappia et al., 1995), and IL-2 production (Calder and Newsholme, 1992; Purasiri et al., 1997). De Caterina et al. (2000) have added DHA to adult saphenous vein endothelial cell cultures activated by cytokines. The result was reduced expression of IL-6 and IL-8.

Animal feeding studies have demonstrated differences in immune system factors between animals receiving ω-3 LCPUFA-rich diets and those receiving ω-3 LCPUFA-free diets; animals consuming ω-3 LCPUFAs show decreased:

1. Macrophage reactive oxygen species production (D'Ambola et al., 1991; Eicher and McVey, 1995; Hubbard et al., 1991; Joe and Lokesh, 1994), cell surface antigen presentation (Huang et al., 1992; Sanderson et al., 1997), TNF-α expression (Billiar et al., 1988; Renier et al., 1993; Wallace et al., 2000; Yaqoob and Calder, 1995a), IL-1β expression (Billiar et al., 1988; Renier et al., 1993; Wallace et al., 2000; Yaqoob and Calder, 1995a), IL-6 expression (Billiar et al., 1988; Renier et al., 1993; Wallace et al., 2000; Yaqoob and Calder, 1995a), and IFN-γ receptor expression (Feng et al., 1999).
2. Monocyte TNF-α and IL-1β expression (Grimm et al., 1994).
3. Natural Killer cell activation (Meydani et al., 1988; Peterson et al., 1998; Sanderson et al., 1995; Yaqoob et al., 1994c).
4. Cytotoxic T lymphocyte activation (Fritsche and Cassity, 1992).
5. Lymphocyte proliferation (Alexander and Smythe, 1988; Fritsche and Cassity, 1992; Fritsche et al., 1991; Jolly et al., 1997; Kelley et al., 1988; Kuratko, 2000; Peterson et al., 1998; Sanderson et al., 2000; Wallace et al., 2001; Yaqoob and Calder, 1995b; Yaqoob et al., 1994a, b) and production of IL-2 and IFN-γ (Wallace, 2001).

Human feeding studies demonstrate similar results to the animal studies at high doses of ω-3 LCPUFAs. These studies also highlight the importance of considering the balance of ω-3/ω-6 LCPUFAs. Three (Endres et al., 1989; Schmidt et al., 1989, 1992) of four studies showed decreased monocyte chemotaxis in populations consuming ω-3 LCPUFA-rich diets; subjects in the study that did not demonstrate a difference between dietary groups (Schmidt et al., 1996) received a relatively lower amount of ω-3 LCPUFAs. Monocyte surface

Table 2
Relationship of ω-3 long-chain polyunsaturated fatty acids with processes involved in inflammation and immunity

Factor or process	Line of evidence		
	In vitro studies	Animal feeding studies	Human feeding studies
IMMUNE SYSTEM CELLS			
MACROPHAGE			
Superoxide/H_2O_2 production		↓ mouse ↓ rat ↓ rabbit	
MCH II (Ia) expression	↓ mouse	↓ mousel ↓ rat	
TNF-α expression		↓ mouse ↓ rat (both ex vivo)	
IL-1β expression		↓ mouse ↓ rat (both ex vivo)	
IL-6 Expression	↓ rat	↓ mouse ↓ rat (both ex vivo)	
IFN-γ receptor expression		↓ mouse	
MONOCYTE			
Superoxide production			NS (low dose)
Chemotaxis			↓ NS (low)
Surface antigen expression (HLA-DR)	↓ human (IFN-γ stim)		↓
TNF-α expression	↓ human	↓ rat (ex vivo)	
IL-1β expression	↓ human	↓ rat (ex vivo)	
IL-6 expression		↓ rat (ex vivo)	
NEUTROPHIL			
Phagocytosis	↓ human		
Chemotaxis			↓ NS
Binding to endothelia			↓
Superoxide production	↓ human		↓ NS
NATURAL KILLER LYMPHOCYTE			
Activation	↓ human	↓ mouse	↓ (healthy, aged 55–75)
		↓ rat (low intake—EPA)	
LYMPHOCYTE			
Antigen expression/presentation	↓ (tetanus toxin stim)		
Cytotoxic T-Lymphocyte activation		↓ mouse	
Proliferation	↓ human (mit. stim)	↓ mouse (low intake)	↓ (♀, healthy, 51–68)
	↓ rodent (mit. stim)	↓ rat (low intake)	↓ (♂)
		↓ rabbit	
		↓ chicken	
Ex Vivo antigen presentation		↓ mouse	
IL-2 Production	↓ human ↓ rat	↓ mouse(low intake)	↓ (♀,healthy)
		↓ pig	↓ (MS patients)
IL-2 Receptor expression		↓ mouse (activ.lymphocyte)	
		↓ rat (active. lymphocyte)	
CYTOKINES			
TNF-α expression			↓ mononuclear
			↓ (low fat diet/oily fish)
Plasma		↓ mouse (endotox stim)	
Serum		↓ rat (burned)	↓ (abdominal surgery)
IL-1β expression			↓mononuclear NS
Plasma		↓ mouse (endotox stim)	
Serum			
IL-6 expression			↓ mononuclear
			↓ (low fat diet/oily fish) NS
Plasma		↓ mouse (endotox stim)	
Serum		↓ rat (burned)	↓ (abdominal surgery)
Serum		↓ rat (burned)	
IL-8 Expression			
IFN-γ production		↓ mouse	↓ (MS, mononuclear)
OTHER PROCESSES			
DTH response		↓ mouse ↓ dog (KLH stim)	
Host vs. graft response		↓ mouse (high levels) ↓rat	
IgE production		↑ rat (ovalbumin stim)	

Note: ↓ = reduction in factor, process, or activity, active. = activated, endotox. = endotoxin, IFN = interferon, IL = interleukin, mit. = mitogen, MCH = major histocomptability complex, MS = multiple sclerosis patient, NS = not statistically significant, stim = stimulation, TNF = tumor necosis factor.

antigen expression was decreased in people consuming high levels of ω-3 LCPUFAs. ω-3 LCPUFA feeding decreased natural killer (NK) lymphocyte activation (Thies et al., 2001) and lymphocyte proliferation (Meydani et al., 1991; Molvig et al., 1991).

Information on the relationship between intake of ω-3 LCPUFAs and production of proinflammatory cytokines is inconclusive from studies in human populations. Five (Caughey et al., 1996; Endres et al., 1989; Gallai et al., 1995; Meydani et al., 1991) of ten (Blok et al., 1997; Cooper et al., 1993; Molvig et al., 1991; Schmidt et al., 1996; Yaqoob et al., 2000) studies demonstrated an inhibitory effect of ω-3 LCPUFA feeding on TNF-α expression. Four (Caughey et al., 1996; Endres et al., 1989; Gallai et al., 1995; Meydani et al., 1991; Virella et al., 1991) of ten (Blok et al., 1997; Cannon et al., 1995; Cooper et al., 1993; Molvig et al., 1991; Schmidt et al., 1996; Yaqoob et al., 2000) feeding studies demonstrated an inhibitory effect on IL-1β expression. Two (Meydani et al., 1991, 1993) of four (Cooper et al., 1993; Schmidt et al., 1996) studies showed an inhibitory effect on IL-6 expression. Two of two studies examining the effect of ω-3 LCPUFAs on IL-2 (Gallai et al., 1995; Meydani et al., 1991) production and one of one study on IFN-γ production demonstrated an inhibitory effect in groups consuming ω-3 LCPUFAs.

There is indirect evidence to support the efficacy of fish oil feeding on inflammatory factors and processes in systemic autoimmune diseases that may coexist with inflammatory eye diseases. Fourteen placebo controlled-double masked fish oil feeding trials in people with rheumatoid arthritis, a prototypical autoimmune disease, demonstrated a beneficial effect of fish oil on clinical outcome measures of joint inflammation (Calder, 2001; Geusens et al., 1994; James and Cleland, 1997; Volker et al., 2000). Animal feeding studies in a rodent model for lupus demonstrated inhibition of proinflammatory cytokine production and induction of anti-inflammatory cytokine production in fish oil-fed groups (Chandrasekar and Fernandes, 1994; Venkatraman and Chu, 1999). In vitro studies on cartilage chondrocytes (cells affected by arthritic inflammation) showed a dose-dependent decrease in expression of COX-2, IL-1β, and TNF-α expression after incubation with fish oil. While these reports do not contain information on ocular inflammation, it is reasonable to assume that similar cell surface and intracellular proteins may be targeted during ocular inflammatory response.

Increased levels of inflammatory mediators are associated with AMD (Penfold et al., 2001), ROP (Yossuck et al., 2001), and DR (Gardner et al., 2002; Joussen et al., 2002). How may proliferative retinopathies be affected by inflammation? DR is characterized partially by an inflammatory component in which leukocyte migration and adhesion occurs within the retinal vasculature; this is preceded by induction of inflammatory (TNF-α, VCAM-1) and redox-sensitive genes (NFκB). TNF-α plays a dominant role in modulating endothelial adhesion molecules. Ischaemia- and inflammation-induced activation of COX-2 regulates the production of VEGF. Both VEGF and TNF-α upregulate intracellular adhesion molecule (ICAM-1) via NFκB and serine/threonine kinase Akt—PI3 kinase—eNOS signaling pathways (Joussen et al., 2002). Hyperglycemia activates PKC, that activates PLA_2 (Williams and Schrier, 1993). Recall that PLA_2 is responsible for releasing esterified AA as a substrate for COX- and LOX-based eicosanoid production. These processes are associated with blood-retinal barrier degradation and manifested in increased vascular permeability.

5.7.3. LCPUFAs are ligands for nuclear hormone receptors involved in signaling pathways

Apart from their effects on AA-eicosanoid metabolism, ω-3 LCPUFAs may operate to influence inflammatory response via modulation of intracellular signaling pathways and transcription factor activation (Miles and Calder, 1998; Yaqoob, 1998). As briefly discussed in Sections 4.4 and 5.4.3, DHA and EPA are natural ligands to a number of nuclear hormone receptors that affect transcriptional activities. Among these are PPAR (Lin et al., 1999) and RXR (de Urquiza et al., 2000). The activated PPAR-γ/RXR heterodimer regulates genes modulating induction of inflammatory signaling pathways (NFκB, MAP kinase pathways, IL-2 secretion) that lead to production of inflammatory mediators. The activated complex also leads to cell proliferation, production of adhesion molecules (VCAM-1, ICAM-1, E- and P-selectins) and modulation of endotheliail-leukocyte adhesion pathways in vascular tissue (Dubuquoy et al., 2002). Jump (2004) provides a comprehensive review of the role and actions of dietary PUFAs in regulation of gene transcription.

DHA is enriched in PS, a negatively charged aminophospholipid involved in serine kinase activation. PKC operates through one such pathway to increase intracellular calcium (an event associated with subsequent activation of PLA_2 and release of the inflammatory phospholipid PAF). PAF is associated with capillary leakage and neutrophil degranulation.

5.8. Neuroactive compounds and cell signaling pathways

Intracellular signaling pathways activate PLA_2 and may influence processes implicated in the pathogenesis of AMD, DR, and ROP. Membrane receptor responses to a number of neuroactive compounds in the retina are modulated by membrane fatty acid concentration and composition. Free DHA affects lipid- and protein-based neuroactive compounds in the retina. A putative, albeit

complex, relationship of DHA with photoreceptor survival exists as this fatty acid is associated with opsin expression (Politi et al., 2001; Rotstein et al., 1998), rhodopsin recycling (Chen et al., 1996), and activation of key cyclic nucleotides involved in phototranduction (Bechoua et al., 2003; Narayanan et al., 2003). Rhodopsin loss is associated with PR death; the degree of rhodopsin bleaching and pre-isomerization rhodopsin content may modulate light-induced rod degeneration (Organisciak et al., 1996). In the following sections we discuss the relationship of LCPUFAs with the retinal actions of cyclic nucleotides, endocannabinoids, glutamate, PAF, dopamine, and ion channel dynamics.

5.8.1. Cyclic nucleotides

Cardiomyocyte adrenergic receptors are a well studied model that has been used to suggest DHA exhibits beta-adrenergic system inhibition in response to ischemia (Delerive et al., 1999). Increases in DHA content of membrane phospholipids reduced 3′,5′-cyclic adenosine monophosphate (cAMP) production by 20% within cardiomyocytes (Grynberg et al., 1996). cAMP is a key intracellular second messenger in catecholamine signaling systems; it may be produced in response to hypoxia. It is formed from ATP by adenylyl cyclase. Adenylate cyclase activity was reduced by EPA and DHA treatment in isolated white adipocytes or in mouse adipocyte plasma membranes (Price and Tisdale, 1998). The authors speculate that EPA operated via an inhibitory guanine nucleotide-binding protein-mediated pathway. DHA was suggested to operate via a direct inhibition of the cyclase catalytic component.

Elevated levels of cAMP have been found in mouse models of inherited retinal degeneration (P347S (Weiss et al., 1995), rd/rd, rds (Nir et al., 2001; Sanyal et al., 1984). Transgenic rats (P23H-1, P23H-3, and S334ter) exhibiting rhodopsin mutations also demonstrate an increase in dark-adapted cAMP levels (Traverso et al., 2002). Dopamine metabolism in response to photostimulation influences key aspects of cAMP production and activation (discussed by Traverso et al., 2002). Light induces dopamine production and release from the inner retina (amacrine and 'inner plexiform' cells) that subsequently activates D2-like/D4-subtype receptors of cells in the photoreceptor layer. This event leads to inhibition of cAMP synthesis and then to decreases in melatonin production. Dopamine receptor agonists exhibit cytoprotective activities in the retina (Nir et al., 2002; Ogilvie and Speck, 2002), while melatonin induces retinal cAMP production; high levels of melatonin are associated with increased risk of photoreceptor loss induced by photic damage (Sugawara et al., 1998; Wiechmann and O'Steen, 1992). As photoreceptors consume large quantities of oxygen delivered via the choroid; loss of photoreceptors may lead to large increases in local oxygen levels, since the choriocapillaris does not regulate oxygen.

5.8.2. Endocannabinoids

LCPUFAs are the substrate for a number of endocannabinoids that may affect neural and vascular retinal function. Cannabinoids (CB) bind to G protein-bound receptors in CNS tissue. Anandamide (*N*-arachidonoylethanolamide, AEA) and 2-arachidonoylglycerol (2-AG) are endogenous ligands derived through hydrolysis of AA by amidohydrolase. Ligands derived from ω-3 LCPUFAs also exist as: (1) *N*-docosahexaenoylethanolamide (DHEA) and 2-docosahexaenoylgylcerol (2-DHG) from DHA (Straiker et al., 1999a). Bovine retina metabolizes and produces 2-AG, AEA, NArPE, 2-DHG, DHEA, and NDHPE, and biosynthetic and hydrolytic enzymes (Straiker et al., 1999b). DHEA and AEA were released from bovine retinal membranes in a time-dependent manner.

Activated CB receptors induce inhibition of adenylate cyclase and N- and P/Q calcium channels. MAP-kinase and K^+ channels are activated with CB1 receptor binding. Activity of CB-based signaling systems may lead to alterations of light sensitivity. Straiker et al. (1999b) have demonstrated that cannabinoid 1 (CB1) receptors are distributed in the outer and inner plexiform layers of vertebrate retina. CB1 receptors were observed in cone pedicules and rod spericals. Within rat retina 2-AG exists as an endogenous CB1 ligand (AEA was not detected) and palmitylethanolamide exists as an endogenous CB2 ligand. Electrophysiology of CB1 activation in tiger salamander demonstrated a CB-modulated depression in currents of voltage-gated L-type calcium channels of retnal bipolar cell axon terminals. Current was restored with SR141716A, a CB1 antagonist (Straiker and Sullivan, 2003). These authors make the conceptual link the CB1 activation may modulate calcium-dependent glutaminergic signaling systems in bipolar and amacrine cells, since glutamate is the main neurotransmitter used at these synapses. They also suggest that, since CB1 receptors are localized on photoreceptor synaptic terminals, that light-induced reduction in glutamate release in these cells may be mimicked by activation with endogenous ligands. There is also evidence to suggest that dopamine acts as neuromodulator of endocannabinoid release (Self, 1999).

5.8.3. Glutamate and PAF

Glutamate is implicated in anoxic and excitotoxic retinal injury. It is also the main excitatory amino acid neurotransmitter used to transduce signals at the initial chemical synapse between photoreceptors and second-order retinal neurons. Post-synaptic glutamate receptors on ON-center bipolar cells are metabotropic in nature; signaling occurs via a cGMP-activated second messenger system. Receptors on OFF-center bipolar cells and

horizontal cells are ionotropic and operate through *N*-methyl-D-aspartate (NMDA) and amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA)/kainate, and sodium ions. Glutamate may act as a cytotoxin by increasing phosphorylation of neurofilament proteins in neuronal cell bodies and axons (Barber, 2003).

PAF is a neuroactive alkyl phospholipid that is capable of modulating glutamate release. As discussed above, PAF increases with PLA_2 activation and PAF accumulation is associated with retinal injury response to photic damage (Reme et al., 1992) and ischemia (Kumar et al., 2003). The $sPLA_2$-OS2 isoform of PLA_2 binds strongly to neural receptors where it acts in vivo as a neurotoxin via activation of glutamate ionotropic receptors (Kolko et al., 2002). Also, in vitro, PAF has exhibited direct neurotoxic actions.

Mitogen-activated protein (MAP) kinase cascades may be activated by stress-sensitive factors (c-Jun N-terminal kinase (JNK) and p38 pathway) and growth factors, (ERK or p42/44 pathway). PAF and glutamate may activate ERK, JNK, and p38 MAP-kinase pathways via activation of NMDA or kainate receptors (DeCoster et al., 1998). Low doses of DHA decreased ERK and JNK activation. p38 activity was not significantly altered by DHA or EPA (Yusufi et al., 2003). In a mouse model of colon cancer DHA reduced localization of the Ras regulatory protein to the plasma membrane and lowered GTP binding and p42/44(ERK)-dependent signaling (Collett et al., 2001).

Wilson (1997) reviews the role of astrocytes in neuroprotection, as they relate to excitotoxicity. Brain astrocytes are less susceptible than oligodendroglia or neurons to cellular damage and death induced by oxidative stress. Astrocytes are also 25-times less sensitive than oligodendrocytes and neurons to glutamate-induced reactive oxygen species-mediated cytotoxicity. Wilson discusses a number of factors that may contribute to cellular life-preserving and neuroprotective actions of brain astrocytes. These include: (1) relatively high cytosolic concentrations of compounds with anti-oxidant properties; (2) the capacity for transport and metabolism of amino acids and glucose; and (3) the capacity for transport of vitamin C and re-reduction of oxidized forms of this compound (dehydroascorbic acid) to its original state.

Lucius and Sievers (1996) observed a cytoprotective effect of astrocytes on axonal degeneration in retinal ganglion cells sustaining exposure to iron, nitric oxide, and the superoxide anion radical. Astrocytes may also act in a cytoprotective role through secondary active transport of glutamate from extracellular fluid. Within the cellular compartment glutamate is converted to glutamine, and via the tricarboxylic acid cycle to lactate. One effect of astrocyte-based lactate release may be increases in local blood flow through dilation adjacent blood vessels (Wilson, 1997).

Most of the DHA in brain is derived from preformed DHA from plasma. DHA can be biosynthesized in brain astrocytes and then accreted to neurons and blood–brain-barrier endothelium (Bernoud et al., 1998). While models of cerebromicrovascular systems demonstrated that bovine brain capillary endothelial cells are capable of biosynthesizing DHA and AA, the greater proportion was accreted from astrocytes (Bernoud et al., 1998). In vitro systems of differentiated brain astrocytes are capable of biosynthesizing DHA from α-LLNA, EPA, and tetracosahexaenoic acid (24:6ω-3); although the rate of biosynthesis was decreased with various interventions to increase extra- and intracellular DHA availability, the process continued (Williard et al., 2001). Because DHA biosynthesis occurred under conditions of DHA abundance, the authors speculate that this process may supply compounds essential for astrocyte-associated actions.

5.8.4. Dopamine

Dopamine is a key neuromodulator in the retina. Local regulation of retinal microcirculation (capillary perfusion) may occur at the capillary level by pericytes (Wu et al., 2001). Neuron-to-capillary molecular signals for local capillary perfusion (regulation of blood flow) may be mediated by dopaminergic amacrine cells. Dopamine may also operate as a molecular signal that integrates neuronal activity with the function of 'pericyte-containing microvasculature.' Wu et al. (2001) applied a perforated-patch configuration of the patch-clamp technique to determine that dopamine is able to activate ATP-sensitive potassium currents in retinal pericytes through a pathway involving D_1 dopamine receptors, adenylate cyclase, and protein kinase A. Djamgoz et al. (1997) review the role of retinal dopamine systems with respect to the aging retina and RP. Fornaro et al. (2002) suggest that neuroleptic drug induced D2/D4 blockade may increase the risk of degenerative retinopathies among psychiatric patients. Animal models of DHA deficiency show reductions in brain dopamine (Wainwright, 2002; Takeuchi et al., 2002; Innis and de La Presa Owens, 2001).

5.8.5. Ionic transport and channel dynamics

ω-3 LCPUFAs may exert cytoprotective actions in their ability to modulate ion transport and channel dynamics. Effects of ω-3 LCPUFAs on ion channel dynamics are attributed mainly to the action of non-esterified (free) fatty acids (Kang and Leaf, 1996). Leaf et al. (1999) and De Caterina et al. (2003) review mechanisms by which ω-3 LCPUFAs may modulate ion currents in voltage-gated ion channels. Fish oil prevents calcium overload in model cell systems by exerting a concentration-dependent inhibition in the activity of L-type calcium channels (Hallaq et al., 1992). EPA

increased activity of the calcium pump enzyme Ca^{2+}/Mg^{2+}-ATPase of cardiac microsomes in a canine model of ventricular arrhythmia (Kinoshita et al., 1994). AA was only effective under conditions of COX-inhibition; this result was believed to be associated with decreases in the AA-COX substrate TXA_2. TXA_2 and some AA-based PGs act to increase intracellular calcium levels. Because specific activities of the sodium pump enzyme Na^+/K^+-ATPase and the NADPH-dependent enzyme cytochrome C reductase did not differ between cardiac microsomes of dogs in either infarction or control groups, Kinoshita et al. (1994) speculated that the observed diet-based membrane substitution of AA with EPA may be a way to reduce severity of ventricular arrhythmias through calcium regulation. Leaf et al. (1999) discuss the inhibitory effect of ω-3 LCPUFAs on the Na^+ current as a voltage-dependent shift from a resting to inactivated state (thereby inhibiting generation of the action potential). These authors also discuss ω-3 LCPUFA inhibition of Cl^- and Na^+ ligand-gated ion channels. The Cl^- channel is cAMP-dependent. We review the relationship of ion channel dynamics with cyclic nucleotide action in Section 4.3.

Neuringer and Jeffrey (2003) review work demonstrating that DHA and AA inhibits L-type Ca^{2+} channel currents in rod inner segments of salamander (Vellani et al., 2000) and then make the conceptual link that voltage-sensitive calcium conductance is involved in the regulation of neurotransmitter release from photoreceptor synapses with horizontal and bipolar cells. While the effects of DHA on the ligand-gated, G-protein-coupled receptors involved photoreceptor outer segment signaling systems have not been investigated, Neuringer and Jeffrey suggest such work may elucidate similar relationships. The effect of rhodopsin activation is to reduce cGMP. As discussed below, DHA may act in a similar capacity and this may be one reason that it is selectively accreted to and retained within photoreceptor outer segments.

cAMP is high in retina of animals with inherited degenerations of the neural retina. Dopamine modulates cAMP and calcium levels. D2/D4 immobilizes Ca^{2+}. Ca^{2+}/calmodulin dampens activity of cyclic nucleotide-gated channels (Broillet and Firestein, 1999). The important issue for the purpose of this review is that DHA reduces cAMP production (Grynberg et al., 1996) and down-regulates genes of cGMP isoforms (phosphodiesterase) (Narayanan et al., 2003). In a human feeding study, marine oil intake reduced cytosolic activity of cyclic nucleotide cAMP– and cGMP–phosphodiesterase (cGMP–PDE) complexes in human peripheral blood mononuclear cells (PBMCs) (Bechoua et al., 2003). There was concomitant increase in activity of these compounds in the particulate fraction of PBMCs; the authors interpret their findings to suggest ω-3 LCPUFAs were able to "favor the translocation" of the cytosolic PDEs to the membrane, which led to decreases rates of cAMP and cGMP hydrolysis.

Neuringer and Jeffrey (2003) review a number of mechanisms by which DHA affects phototransduction. As a major component of photoreceptor disc phospholipid membranes, the bond structure and acyl chain length of DHA imparts a number of biophysical properties that enhance lipid–protein interactions; these include high lateral compressibility (free volume) and low membrane order (Litman and Mitchell, 1996). As rhodopsin is isomerized by incident light, it increases in volume. The nature of the DHA-rich membrane environment facilitates lateral diffusion of the activated chromophore (all-*trans* retinol) and binding with key proteins in the phototransduction pathway (Niu et al., 2001). As the concentration of membrane DHA increases, the energy expended in rhodopsin activation decreases (Brown, 1994). Because it has high affinity to a lipid binding domain on IRBP, DHA may be involved in retinoid transport from the RPE to the photoreceptor outer segments; this is an essential step in the reconstitution rhodopsin to a form that renders it receptive to light energy (Chen et al., 1996, 1993).

5.9. Developmental processes

Pieri (2000) presents a brief overview of current theories of aging; he states: Aging can be defined as a multistep, time-dependent phenomenon characterized by the decreased capability of the individual cell, tissue, organ, and whole organism to respond to exogenous and endogenous insults from either physical, chemical, or biological agents. Developmental processes related to aging affect retinal structure and function. We focus here on tissue and changes in the chororiocapillaris–Bruch's membrane–RPE–photoreceptor interface, as this region is primarily affected in AMD.

5.9.1. Aging and the chororiocapillaris–Bruch's membrane–RPE—photoreceptor complex

We discuss retinal vascular networks in Section 5.4.1. Bruch's membrane is a pentalaminar choroidal structure that separates the RPE from the choriocapillaris. Layers include: the basement membrane of the RPE (composed of interwoven collagen and elastin); an inner collagenous layer that may bind the RPE to the choroids; an elastic layer that functions as the primary structural support; an outer collagenous layer; and the basement membrane of the choriocapillaris (Hogan et al., 1971). The diffusion characteristics of this critical structure permit exchange of nutrients and metabolites between the choricapillaris and the RPE, thus supporting the metabolic needs of the bioactive neural retina. Age-related changes in Bruch's membrane are associated with reduction in efficiency and effectiveness of diffusion. Changes include thickening (lengthening of diffusional path length) and decreases in

collagen solubility. The concomitant age-related reduction in density of the choriocapillaris may likewise alter diffusion characteristics (reviewed in Ambati et al., 2003).

There may be age-related changes in the specific activities of lysosomal enzymes operating in the RPE. Boulton et al. (1994) have observed that acid phosphatase and cathepsin D activities increase with age in human macula; they state that this condition may be an adaptive response to alterations in hydrodynamics and exchange rates between RPE and the choroid. Cathepsin D is a key enzyme in opsin proteolysis (Rakoczy et al., 1999). Enzyme activities may not be sufficient to handle an increased solute load and may result in deposition of macromolecules that impede diffusion. Ambati et al. (2003) suggest an opposite direction of relationship and cite work on a transgenic mouse expressing and inactive form of cathepsin D. Retinas of these animals are characterized by progressive degeneration of photoreceptors, hypertrophic RPE cells with autofluorescent deposits, and basal laminar and linear deposits. Aging is also associated with a decrease in the specific activity of a number of glycosidases in macular regions of fresh donor eyes; these include α-mannosidase, β-galactosidase, *N*-acetyl-β-glucosaminidase, and *N*-acetyl-β-galactosaminidase (Cingle et al., 1996). The authors note that a large proportion of retinal oligosaccharide chains are galactosylated and that mannose and *N*-acetyl-glucosamine are major carbohydrate monomers of these chains in human rhodopsin. Elner (2002) has examined the relationship of LCPUFA intake with lysosomal acid lipase activity in primate RPE cells. RPE cells of monkeys fed fish oil show increased lysosomal acid lipase activity; the author speculates that this condition may increase hydrolysis of intralysosomal RPE lipids and prevent development of AMD by reducing lipofuscin accumulation and RPE oxidative damage.

Cytoarchitecture of RPE cells changes from hexagonal to non-hexagonal with increasing age (Watzke et al., 1993); these authors suggest that such morphologic change is manifested in functional alterations that drive AMD pathology. Macular RPE cell density has been reported to decrease with age (Dorey et al., 1989; Panda-Jonas et al., 1996); others have not observed this condition (Gao and Hollyfield, 1992). Harman et al. (1997) observed higher RPE cell density in aged retinas than in than young retinas from corneal donor eyes. Del Priore et al. (2002) observed similar density of foveal RPE cells in older and younger cadaver eyes, but a greater density of apoptotic cells in older eyes. Most apoptotic cells in this group were located in the fovea. Within the neural retina, cones may be less susceptible to age-related loss than foveal cells in the ganglion cell layer and rods (Gao and Hollyfield, 1992). Jackson et al. (2002) review age related photoreceptor loss in detail and speculate that rod dysfunction in early AMD may lead to macular cell loss via alterations of retinoid translocation within the RPE/Bruch's membrane complex.

5.9.2. Aging and oxidative stress

Pieri (2000) states that the kinetics of aging are determined by multiple causes and suggests that free radical theory of aging as one supported by evidence from the field of free radical biochemistry. This theory has evolved from its original form (oxygen is a causal factor of aging) to the oxidative stress theory (age-related injury in cellular systems may result from an insufficient anti-oxidant potential and excessive oxidative stress that coexists with insufficient damage-repair and detoxification activity). The reformulated theory (Yu and Yang, 1996) recognizes:

1. The diverse physiological origin of reactive species. Species are derived from oxygen, glucose, protein, DNA, nitric oxide, aldehyde.
2. The value of assessing the balance of oxidants to anti-oxidant defense systems. Defense processes and factors include cellular compartmentalization, DNA repair, anti-oxidant enzymes, anti-oxidant vitamins, other compounds with anti-oxidant properties.
3. The various types of reactions between reactive oxygen species and other bioactive molecules. These include damage, damage-repair, detoxification.

Mitochondrial aging involves alterations in membrane composition and fluidity that are purported to negatively affect biophysical response in energy production. Study of this topic is germane to issues discussed throughout this work because the bulk of free radicals are derived from mitochondrial respiration. Aging membranes become less fluid and fluidity is determined largely in part by membrane lipid concentration and composition (phospholipid species, fatty acid acyl chain length, number of double bonds, position of the first double bond from the methyl terminal, phospholipids-to-cholesterol ratio). Mitochondria produce reactive oxygen species that target membrane lipids.

Beatty et al. (2000) list changes in oxidant load, elastin, collagen, and mitochondrial and nuclear DNA as putative pathogenic factors associated with age-related oxidative retinal damage. These authors observe that exogenous and endogenous retinal defense systems are compromised with advancing age. Age-related morphological and functional changes in retina include reduction in macular cone density (Farber et al., 1985) and loss of sensitivity of short-wavelength sensitive cones (Werner et al., 1989). It is interesting to note that the inner fovea is normally less susceptible to photic damage than adjacent areas (Haegerstrom-Portnoy, 1988). Morphological change also occurs within RPE

cells; lipofuscin accumulation is relevant issue in age-related eye disease and ocular lipid metabolism.

5.9.3. Lipofuscin

Lipofuscin is the term for a family of short-wavelength-photoreactive autofluorescent lipid–protein complexes that form deposits within lysosomes of post-mitotic neural (and other) cells. Lipofuscin is a marker of cellular senescence. Lipofuscin is also the major fundus fluorophore; it accumulates in RPE across the life-span to fill as much as 20% of total cytoplasmic volume by 80 years-of-age. Lipofuscin granules are uniform in size and concentrated most densely within the posterior pole (basal half) of RPE cells. Components of RPE lipofuscin are metabolites of photoreceptor phagocytosis (reviewed in Beatty et al., 2000; Winkler et al., 1999). High levels of lipofuscin may be associated with RPE and photoreceptor degeneration. Beatty et al. (2000) review possible mechanisms by which lipofuscin may alter RPE cell function. One is via a physical reduction in the volume of cytosol available to support basic cellular functions. Also, in human RPE cells, lipofuscin operates as a photoinducible generator of the superoxide anion, singlet oxygen, hydrogen peroxide, and lipid peroxides (Boulton et al., 2001; Davies et al., 2001; Rozanowska et al., 1998); the compound has highest potency at wavelengths in the blue spectrum (400–520 nm). A third mode of action is in the release of lysomotropic amines. *N*-retinylidene-*N*-retinylethanolamine (A2E), a pyridinium bis-retinoid, is the major constituent of lipofuscin (Wolf, 2003) and has been demonstrated to affect intra- and extracellular processes of lysosomal degradation (Holz et al., 1999; Bergmann et al., 2004).

The pathways of A2E biosynthesis are the subject of recent reviews (Liu et al., 2000; Mata et al., 2000). *N*-retinylidene PEA is formed via condensation in a Schiff base reaction from all-*trans*-retinaldehyde and PEA. All-*trans*-retinaldehyde is the isomerized chromophore of visual pigment (11-*cis*-retinaldehyde). PEA is present as a structural component of the photoreceptor outer segment disk membranes. *N*-retinylidene PEA then reacts with all-*trans*-retinaldehyde in another condensation reaction to yield a bis-retinoid within the rod outer segment; this compound is oxidized to form a pyridinium salt in the RPE. Finally, the pyridinium salt is stabilized by hydrolysis of its phosphate ester by phospholipase D. Liu et al. (2000) suggest that the fatty acid profiles of PEA constituent of A2E contain DHA and palmitic acid (16:0) or stearic acid (18:0).

5.9.4. Astrocytes

Relative to subjects younger than 60 years-of-age, there were reductions in the number of astrocytes covering retinal capillary networks in the ganglion cell layer and in the number of processes from astrocytes covering radial papillary capillaries in the nerve fiber layer (Ramirez et al., 2001). There were increases in the number of cytoplasmic organelles (indicating increased cellular activity) and in the number of reactive astrocytes. Reactive astrocytes of eyes of people with AMD had relatively higher concentrations of lipofuscin than astrocytes from eyes of people without AMD.

Relative to people over 60-years-of-age without AMD, people with AMD exhibited a thickening of the basal membrane of the retinal capillary bed/inner limiting membrane. A thick layer of collagen abutted astrocytes and Müller cells. People with AMD had many reactive and hypertrophic astrocytes with increased concentrations of lipofuscin and secondary lysosomes. Some of these astrocytes had trunk-shaped processes that crossed the inner limiting membrane and encroached the vitreous humour in a parallel orientation to the ILM. These processes were characterized by microvilli interfacing with vitreous humour in areas devoid of basal membrane. These cells thus share ultrastructural homology with epiretinal membranes. Ramirez et al. (2001) raise the possibility that such a system may be one adaptive response in creating an alternative system of nutrient and energy substrate transport within areas of retinal tissue exposed to extensive ischemia that expends metabolic reserve.

5.9.5. LCPUFAs affect factors and processes implicated in aging

How may LCPUFA status be affected by age, and how may LCPUFA status be expected to affect bioactive molecules influenced by age-related processes? Rotstein et al. (1987) demonstrated that aging rats show decreases in retinal DHA concentrations. Dietary DHA was effective in repleting tissue stores. Since biosynthetic enzyme systems were operational, the authors concluded that age-related decreases in tissue status were related to low intake of DHA. Similar results have been found in hippocampus (Favrelere et al., 2000; Delion et al., 1997) and frontal cortex (Delion et al., 1996) of rats fed diets containing α-LLNA.

Balazy and Nigam (2003) review characteristics of age-related increases in lipid peroxidation. Membranes from elderly subjects contain higher concentrations of esterified isoprostanes and hydroxylated fatty acids than do membranes of young subjects. Oxidation of AA has a higher likelihood to occur among aged populations than does oxidation of other LCPUFAs. The AA-autooxidation product, isoprostaglandin 8-*iso* PGF_{2a}, demonstrates increases as high as 50-fold, in aged rats relative to young rats (Morrow and Roberts, 1997).

Retinal LCPUFA status may affect A2E concentration. All-*trans*-retinaldehyde and PEA are necessary for production of A2E. In photoreceptor outer segments PEA is a DHA-rich moiety. DHA may be involved in reducing photobleached (all-*trans*) retinaldehyde to a

less cytotoxic 11-*trans*-retinol through IRBP mediated transport mechanisms (Chen et al., 1996). In many cases DHA composes *sn*-1 and *sn*-2 fatty acids of the outer segment disk phospholipids. Results of Liu et al. (2000) suggest that the PEA component of A2E commonly contains DHA and a 16- or 18-carbon saturated fatty acid. As enzymatic systems driving biosynthesis of LCPUFAs from EFA do not appear to be compromised with age, it may be that DHA of di-DHA PEA species are of dietary origin and mono-DHA species are of cellular origin. We consider this possibility based upon a study in mice fed a high DHA diet from 17- to 71-weeks-of-age (Du et al., 2003). Lipofuscin concentrations in brains of these animals were equivalent to those from animals fed a low ω-3 LCPUFA diet. We acknowledge that it may not be appropriate to extrapolate these findings to make assumptions about retinal lipofuscin, since the retina has a unique fatty acid composition and exposure profile.

6. Role of LCPUFAs in structure and function of vascular retina

A rapidly amassing evidence base suggests ω-3 LCPUFAs exert anti-angiogenic and -vasculogenic properties through modulation of processes involved in intracellular signaling, activation of transcription factors, and production of inflammatory mediators. Since ω-3 LCPUFA tissue status is modifiable by and dependent on dietary intake from foods that are not commonly consumed in the Western diet, these nutrients may be reasonable choices for diet- or supplement-based interventions to prevent neovascular (NV) retinal diseases. ω-3 LCPUFAs may also show merit as complementary therapies with COX (Masferrer et al., 2000) inhibitors and LOX metabolites (Hong et al., 2003; Serhan et al., 2000a, b; Serhan and Oliw, 2001). Selective COX-2 inhibitors are effective in preventing experimentally induced retinal neovascularization (Wilkinson-Berka et al., 2003) and production of factors involved in this event (Ayalasomayajula and Kompella, 2003; Joussen et al., 2002). COX-2 inhibitors may offer enhanced safety relative to other protective agents that simultaneously suppress COX-1 metabolite-based vasoregulatory activity.

6.1. *LCPUFAs affect pathogenic processes implicated in neovasculaization*

ω-3 LCPUFAs have the capacity to modulate activation of a number of factors and processes implicated in retinal neovascularization, inflammation of retinal vasculature, and alterations in retinal capillary ultrastructure and integrity. ROP and DR share common pathologic characteristics of fibrovascular proliferative expansion in the intraretinal capillary networks that normally nourish highly active neural retinal tissue. Vascular pathology in AMD is localized mainly within the choriocapillaris. Increased microvascular density is frequently accompanied by alterations in the integrity of the capillary walls. Vascular permeability may lead to abnormal vascular remodeling, capillary leakage, inflammation, and thrombosis. These processes are manifested in vitreous hemorrhage, fibrovascular scarring, mechanical stress, and subsequent retinal detachment. Dietary composition and balance of LCPUFA intake may modulate the balance and activity of angiogenic and anti-angiogenic factors.

By what mechanisms may LCPUFAs modulate factors and processes involved in retinal neovascularization? Addressing this question requires a basic understanding of neovascularization. In this work we have applied the term neovascularization to represent angiogenesis and/or vasculogenesis. Angiogenesis is the growth of new capillaries from existing vessels. Vasculogenesis involves differentiation of hemangioblasts or endothelial progenitor cells; although vasculogenesis occurs mainly in retinal development, it may occur in mature systems (reviewed in Das and McGuire, 2003). Nevascularization may proceed in a systematic manner; we have adopted the following framework to organize extant reports examining the relationship of LCPUFAs with this process:

1. Some chemical or physical insult induces damage of vascularized tissue.
2. Diseased tissue generates and releases lipid- and protein-based angiogenic factors.
3. Growth factors bind to and activate receptors on endothelial cells that compose existing blood vessels.
4. Activated complexes on endothelial cells send signals to the nucleus. This event activates signaling molecules and digestive enzymes.
5. Endothelial-cell-derived enzymes alter the integrity of the basement membrane pericytes that form and stabilize vessel walls, leading to vascular permeability.
6. Endothelial cells proliferate and migrate through the basement membrane toward growth factors.
7. Cellular adhesion molecules or integrins act as mechanical stimul to pull the vessel sprout forward.
8. MMPs are produced and dissolve tissue (extracellular matrix) in front of developing vessel tip.
9. Endothelial cells form blood vessel tubes. Tubes to establish functional loops.
10. Pericytes structurally support and stabilize new blood vessels.

6.1.1. *Production and release of angiogenic factors*

We review lipid- and protein-based angiogenic molecules in this section. Lipid-based molecules are

cyclooxygenase (COX) and LOX metabolites of LCPUFAs. Protein-based molecules include cytokines and growth factors.

6.1.1.1. Lipid-based molecules (eicosanoids). Eicosanoids are bioactive lipid-based molecules that modulate vascular function via intra- and intercellular signaling pathways. They are metabolized from 20-carbon LCPUFAs that have been released from membrane stores by membrane-based PLA_2 activity. Conversion from LCPUFA to prostanoids (PGs), TXs, prostacyclin (PGI_2), hydroxyeicosatetranoic acids (HETE) or LTs occurs through catalysis by COX or LOX. AA serves as the primary substrate in the COX pathway for 2-series PGs and TXs. COX exists in two forms: (1) endogenous (constitutive, COX-1), and (2) mitogen-, cytokine-, or endocrine-inducible (COX-2). AA also serves as the primary substrate in the LOX pathway to yield 5-, 12-, or 15-HETE and 4-series LTs. EPA is the substrate for 3-series PGs and TXs; it is metabolized by LOX to form 5-series LTs. Figs. 6 and 7 contain details on eicosanoid production for AA- and EPA-derived metabolites.

AA-based cyclooxygenase and LOX metabolites are extremely potent at low concentrations and act as autocrine hormones through G-protein mediated signaling pathways to increase cAMP levels at the site of biosynthesis. The most potent AA-derived angiogenic eicosanoids are PGE_2, PGI_2, TXA_2, and LTB_4. PGE_2 increases vasodilation and augments vasodilatory effects of bradykinin and histamine. PGI_2 induces vasodilation and cAMP production; it inhibits leukocyte and platelet aggregation. TXA_2 induces platelet aggregation and vasoconstriction. LTB_4 induces vascular permeability. Alterations in the integrity of capillary walls lead to vascular leakage and recruitment of immune system cells capable of producing inflammatory mediators and angiogenic growth factors. The existing instability of the capillary basement membrane then permits out-migration of activated vascular endothelial cells.

COX-catalyzed AA-metabolites induce vascular endothelial cell migration and tube formation (vs. endothelial cell proliferation) (Rose and Connolly, 2000). The AA-derived LOX product 12-HETE promotes tumor angiogenesis by inducing vascular endothelial cell migration and mitogenic activity in microvascular endothelial cells (Nie et al., 1998; Tang et al., 1995a). 12-HETE also enhances expression of cell surface integrin $\alpha_v\beta_3$ (Tang et al., 1995b; Liu et al., 1991). AA-derived LTs affect the production of TNF-α (Talvani et al., 2002).

Evidence exists to support the idea that ω-3 LCPUFAs alter both substrate and enzymes involved in the production of vasoregulatory eicosanoids. Nude mice with human breast cancer xenografts and fed ω-3 LCPUFA-rich diets showed displacement of AA from tumor cell membranes, a reduction in PGE_2 and 12-HETE (Rose and Connolly, 1999a; Rose et al., 1995), loss of COX-2 mRNA expression (Badawi et al., 1998), and reduction in COX-2 and COX-1 immunoreactive protein (Hamid et al., 1999). Both EPA and DHA inhibit COX-2 and COX-1 induction of PG biosynthesis (Ringbom et al., 2001). There may be a link between this

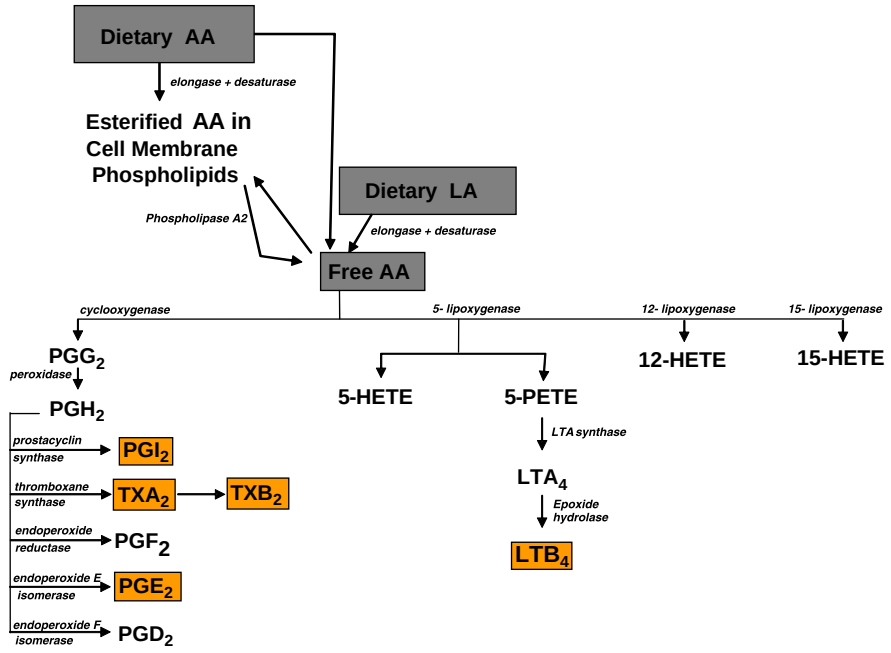


Fig. 6. Biosynthetic pathways for arachidonic acid (AA)-derived eicosanoids. Prostaglandins (PG), prostacyclin (PEI_2), and thromboxanes (TX) are produced through the cyclooxygenase pathway. Leukotrienes are produced through the 5-lipoxygenase pathway. LA = linoleic acid.

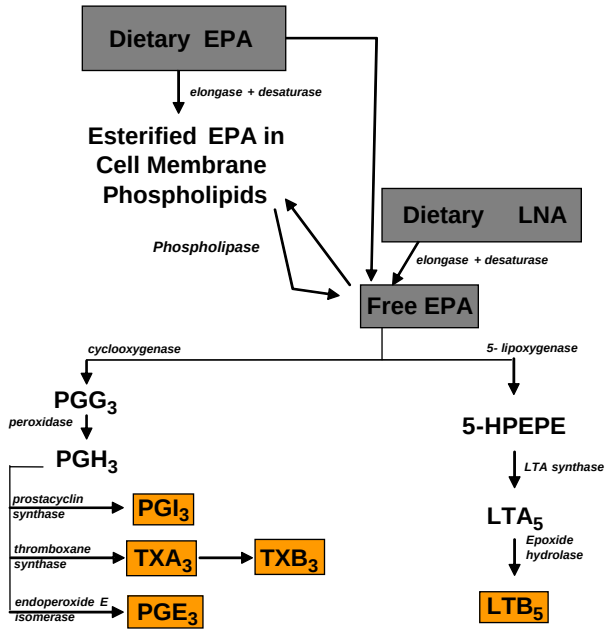


Fig. 7. Biosynthetic pathways for eicosapentaenoic acid (EPA)-derived eicosanoids. See caption for Fig. 6. LNA = α-linolenic acid.

pathway, growth factor expression, and angiogenesis. The selective COX-2 inhibitor celecoxib (SC-58635) is capable of regulating production of VEGF and VEGF receptors in animal models of retinal neovascularization (Ayalasomayajula and Kompella, 2003; Ozaki et al., 2002). This agent is also effective in suppressing FGF-2-induced corneal angiogenesis in rats (Masferrer et al., 2000). Calder (2001) discusses the ways by which ω-3 LCPUFAs may reduce the effect of AA-derived angiogenic eicosanoids:

1. Displacing stores of the ω-6 substrate (AA) available for catalysis in membrane phospholipids.
2. Inhibiting activation of PLA_2, the enzyme necessary to release the substrate for metabolism.
3. Competing effectively for biosynthetic eicosanoid synthases and peroxidases used for series-2 prostanoid and series-4LT production.
4. Providing substrate for production of less-bioactive eicosanoids of the 3-series (PG and TX) and the 5-series (LT).

What evidence exists to link eicosanoid metabolism with ischemia-induced retinal neovascularization? Concentrations of bovine retinal endothelial cell TXA_2 and PGI_2 increased linearly with increasing glucose feeding in an in vitro system designed to examine pathologic mechanisms in DR; however, the effect of high glucose on retinal endothelial cell PGE_2 was negligible (Johnson et al., 1999; Sone et al., 1996). Streptozotocin kills pancreatic islet B cells to produce hypoinsulinemia. Altered retinal eicosanoid metabolism was observed in a rat model of streptozotocin-induced diabetes (Johnson et al., 1999). Retinal vascular PGE_2 and PGI_2 was present in greatest concentrations among streptozotocin-diabetic animals with no glycemic control, as compared to diabetic animals with insulin replacement. The streptozotocin-diabetes rat model was also used to demonstrate increased production of TXB_2 in untreated diabetic animals (de la Cruz et al., 1998) 2-series TXs also appear to be involved in oxidative stress-induced ocular vasoconstriction that may induce neovascularization of ROP (Chemtob et al., 1995).

AA-derived LTB_4 affects the production of TNF-α (Talvani et al., 2002)· TNF-α is detectable in retinal vascular tissue and extracellular matrix of fibrovascular tissue in people with proliferative diabetic retinopathy (PDR) (Limb et al., 1996). A TNF-α gene polymorphism in the major histocompatibility complex (MCH) of people with non-insulin dependent diabetes mellitus (NIDDM) is associated with PDR; these results were interpreted to represent a form of genetic susceptibility (Hawrami et al., 1996). Vitreous levels of soluble TNF-α receptors were higher in eyes of with PDR than in eyes of healthy people without diabetes (Limb et al., 2001).

People with insulin-dependent diabetes mellitus (IDDM) and PDR have higher concentrations of soluble TNF-α receptors than: (1) people with IDDM who do not have PDR, and (2) healthy controls. Altered metabolism of active TNF-α was also observed in IDDM subjects with PDR (Limb et al., 1999). TNF-α production and expression is also modified in ischemia-induced retinopathy (Yossuck et al., 2001). Certain eicosanoids play an important role in angiogenesis as activators of cytokine producing cells. These are reviewed in the following section.

6.1.1.2. Protein-based molecules (cytokines and others). A number of protein-based angiogenic factors and their signaling pathways are affected by and affect LCPUFAs, eicosanoids, and PLA_2, COX, and LOX activities. These factors are classified as cytokines and growth factors and include VEGF, TGF-β, TNF-α, angiogenin, angiopoietin-1, FGF, follistatin, G-CSF, IL-8, leptin, and PDGF-BB.

Cytokines are regulatory proteins that function as chemical messengers within the acquired immune system and between natural and acquired immune systems; one mechanism by which cytokines operate is as ligands for cell surface receptors that induce cellular growth, development, and activity related to vascular remodeling. Cytokines are relevant for the purposes of this review since they can induce capillary leakage and leukocyte migration. The LOX-5 catalyzed AA-metabolite (LTB_4) directly increases the production of the proinflammatory cytokine TNF-α (Talvani et al., 2002); through its effect on this factor, LTB_4 indirectly increases production other proinflammatory cytokines

(IL-1, IL-2, IL-8, IL-6, IFN-γ) and the inflammatory lysophospholipid PAF. Fig. 4 illustrates these relationships.

Angiogenic growth factors are a class of molecules that may operate directly or indirectly on endothelial cells. Direct activity occurs via endothelial cell-surface receptors to induce endothelial cell migration and proliferation. This is the case with macrophage- and mast cell-derived polypeptide growth factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). Indirect mechanisms operate via induction of chemotaxis in macrophages that subsequently secrete VEGF and bFGF. This is the case of the eicosanoids reviewed in Section 6.1.1.1.

VEGF is a 45-kDa glycoprotein produced in the neural retina by astrocytes. It has specificity to several tyrosine kinase receptors (Flk-1, Flt-1) and has been identified as a primary inducer of PDR (Aiello and Wong, 2000). VEGF plays an essential role in induction of: endothelial cell migration and proliferation, microvascular permeability, endothelial cell release of metalloproteinases and interstitial collagenases, and endothelial cell tube formation (Ferrara and Davis-Smyth, 1997). This compound has been also shown to stimulate capillary meshwork formation in vivo (Yang et al., 1998) and has thus been implicated as a proangiogenic factor in ischemia-induced proliferative retinopathies (Aiello, 1997; Lu and Adamis, 2002; Smith, 2002).

Evidence from human and animal studies on ROP indicate that pathogenesis is both VEGF- and insulin-like growth factor (IGF) 1-dependent (Hellstrom et al., 2001; Smith et al., 1997; Smith et al., 1999). ROP has been modeled as a two-stage process in which a period of hyperoxia-induced vasoobliteration of first occurs as a result of supplemental oxygen for pulmonary insufficiency. As metabolic demands increase within the developing retina, the oxygen balance is shifted to a hypoxic state. This process is associated with VEGF upregulation (Pierce et al., 1995, 1996) and characterized anatomically by proliferation of retinal vascular tissue. Alterations of IGF-1 are associated both with preterm birth and phase I ROP (Hellstrom et al., 2001; Smith et al., 1999); IGF-1 modulates VEGF-induced activation of mitogen-activated protein kinase (MAPk). MAPk is a factor involved in endothelial cell proliferation. Low levels of IGF-1 inhibit VEGF-induced activation of protein kinase B (Akt). The Akt/PI-3 kinase signaling system modulates processes controlling vascular endothelial cell survival and activation, as well as leukocyte migration and adhesion.

How do LCPUFAs influence VEGF-dependent signaling systems? LCPUFAs affect VEGF-specific tyrosine kinase receptor activation and expression. EPA affected VEGF-induced proliferation of bovine carotid artery (BAE) endothelial cells by inhibition of the fetal liver tyrosine kinase 1 (Flk-1) receptor, a factor essential for the mitogenic processes of endothelial cell differentiation and proliferation. Neither DHA nor AA affected this system. Neither EPA, DHA, nor AA affected the VEGF-induced proliferation of endothelial cells mediated via the *fms*-like tyrosine kinase receptor (Flt-1), a factor involved in vessel construction (Yang et al., 1998). Unlike VEGF-, basic fibroblast growth factor (bFGF)-induced endothelial cell proliferation was not inhibited by EPA, DHA, or AA. However, EPA inhibited VEGF-induced activation of MAPk. bFGF-induced MAPk activation was not altered by EPA.

EPA down-regulated expression of Flk-1 receptors in a dose-dependent manner and up-regulated Flt-1 receptor expression (Yang et al., 1998). It also inhibited endothelial cell tube formation in vitro (Kanayasu et al., 1991a). In another experiment EPA down-regulated VEGF-specific tyrosine kinase receptor expression and suppressed the VEGF-induced tube-forming activity of endothelial cells in a dose-dependent manner. EPA-treated endothelial cells had low MAPk activity relative to comparison cells that were not incubated with EPA. The mechanism of VEGF receptor down-regulation is believed to occur at the tyrosine kinase NFκB site because EPA treatment caused suppression of NFκB activation. NFκB is a nuclear transcription factor that up-regulates COX-2 expression, intracellular adhesion molecule ICAM, and nitric oxide synthase (von Knethen et al., 1999).

ω-3 LCPUFAs may influence activation of IGF-1 pathways. Burn patients have low levels of IGF-1. In a study using nutritional support in a 15% fat diet with fish oil (50% of total dietary lipids from fish oil vs. 15% and 30% fat without fish oil), subjects returned to normal serum IGF-1 concentrations by 30 days post-intervention. Subjects in the no fish oil groups were consistently lower on this factor and did not approach normal values within the first 28 days of the study (Abribat et al., 2000). IGF-1 and VEGF interact in ROP via the Akt/PI-3 kinase signaling pathway, a serine/threonine kinase activated system. DHA enriched Neuro 2A cells survived staurosporine-induced apoptotic insult in via inhibition of normally observed decreases in Akt phosphorylation and activity (Akbar and Kim, 2002). Akt activity is also essential for vascular endothelial cell survival (Hellstrom et al., 2001).

The findings described above suggest that ω-3 LCPUFAs are capable of influencing growth factor mediated pathways in neovascularization. Retinal vascular endothelial cells interface with tight connections known as gap junctions. Hypoxia/reperfusion (H/R) injury sustained in ROP and DR compromises the integrity of the endothelial gap junctions and alters intercellular communication. Incubation of human vascular endothelial cells with EPA protected against

Table 3
Angiogenic factors affected by LCPUFAs, eicosanoids, cyclooxygenase, and lipoxygenase

Molecule	Reference
Vascular endothelial growth factor (VEGF)	Tsuji et al. (2003), Zhang et al. (2002), Tevar et al. (2002), Murota et al. (2000), Rose and Connolly (1999a,b), Yang et al. (1998), Mukutmoni et al. (2001), Mukutmoni-Norris et al. (2000)
Transforming growth factor-β (TGF-β)	Yu et al. (2003), Hida et al (2003)
Tumor necrosis factor-α (TNF-α)	Trebble et al. (2003), Mickleborough et al. (2003), Babcock et al. (2002), Kielar et al. (2003)
Angiogenin	Bicknell and Vallee (1989)
Angiotensin II	Kohno et al. (2000), Diep et al. (2002)
Fibroblast growth factors: acidic (aFGF)/basic (bFGF)	Murota et al. (2000), Kanayasu (1991)
Follistatin	Liu et al. (2002)
Granulocyte colony-stimulating factor (G-CSF)	Nakata et al. (2003)
Interleukin-8 (IL-8)	De Caterina et al. (2000)
Leptin	Cammisotto et al. (2003), Reseland et al. (2001)
Platelet-derived growth factor-BB (PDGF-BB)	Nitta et al. (1998), Hida et al. (2003), Kohno et al. (2000)

gap junctional intercellular communication injury after H/R challenge. This effect was mediated by inhibition of tyrosine kinase activation and hence provides a putative link to the VEGF receptor (Morita et al., 2001). Table 3 displays information on studies examining relationships between LCPUFAs and protein-based angiogenic factors.

6.1.2. Growth factors bind to receptors on endothelial cells

Yang et al. (1998) observed that Flk-1 expression was inhibited in EPA-treated in bovine carotid artery endothelial (BAE) cells. Flt-1 expression was increased.

6.1.3. Endothelial cells are activated and send signals to the nucleus for production of signaling molecules and enzymes

EPA selectively inhibited VEGF-induced, but not bFGF-induced activation of MAP kinase (Yang et al., 1998). Members of the ERK MAP kinase family respond to proliferative and mitogenic stimuli, regulate changes in transcription, and are associated with cellular differentiation and proliferation. The c-Jun amino terminal kinas (JNK) and p38 MAPK pathways may be activated by exposures associated with retinal disease; these include hypoxia, ultraviolet light, inflammatory cytokines, osmotic shock, or environmental stress (Kumar et al., 2003). Bogatcheva et al. (2003) discuss the role of MAPKs in endothelial barrier function.

6.1.4. Enzymes digest the basement membrane

Incubation of human vascular endothelial cells with EPA protected against gap junctional intercellular communication injury after hypoxia/reperfusion challenge. The effect was mediated by inhibition of tyrosine kinase activation (Morita et al., 2001). EPA inhibits urokinase-type plasminogen (uPA) activator activity (du Toit et al., 1996). uPA catalyzes conversion of plasminogen to plasmin. Plasmin is a serine proteinase that is involved in conversion of fibrin to soluble forms. uPA is implicated in retinal neovascularization and may affect cell associated proteolytic activity (McGuire et al., 2003).

6.1.5. Endothelial cells divide and migrate through basement membrane toward growth factors

ω-3 LCPUFAs prevent serotonin-induced endothelial cell proliferation (Pakala et al., 1999). Bovine aortic endothelial cells, treated with 0–5 μg/ml EPA for 48 h, displayed a dose-dependent suppression to VEGF (0.2 nM)-induced proliferation (Yang et al., 1998). EPA suppressed and serum-induced endothelial cell migration in bovine endothelial cells (Kanayasu et al., 1991a). When bovine carotid artery endothelial cells were cultured between collagen gels, EPA inhibited cell growth in a dose-dependent manner; maximum inhibition existed at 2.5 μg/ml (Kanayasu et al., 1991b). Prostacyclin (PGI_2) is the main prostanoid in most vascular systems. PGI_2 regulates proliferation of vascular endothelial cells. Changes in the fatty acid composition of membrane phospholipids induced by incubation of human umbilical vein endothelial cells (HUVEC) in serum-free medium with allogeneic peripheral blood lymphocytes with EPA or DHA resulted in reduced basal PGI_2 production (Dominguez et al., 2001).

6.1.6. Adhesion molecules, or integrins ($\alpha_v\beta_3$, $\alpha_v\beta_5$) help pull the blood vessel sprout forward

DHA incubation of human adult saphenous vein endothelial cell cultures reduced endothelial expression of VCAM-1, E-selectin, ICAM-1, IL-6 and IL-8 after challenge with IL-1, IL-4, TNF-a, or bacterial endotoxin (De Caterina et al., 2000). Primary HUVECs

activated with IL-1β will yield ICAM-1, E-selectin and VCAM-1 transcripts. Both EPA (65 μM) and DHA (65 μM) attenuate induction of these adhesion molecules after challenge with IL-1β (Collie-Duguid and Wahle, 1996).

6.1.7. *MMP are produced to dissolve the tissue in front of the sprouting vessel tip in order to accommodate it. As the vessel extends, the tissue is remolded around the vessel*

LCPUFAs affect activity of MMP activation. MMPs are a family of neutral zinc endopeptidases. They are usually secreted as pro-enzymes and activated in extracellular areas. MMPs modulate aspects of angiogenesis and inflammation. They also affect tissue remodeling through: degradation of specific extracellular matrix components; destruction of cell surface proteins, cytokines, proteinase inhibitors; and activation or release of signaling molecules and proteinases. Expression of most MMPs is induced by cytokines (including TNF-α, IL-1), growth factors (including VEGF, bFGF), and reactive oxygen species. Endogenous tissue inhibitors of metalloproteinases (TIMPs) bind non-covalently to the zinc-dependent active site of MMPs to regulate their activity.

MMP-2 (Gelatinase A, 57-kDa) and MMP-9 (Gelatinase B, 92-kDa) operate against type IV collagens and laminins that compose vascular endothelial basement membranes. MMP-2 is endogenous to normal human vitreous. It binds on vascular endothelial cell surfaces to $\alpha_v\beta_3$ integrin during the process of capillary tube formation. MMP-9 is constitutively produced in the retinal ganglion cell layer. These MMPs exist in the IPM and are implicated in the pathogenesis of ROP, PDR, and Sorsby's fundus dystrophy (Sivak and Fini, 2002). MMP-2 and MMP-9 expression was elevated in C557Bl/J6 mice with ischemia-induced retinal neovascularization, relative to animals reared under normal conditions. TIMP-1 (MMP-9 inhibitor) and TIMP-2 (MMP-2 inhibitor) were decreased within these same animals, indicating that these extracellular proteinases are important factors in retinal angiogenesis (Majka et al., 2001). The mechanism by which MMPs are up-regulated occurs via soluble mediators TNF-α and VEGF (and not directly, via the hypoxic insult introduced in the ischemic challenge) (Majka et al., 2002).

What is the relationship between LCPUFAs and MMP activity? Serum MMP-2 and -9 activity was decreased in female CD-VAF rats fed DHA-containing diets with various compositions of EFAs and LCPUFAs, relative to a comparison group fed DHA-free diets (Harris et al., 2001). CDF_1 and BALB/c mice receiving diets enriched in DHA had significantly lower MMP-9 activity in tumor tissue extract than animals fed an LCPUFA-free diet. MMP-9 activities were also significantly lower in animals consuming DHA + oleic acid and DHA + LA, relative to controls fed a DHA-free diet. Gelatinolytic (MMP-2 and -9) activities were not significantly different between controls and animals consuming oleic acid, LA, EPA, and EPA + DHA enriched diets (Suzuki et al., 1997). EPA treatment in nude mice prevented development of lung metastases from MDA-MB-435 human breast cancer cell mammary fat pad solid tumors. Incubation with 0.25–1.0 μg/ml resulted in a dose-related inhibition of cultured MDA-MB-435 cell 92-kDa type IV collagenase (MMP-9) mRNA expression (Liu and Rose, 1995).

6.1.8. *Sprouting endothelial cells form tubes*

When the endothelial cells isolated from bovine carotid artery were treated for 2 days with 1.0–5.0 μg/ml EPA the inhibition of tube formation was dose-dependent. At 5.0 μg/ml of EPA the inhibition reached 76%. AA increased tube formation, and DHA had no effect (Kanayasu et al., 1991a, b). DPA (22:5ω-3) suppressed tube-forming activity in endothelial cells induced by VEGF (Tsuji et al., 2003). Endothelial cells (cultured in collagen gel) treated with 0.5–5 μg/ml EPA (EPA, 20:5ω-3) for 48 h displayed a dose-dependent suppression of tube formation, VEGF-induced proliferation, and activation of p42/p44 MAP kinase but not bFGF-induced ones (Murota et al., 2000).

7. Retinal diseases of public health significance

The factors and processes that activate PLA_2, COX, LOX, and the bioactive molecules discussed above are associated with a number of retinal diseases of public health significance. These diseases usually manifest both vascular- and neural pathology. Here we discuss DR, AMD, and ROP.

7.1. *Diabetic retinopathy*

DR is the most common retinal disease in US residents aged 18–55 years. Researchers at the Centers for Disease Control and Prevention (CDC) approximate that of the 16 million people in the US living with diabetes, one-in-three is undiagnosed. Prevalence of DR in the US among persons over 18 years-of-age is 2.5%. This amounts to approximately 5.3 million with some form of DR; 24,000 people are blinded each year by the disease. PDR may be present in more than half of those who have had type 1 diabetes for 15 years; the value is approximately 10% for those with type 2 diabetes sustaining the same duration of disease. Researchers from the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) report that, after 10 years of follow-up, 20% of subjects with type 1 diabetes had macular edema; incidence in people with type 2 diabetes was 25% and 14%, respectively, for those who were using insulin and those who were not (Klein et al., 1995).

DR exhibits aspects of both reactive neovascularization and neural degeneration that may be modulated by ω-3 LCPUFAs. Vascular and neural pathology in DR is discussed in the next sections.

7.1.1. DR and the vascular retina

Frank (2004) reviews the clinical and histopathological manifestations of DR with a focus on pathogenic mechanisms in the vascular retina. DR affects both vascular tissue and blood, resulting capillary occlusion, which is a major feature of the disease. DR is characterized by alterations in the integrity of retinal capillaries, vascular leakage, neovascularization, and hemorrhage. Microaneurysms and dot intraretinal hemorrhages exist early in the history of DR. As the disease becomes more severe, retinal microvascular circulation is compromised, resulting in ischemia that is manifested as increases in size and number of intraretinal hemorrhages; cotton wool spots may appear at this time. Proliferating new vessels may break through inner limiting membrane into the vitreous, hemorrhage, and eventually lead to tractional or serous retinal detachment. Macular edema is a complication of DR related to breakdown of the blood-retinal barrier. In this condition, plasma leaks from retinal microvasculature; upon resorption, lipid and lipoprotein precipitates result in formation of hard exudates.

Pericytes are mesenchymal-like cells of microvascular networks that encase retinal capillary endothelial cells. Pericyte dropout occurs in early stages of DR and is associated with subsequent capillary endothelial cell loss. Apoptotic cell death is speculated operate in both instances (Mizutani et al., 1996). Pericytes may act as local regulators of capillary blood flow; evidence to support this concept includes presence of dilated capillaries in pericyte-free tissue, high pericyte concentration of smooth muscle alpha actin (a contractile filament), the presence of pericyte endothelin (ET)-1 (ET-1 is a strong vasoconstrictor secreted by retinal endothelial cells) receptors. In addition to this autoregulatory role, pericytes may also regulate endothelial cell growth and proliferation, as in vitro co-culture systems used by Orlidge and D'Amore (1987) have demonstrated a contact-dependent inhibition of endothelial cell multiplication (reviewed in Hirschi D'Amore, 1997).

Retinal circulation is able to 'autoregulate' on the basis of local and systemic signals. People with diabetes show dysfunction in this capacity. The blood-retina-barrier (BRB) is composed of tightly joined endothelial cells that separate the neural retina from the circulatory system and its constituent inflammatory cells and proteins. Astrocyte processes encase blood vessels within the nerve fiber layer, and through an unknown mechanism, initiate expression of the tight junction protein ZO-1. Diabetes and its complications are associated with altered astrocyte function and morphology. Müller cell function is likewise affected. A major function of Müller cells is to regulate glutamate metabolism. Impaired capacity to reduce this amino acid signaling molecule to glutamine may result in accumulation to subsequent neurotoxicity (Gardner et al., 2002).

7.1.2. DR and the neural retina

Barber (2003) suggests that chronic neural degeneration is a critical component of DR and is inextricably linked with vascular pathology. Steptozotocin (STZ)-induced diabetes in rats is associated with elevated apoptosis in the retina, decreased numbers of ganglion cells (and thinning of inner retina) (Barber et al., 1998; Zeng et al., 2000), and retinal nerve fiber layer thickness (Lopes de Faria et al., 2002). Neurofilament proteins are essential structural molecules of cell bodies and axons. Elevated rates of phosphorylation in neurofilament proteins detrimentally affect cell viability. People with diabetes (McLean, 1997), the diabetic BB rat, and the STZ-induced diabetic rat (Fernyhough et al., 1999) exhibit aberrant neurofilament phosphorylation and decreased neurofilament mRNA in sensory neurons of the peripheral nervous system. Barber (2003) has considered the possibility that cytoskeletal protein in retinal ganglion cells will be influenced in similar ways. Humans with diabetes (Mizutani et al., 1998) and rats treated with STZ (Lieth et al., 1998; Rungger-Brandle et al., 2000) exhibit increased expression of intermediate filament glial fibrillary acidic protein in Müller cells (GFAP), a marker for neural degeneration. Li et al. (2002) observe GFAP localization starts at the endfoot (in the vitreo-retinal border) and moves throughout the entire length of the Müller cells with increasing duration of diabetes. In the STZ-induced diabetic rat GFAP expression within astrocytes did not increase substantially, relative to levels in non-diabetic control animals (Li et al., 2002); in one study astrocyte GFAP was reduced (Barber et al., 2000). Glutamate is an excitatory amino acid that is involved in neural toxicity. Glutamate metabolism and transport may be altered in diabetes, leading to a state of chronic glutamate excitotoxicity (reviewed in Barber, 2003).

7.1.3. Putative role of ω-3 LCPUFAs in modulating factors and processes implicated in pathogenesis of DR

Bhathena (2000) reviews the issues dietary fatty acids and fatty acid metabolism in diabetes. He states that glucose metabolism is closely linked to lipid metabolism; insulin is a key factor modulating these processes. Tissue lipid profiles of people with diabetes are characterized by lower than normal concentrations of LCPUFAs, and higher than normal concentrations of monounsaturated and saturated fatty acids. Diabetes is also characterized by increased lipolysis and altered lipogenesis which leads

Table 4
Etiologic factors for diabetic retinopathy and putative actions of ω-3 LCPUFAs

Factor/process	Putative pathogenic mode of action	Putative action of $\omega-3$ LCPUFAs
INFLAMMATORY MOLECULES	↑ leukocyte adhesion → ischemia BRB breakdown → macular edema	EPA and DHA⊣ eicosanoids cytokines (Section 5).
PKC	PKC → VEGF DAG → PKC → vascular abnormalities	EPA⊣ DAG (Kuroki et al., 1998)
ROI	Lipid, protein, and amino acid damage	EPA/DHA⊣in vivo oxidants (Mori et al., 2003)
NO	NO → ROI NO → VEGF	DHA⊣ iNOS expression (Komatsu et al., 2003) DHA⊣ NFkB (Komatsu et al., 2003)
VEGF	VEGF → BRB breakdown → macular edema VEGF → endothelial proliferation → neovasc.	EPA⊣ VEGF-ind. proliferation (Yang et al., 1998)
PERICYTE DAMAGE	Pericytes modulate endothelial cell growth and proliferation in vitro via cell-cell contact.	EPA/DHA⊣ pericyte degen. (de Wilde et al., 2002)
ET-1	ET-1 → vasoconstriction. Retinal microvascular pericytes contain ET-1 receptors.	DHA⊣ ET-1-ind. Ca^{2+} increase (Rinaldi et al., 2002) EPA⊣ ET-1 production (Nitta et al., 1998)
AGE	AGE⊣inactivate enyzmes → pericyte survival AGE → protein glycosylation⊣cell survival	$\omega-3$ LCPUFAs⊣AGEs (El-seweidy et al., 2002)
ENDOTHELIAL CELL DAMAGE	Endothelial cells → retinal capillary perfusion.	
Haemodynamics	↓ autoregulation → ΔO_2 conc. → endothelium.	DHA⊣ collagen aggregation (Agren et al., 1997) DHA⊣ TXB2 (Woodman et al., 2003)
	Platelet activity → occlusion RBC aggregation → occlusion	DHA⊣ PAF-ind. platelet activity (Mayer et al., 2002) DHA⊣ platelet aggregation (Akiba et al., 2000) DHA⊣ RBC aggregation (Agren et al., 1997)
Glucose toxicity	Hypoglycemia → Δmito. metabolism Hypoglycemia → ROI	DHA → glucose absorption (Onuki et al., 2000)

Note: AR = adose reductase, AGE = advanced glycation end products, BRB = blood retina barrier, conc. = concentration, DAG = diacylglycerol, ET-1 = endothelin-1, GH = growth hormone, ind. = induced, IGF-1 = insulin-like growth factor-1, iNOS = inducible nitric oxide synthase, PEDF = pigment epithelium derived Factor, PKC = protein kinase C, ROI = reactive O_2 intermediate, ROS = reactive O_2 species, VEGF = vascular endothelial growth factor. ↑ = enhancement, increased expression, or activation; Δ = change; ↓ = reduction; ⊣ = decreased expression, inhibition or deactivation; → = factor or process leads to outcome.

to increased plasma concentrations of unesterified fatty acids. Table 4 displays key factors and processes implicated in pathogenesis of DR, the possible pathogenic actions, and the mode of action of ω-3 LCPUFAs on the factor or process.

7.2. Age-related macular degeneration

AMD is the leading cause of vision loss in people aged 65 years or more. In the US the number of people in this age group is projected to increase by 53% from 35 million in 2001 to 53 million in 2020. This demographic shift will lead to increases in the prevalence of age-related eye disease. More than 1.6 million Americans aged 50 or older have sight-threatening forms of AMD.

Early forms of AMD are characterized by deposits of extracellular material between the basal lamina of the RPE and the inner collagenous layer of Bruch's membrane. These deposits are known as drusen. Drusen are protein–carbohydrate–lipid complexes present in most people diagnosed with AMD; Hageman et al. (2001) provide an informative overview of drusen ultrastructure and biogenesis. There is a substantially increased risk of progression to advanced AMD among people with certain drusen characteristics (Abdelsalam et al., 1999).

Advanced forms of AMD are characterized by geographic atrophy (GA)/RPE depigmentation, RPE detachment, and/or choroidal neovascularization (CNV). While approximately 90% of people with advanced AMD have the atrophic the form of the disease, the burden of AMD-related blindness is attributed mainly (~90%) to the NV form. Advanced AMD exhibits aspects of both reactive neovascularization and cellular degeneration that may be modulated by ω-3 LCPUFAs. Vascular and neural pathology in AMD is discussed in the next sections.

7.2.1. Advanced AMD and CNV

Ambati et al. (2003) review morphologic forms of AMD and the natural history of the disease. NV AMD is characterized by CNV: growth of new vessels (vs. angiogenesis or sprouting from existing choroidal vessels) in the primary capillary bed of the choroid, the choriocapillaris. NV networks may break through Bruch's membrane and the RPE to occupy the subretinal space. Hemorrhage may occur either in sub-RPE or subretinal tissue leading to exudation of blood,

serum, and lipids that may eventually induce reactive fibrogliosis that results in a disciform scar.

7.2.2. Advanced AMD and degeneration of RPE and neural retina

The RPE is a melanin-rich monolayer of cells derived from neuroectoderm. The RPE is located between the choriocapillaris and the sensory retina; cells are polarized with infoldings that abut Bruch's membrane at the basal region and microvilli that project between photoreceptor outer segments at the apical region. At apical regions the tight junctions existing between neighboring cells render the RPE–photoreceptor interface impervious to diffusion of macromolecules. Apical villar processes contain melanin granules. The basal region contains a nucleus, "metabolic and synthetic organelles", outer segment phagosomes, and oil droplets. RPE cells act in a homeostatic role within the outer retina where they form constituents of the BRB, regulate transport of metabolites and nutrients through the IPM, absorb scattered light, and exclusively phagocytize the shed tips of photoreceptor outer segments.

GA is a condition characterized as confluent areas ($\geqslant 175\,\mu m$ in diameter) of RPE death and photoreceptor atrophy. Frequently the cone-dense fovea is spared until late in the course of disease. In some instances the choriocapillaris exhibits atrophic regions. Dunaief et al. (2002) have demonstrated in eyes of people with GA that RPE, photoreceptors, and inner nuclear layer cells die by apoptosis.

Activities of macro- and microglia are altered in advanced AMD. The two major types of retinal macroglia are astrocytes and Müller cells. Astrocytes are glial cells whose cell bodies and processes are found almost exclusively in the nerve fiber layer; processes surround blood vessels adjacent to ganglion cell bundles. They are most concentrated in the optic nerve head and do not exist within the fovea or ora serrata. Morphology of astrocytes changes from an elongated structure in the central retina to a symmetrical stellate structure in eccentric regions (Schitzer, 1988). Astrocyte bodies and processes ensheathe retinal ganglion cell axons, adjacent blood vessels, and act as constituents of the blood-retinal-barrier. They are in close contact with retinal endothelial cells, fibroblasts, and mesenchymal cells. Outside of this structural role, they are speculated to provide energy substrates to adjacent neurons, modulate ionic homeostasis of extracellular K^+, and regulate metabolism of GABA (Kolb et al., 2004).

Müller cells are cells of neuroepithelial origin with cell bodies located in the inner nuclear layer; they send irregular processes to the outer- and inner limiting membranes. Ramirez et al. (2001) examined retinas of 6 eyes from people > 70 year-of-age with histopathologic findings representative of AMD. Morphology and distribution of astrocytes and Müller cells in these samples was different than those from 8 "normal" eyes from elderly donors without histopathologic indication of any eye disease. The AMD samples contained large numbers of reactive and hypertrophic astrocytes in the ganglion cell layer. These cells contained high concentrations of lipofuscin and secondary liposomes. There were also GFAP positive processes from cells with ultrastructural characteristics to astrocytes and Müller cells that crossed from the inner retina into the vitreous to form membranous structures. The authors speculate that astrocytes had migrated to the vitreous in an effort to obtain metabolic substrates.

Microglia are stellate cells of mesodermal origin that surround inner retinal vessels. They are found in all retinal layers. Upon activation, microglia migrate to the site of tissue damage and act as phagocytes; they may also secrete cytotoxic molecules that affect healthy cells around the damaged area. Gupta et al. (2003) have observed activated microglia in the outer nuclear layer of retinas from 3 donors with GA. These cells contained rhodopsin-positive cytoplasmic inclusions and were located in areas of primary rod cell death. The authors observe that GA is characterized by a 'leading edge of photoreceptor apoptosis [that is] tailed by degeneration of adjacent photoreceptors' and offer the explanation of microglial activation to explain why loss of rods in the parafovea may precede central GA.

7.2.3. Putative role of ω-3 LCPUFAs in modulating factors and processes implicated in pathogenesis of AMD

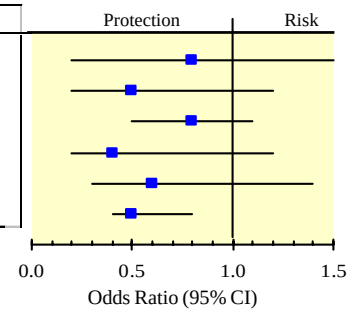
Extant epidemiologic studies examining the relationship of ω-3 LCPUFA or fish intake with prevalent advanced AMD suggest a protective relationship (Table 5).

Odds of AMD (with visual loss of 20/30 or worse in at least 1 eye) decreased with increasing intake of DHA, tuna, and total fish in a prospective sample from the Health Professionals Follow-up Study and the Nurses' Health Study (Cho et al., 2001). The relationship of DHA did not persist (OR for highest vs. lowest of DHA intake = 0.8; 95%CI, 0.5–1.1) when modeled simultaneously with intake of other dietary lipids, although the OR favored protection. The Dietary Ancillary Study of the Eye Disease Case Control Study (EDCCS) reported results from 349 participants with NV AMD (Seddon et al., 2001). The authors report a trend for decreasing odds of NV AMD with increasing amounts of ω-3 LCPUFA and fish intake. The relationship of ω-3 LCPUFAs with AMD was restricted to subjects with low LA intake. ORs for ω-3 LCPUFAs or fish for the highest vs. lowest quintiles were 0.6 (95%CI, 0.3–1.4) and 0.6 (95%CI, 0.3–1.1), respectively, and trends were statistically significant.

Three population-based studies examined the relationship of fish intake with 'late' age-related maculopathy (ARM) as classified by the Wisconsin

Table 5
Multivariable Odds Ratios for Neovascular AMD and late ARM

	Study Characteristics			
Study/Pub. year	Sampling Design	Exposure (high v. low)	Outcome	Cases
BDES 1995	Population-based	Fish	Late ARM	30
BMES 2000	Population-based	Fish	Late ARM	46
NHS/HPFU 2001	Prospective	LCPUFA	NV AMD	—
NHANES 2001	National Survey	Fish	Late ARM	9
EDCCS 2001	Case-Control	LCPUFA	NV AMD	349
AREDS 2003	Case-Control	LCPUFA	NV AMD	657



Note: BDES = Beaver Dam Eye Study; BMES = Blue Mountains Eye Study; NHS/HPFU = Nurses Health Study/Health Professionals Follow-up; NHANES = National Health and Nutrition Survey; EDCCS = Eye Disease Case-Control Study; AREDS = Age-Related Eye Disease Study. Late ARM = Geographic Atrophy or neovascular age-related macular degeneration; NV AMD = neovascular age-related macular degeneration.

Age-Related Maculopathy Grading System (Klein et al., 1991, 1992); in this system, late ARM represents NV AMD or GA. Late ARM was not associated with fish intake in the Beaver Dam Eye Study (BDES), although the range of intake was less than in subsequent reports (Mares-Perlman et al., 1995). Odds of late ARM relative to 1 or less serving of fish per month decreased as frequency of fish consumption increased among people aged 60 or older in the Third National Health and Nutrition Examination Survey (NHANES). Results were not statistically significant (OR = 0.4; 95%CI, 0.2–1.2) (Heuberger et al., 2001), as in previous studies the OR favored protection. Participants consuming 1 or more servings of fish per month were less likely to be diagnosed with late ARM than participants consuming less than 1 serving per month, in the Blue Mountains Eye Study (BMES) (Smith et al., 2000). In this study the relationship of fish intake with late ARM was non-monotonic. In most cases the relationship of fish intake with late ARM was not statistically significant. The exception was for the group consuming 1–3 servings per month (OR = 0.2; 95%CI, 0.1–0.6).

The relationship of lipid intake with incident AMD has been examined in a recent report (Seddon et al., 2003). Among subjects who reported lowest amounts of LA, those who also reported consuming two or more servings of fish per week were less likely to progress to advanced AMD, relative to those subjects reporting consumption of less-than-one serving of fish per week (OR = 0.4; 95%CI, 0.1–0.95).

Many pathogenic factors for DR (Section 7.1) are also implicated in AMD, although the primary vascular network affected in AMD is the choriocapillaris. Table 6 contains information on the putative actions of ω-3 LCPUFAs on such factors.

7.3. Retinopathy of prematurity

ROP is the leading cause of blindness and visual impairment during infancy. Incidence is highest among very low birthweight infants. Approximately 90% of infants weighing less than 750 g at birth develop some form of ROP; approximately 13% of these reach threshold for treatment. The percentages are 78% (incident ROP) and 45% (threshold ROP) for infants weighing 750–999 g. Among infants born at $\leqslant$1250 g, approximately 8% develop threshold ROP. Among untreated infants with severe ROP, half will progress to retinal detachment. Approximately 420 US-born infants lose vision each year from ROP; many others sustain visual impairment across the life-span. ROP exhibits aspects of both reactive neovascularization and cellular degeneration that may be modulated by ω-3 LCPUFAs. We discuss vascular and neural pathology in ROP within the next sections.

7.3.1. ROP and the vascular retina

Severe forms of ROP involve vascular leakage and remodeling that may result in fibrovascular proliferation, scarring, retinal traction, and detachment. Intraretinal microvascular networks begin development around the 16th week of gestation. The nasal retina is fully vascularized by 32 weeks of gestation. The temporal retina is fully vascularized around 40 weeks of gestation. In preterm infants a dense ridge of spindle cells accumulates between vascularized (posterior) and non-vascularized (anterior) retinal regions. Gariano (2003) suggests that these spindle cells are astrocytes that enter at the optic nerve prior to the intraretinal vessels; these spindle cells may operate establish a framework for vascular pericytes and endothelial cells.

Preterm infants may receive supplemental oxygen for pulmonary insufficiency. This intervention is associated with hyperoxia-induced apoptotic obliteration of the developing and existing retinal microvasculature. As neural systems develop in avascular retinal areas, there is a concomitant increase in metabolic load that results in hypoxia at greater-than-physiological levels. This condition leads to ischemia and is associated with activation of hypoxia-inducible factor (HIF)-1. HIF-1α

Table 6
Etiologic factors for age-related macular degeneration and putative actions of ω-3 LCPUFAs

Factor/process	Putative pathogenic mode of action	Putative action of ω-3 LCPUFAs
NEOVASCULARIZATION		
VEGF	VEGF → vascular endothelial cell proliferation VEGF → vascular endothelial cell migration VEGF → vascular endothelial cell survival VEGF → vascular endothelial cell permeability VEGF → uPA + tPA → ECM degradation VEGF → MMP → ECM degradation VEGF ↔ NO → vascular hyperpermeability VEGF → ICAM-1	EPA ⊣ VEGF KDR (Murota et al., 2000) ω-3 LCPUFA ⊣ VEGF KDR (Tsuji et al., 2003) EPA ⊣ VEGF FLK-1 (Yang et al., 1998) EPA ⊣ urokinase activity (du Toit et al., 1996) DHA ⊣ MMP-2 (Harris et al., 2001) DHA ⊣ ICAM-1 (De Caterina et al., 2000) DHA ⊣ VCAM (De Caterina et al., 2000)
Angiotensin	Ang1 → pericyte recruitment → vascular integrity Ang2 ⊣ Ang1 Ang2 + VEGF → neovascularization	DHA ⊣ Ang-2 (Diep et al., 2002)
Nitric oxide	NO → integrin $\alpha_v\beta_3$ → endothelial cell migration NO → integrin $\alpha_v\beta_3$ → endothelial cell different. VEGF ↔ NO → vascular hyperpermeability VEGF → eNOS → NO → VEGF eNOS → vasoobilterartion eNOS → vitreous neovascularization iNOS ⊣ VEGF receptor expression	DHA → iNOS (Hirafuji et al., 2002, 2003)
Extracellular matrix	Integrins + ECM proteins → endothelial migration TNF-α → $\alpha_v\beta_3$ → endothelial cell migration MMP → ECM degradation TIMP-3 ⊣ VEGF-ind. endothelial cell migration	DHA ⊣ TNF-α (Baldie et al., 1993) DHA ⊣ MMP-2 (Harris et al., 2001) ω-3 LCPUFA → TIMP (Curtis et al., 2002)
OXIDATIVE STRESS		
Reactive O_2 intermediates	Membrane lipid peroxidation Mitochondrial DNA damage H_2O_2 → p53/p21 → apoptosis H_2O_2 ⊣ bcl-2 ⊣ apoptosis tBHP → caspase → mito. damage → apoptosis H_2O_2 → VEGF in RPE → apoptosis	Fish oil → mitochond. fct (Demaison et al., 1994) Fish oil → ATP metabolsim (Grynberg et al., 1996) DHA → bcl-2 (Rotstein et al., 2003) DHA ⊣ caspase-3 (Kim et al., 2001) DHA → Akt/PI3 pathway (Akbar and Kim, 2002)
Hypoxia	Hypoxia → VEGF in RPE Hypoxia → Ang2	See VEGF and Ang Sections of Table (above)
Lipofuscin/A2E	Short wavelength light-induced RPE apoptosis A2E ⊣ photoreceptor lysosomal degradation Physical disruption of RPE cytoarchitecture	DHA → IRBP (Chen et al., 1996)
AGE/RAGE	AGE → RPE aging genes AGE → VEGF in RPE	ω-3 LCPUFA ⊣ AGE (El-seweidy et al., 2002)
HEMO-HYDRODYNAMIC Δ		
Bruch's membrane	Thickening ⊣ diffusion ⊣ choroidal perfusion	DHA → IRBP (Chen et al., 1996) DHA ⊣ collagen aggregation (Agren et al., 1997)
Choriocapillaris	↓ diameter ⊣ choroidal perfusion → transport ↓ density ⊣ choroidal perfusion → RPE transport	DHA → vascular compliance (Knapp, 1997)
Neutral fat deposits	Modification of diffusion characteristics of BM	DHA → IRBP (Chen et al., 1996)
RPE SENESCENCE		
AGE	AGE ⊣ gene expression lysosomal enzymes AGE shortened chromosomal telomeres	ω-3 LCPUFA ⊣ AGE (El-seweidy et al., 2002)
β-galactosidase	↓ proteolytic activity → RPE deposits	ω-3 LCPUFA → galactosidase (Kaur et al., 1996)

Note: The conceptual structure of this table was partially adapted from Ambati et al. (2003). AGE = advanced glycation end products; Ang = Angiopoietin; bFGF = basic fibroblast growth factor; BM = Bruch's membrane; eNOS = endothelial nitric oxide synthase; ECM = extracellular matrix; H_2O_2 = hydrogen peroxide; iNOS = inducible nitric oxide synthase; ICAM = intracellular adhesion molecule; IRBP = interphotoreceptor binding protein; KDR = VEGF receptor-2, kinase insert domain-containing receptor; MMP = matrix metalloproteinase; mitochond. fct = mitochondrial function; NO = nitric oxide; PEDF = pigment epithelium derived factor; ROI = reactive O_2 intermediates; RAGE = receptor for advanced glycation end products; RPE = retinal pigment epithelium; SWL = near-UV light; TIMP = tissue inhibitor of matrix metalloproteinase; tPA = tissue-type plasminogen activator; uPA = urokinase-type plasminogen activator; VEGF = vascular endothelial growth factor; Δ = change; ↓ = reduction; ⊣ = decreased expression, inhibition or deactivation; → = factor or process leads to outcome.

modulates transcription of VEGF- and other redox-sensitive genes for growth factors, proteinases, and cell-surface molecules; reactive neovascularization follows. In advanced ROP, NV turfs and fibrovascular structures may beak through the internal limiting membrane. Smith (2002) describes the role of bioactive molecules in modulation vasoobliterative and vasculogenic phases of ROP.

7.3.2. ROP and the neural retina

Fulton et al. (1999) observed disorganized and shortened rod outer segments within an infant rat model of ROP. Relative to animal raised in room air, attenuated photoresponses were measured in oxygen-exposed animals; results were attributed to variable and higher alterations in rhodopsin absorbance. A feline model of ischemia-induced ROP showed retinal astrocyte degeneration under exposure to hypoxic conditions that were induced upon introduction of animals to room air after a 4-day period of hyperoxia (Stone et al., 1996). Gu et al. (2002) used a mouse model of ischemia-induced ROP to demonstrate that astrocytes and Müller cells can sustain a period of hyperoxia and may be key factors in recovery from hypoxic insult.

Gariano (2003) reviews work examining mechanisms of retinal vascular development and states that retinal astrocytes may act as the key "cellular link" between a hypoxic condition and the process of neovascularization. He reviews literature indicating astrocytes demonstrate the capacity to: respond to neuronal signaling systems; transmit information in response to such stimulation; and synthesize and secrete VEGF in response to hypoxia. Evidence supporting the role of astrocytes in vascular development is strengthened on the basis that astrocyte-free areas are avascular.

7.3.3. Putative role of ω-3 LCPUFAs in modulating factors and processes implicated in pathogenesis of ROP

Maternal-fetal accretion of LCPUFAs and mineral cofactors of anti-oxidant enzymes (Zn, Cu, Mn) that protect the integrity of LCPUFAs occurs mainly in the third trimester of gestation. Prenatally, tissue status will depend on maternal preconceptional and prepartum lipid stores, maternal dietary choice, and materno-fetal tissue accretion rates. Lipolysis of subcutaneous maternal EFA/LCPUFA stores and fetal accretion of ω-3 EFA/LCPUFAs takes place throughout pregnancy, but mainly during the third trimester. Dietary DHA is more likely to be efficiently transferred from the mother to the fetus or young infant than the DHA that is synthesized from ω-3 EFAs. Because metabolites of the ω-6 EFA family compete with those of the ω-3 family for desaturation and elongation enzymes, the dietary balance of ω-6/ω-3 can also affect maternal and infant DHA biosynthesis.

VEGF is a key growth factor implicated in the vascular pathogenesis of ROP (Smith et al., 2000). The discussion in DR and AMD sections of this review on the relationship of LCPUFAs with VEGF are germane to this issue. Work implicating DHA as a neurotrophic and survival factor in model systems of developing photoreceptors may elucidate important mechanisms in ROP pathogenesis (Politi et al., 2001; Rotstein et al., 2003, 1996, 1997, 1998). Observations on the role astrocyte fuction in ROP are also important to consider, as these cells have the capacity to supply DHA from α-LLNA precursors (Bernoud et al., 1998; Williard et al., 2001).

8. Summary and future directions

The importance of LCPUFAs in the retina is indicated by the efficient conservation and use of these 'easily oxidized' lipids in areas that are both highly susceptible to oxidative stress and chonically exposed to conditions that facilitate production of reactive oxygen species (Gordon and Bazan, 1997). Conditions associated with activation of enzymes essential for liberating and moblizing LCPUFAs from tissue stores and then converting these comounds to eicosanoids are also associated with activation of vaso- and neuroactive compounds implicated in pathogenesis of retinal diseases of public health significance. Within this context, it is important to recognize that modulating intake of LCPUFAs and EFAs is a feasible approach to modifying tissue stores.

New and emerging concepts related to the content of the work we present should focus on the interrelationship of LCPUFAs with neural and vascular structure and function. Dietary lipids demonstrate potent actions in inter- and intracellular communication that extend beyond the traditional conception of these molecules as energy substrates and essential structural molecules in the retina. Techniques for in vivo imaging and high throughput biochemical analyses are under development; these factors should increase feasibility of clinical trials and applied research in humans. The role of ω-3 LCPUFAs in visual signal transduction is being elucidated with advances in analytic methods and modeling tools in structural biology and biophysics. We now have the capacity to examine putative modulatory role of ω-3 LCPUFAs in neovascularization within microvascular systems. While much has been gleaned from studies on the immunoregulatory actions of ω-3 LCPUFAs, there are still many opportunities for examining the relationship of these nutrients with neuro- and vascular inflammatory etiology of the diseases discussed in this work.

Our review leads us to the conclusion that it is essential to consider the nature of processes linking

vascular and neural systems in retinal health and disease. LCPUFAs operate as key structural and signaling molecules and likewise affect and are affected by key compounds with known vaso-, neuro-, and immunomodulatory actions. As lipid-dependent signaling mechanisms are elucidated, more knowledge will be available to guide researchers in discovery of key pathways driving retinal response to developmental, demographic, environmental, and metabolic factors. To the extent that retinal tissue status of LCPUFAs is dependent upon and modifiable by diet, we may eventually arrive at some reasonable understanding of whether alterations in dietary or cellular lipid composition or modulation of phospholipase/cyclooxygenase/LOX activity may be effectively used as a preventive intervention against retinal disease.

Acknowledgements

We would like to acknowledge Drs. Norman Salem, Martha Neuringer, Burton Litman, and Joseph Hibbeln for generously discussing practical and theoretical aspects of their research and other applied research on LCPUFAs in human health and disease. I would like to thank Dr. Frank Sacks for first exposing me to work describing innovative mechanistic approaches for investigating the functions and actions of LCPUFAs in living systems (JPSG).

References

Abdelsalam, A., Del Priore, L., Zarbin, M.A., 1999. Drusen in age-related macular degeneration: pathogenesis, natural course, and laser photocoagulation-induced regression. Surv. Ophthalmol. 44, 1–29.

Abribat, T., Nedelec, B., Jobin, N., Garrel, D.R., 2000. Decreased serum insulin-like growth factor-I in burn patients: relationship with serum insulin-like growth factor binding protein-3 proteolysis and the influence of lipid composition in nutritional support. Crit. Care Med. 28, 2366–2372.

Agren, J.J., Vaisanen, S., Hanninen, O., Muller, A.D., Hornstra, G., 1997. Hemostatic factors and platelet aggregation after a fish-enriched diet or fish oil or docosahexaenoic acid supplementation. Prostaglandins Leukot. Essent. Fatty Acids 57, 419–421.

Aguirre, G.D., Acland, G.M., Maude, M.B., Anderson, R.E., 1997. Diets enriched in docosahexaenoic acid fail to correct progressive rod-cone degeneration (prcd) phenotype. Invest. Ophthalmol. Vis. Sci. 38, 2387–2407.

Aiello, L.P., 1997. Clinical implications of vascular growth factors in proliferative retinopathies. Curr. Opin. Ophthalmol. 8, 19–31.

Aiello, L.P., Wong, J.S., 2000. Role of vascular endothelial growth factor in diabetic vascular complications. Kidney Int. Suppl. 77, S113–S119.

Akbar, M., Kim, H.Y., 2002. Protective effects of docosahexaenoic acid in staurosporine-induced apoptosis: involvement of phosphatidylinositol-3 kinase pathway. J. Neurochem. 82, 655–665.

Akiba, S., Murata, T., Kitatani, K., Sato, T., 2000. Involvement of lipoxygenase pathway in docosapentaenoic acid-induced inhibition of platelet aggregation. Biol. Pharm. Bull. 23, 1293–1297.

Alexander, N.J., Smythe, N.L., 1988. Dietary fat modulation of in vitro lymphocyte function. Ann. Nutr. Metab. 32, 192–199.

Ambati, J., Ambati, B.K., Yoo, S.H., Ianchulev, S., Adamis, A.P., 2003. Age-related macular degeneration: etiology, pathogenesis, and therapeutic strategies. Surv. Ophthalmol. 48, 257–293.

Anderson, R.E., 1970. Lipids of ocular tissues. IV. A comparison of the phospholipids from the retina of six mammalian species. Exp. Eye Res. 10, 339–344.

Anderson, R.E., Maude, M.B., Alvarez, R.A., Acland, G.M., Aguirre, G.D., 1991. Plasma lipid abnormalities in the miniature poodle with progressive rod-cone degeneration. Exp. Eye Res. 52, 349–355.

Anderson, R.E., Maude, M.B., Alvarez, R.A., Acland, G., Aguirre, G.D., 1999. A hypothesis to explain the reduced blood levels of docosahexaenoic acid in inherited retinal degenerations caused by mutations in genes encoding retina-specific proteins. Lipids 34 (Suppl), S235–S237.

Anderson, R.E., Maude, M.B., Bok, D., 2001. Low docosahexaenoic acid levels in rod outer segment membranes of mice with rds/peripherin and P216L peripherin mutations. Invest. Ophthalmol. Vis. Sci. 42, 1715–1720.

Anderson, G.J., Connor, W.E., 1988. Uptake of fatty acids by the developing rat brain. Lipids 23, 286–290.

Ando, M., Sanaka, T., Nihei, H., 1999. Eicosapentanoic acid reduces plasma levels of remnant lipoproteins and prevents in vivo peroxidation of LDL in dialysis patients. J. Am. Soc. Nephrol. 10, 2177–2184.

Antonetti, D.A., Lieth, E., Barber, A.J., Gardner, T.W., 1999. Molecular mechanisms of vascular permeability in diabetic retinopathy. Semin. Ophthalmol. 14, 240–248.

Appel, L.J., Miller III, E.R., Seidler, A.J., Whelton, P.K., 1993. Does supplementation of diet with 'fish oil' reduce blood pressure? A meta-analysis of controlled clinical trials. Arch. Int. Med. 153, 1429–1438.

Archer, S.L., Green, D., Chamberlain, M., Dyer, A.R., Liu, K., 1998. Association of dietary fish and n-3 fatty acid intake with hemostatic factors in the coronary artery risk development in young adults (CARDIA) study. Arterioscler Thromb. Vasc. Biol. 18, 1119–1123.

Aveldano, M.I., Sprecher, H., 1987. Very long chain (C24 to C36) polyenoic fatty acids of the n-3 and n-6 series in dipolyunsaturated phosphatidylcholines from bovine retina. J. Biol. Chem. 262, 1180–1186.

Ayalasomayajula, S.P., Kompella, U.B., 2003. Celecoxib, a selective cyclooxygenase-2 inhibitor, inhibits retinal vascular endothelial growth factor expression and vascular leakage in a streptozotocin-induced diabetic rat model. Eur. J. Pharmacol. 458, 283–289.

Babcock, T.A., Helton, W.S., Hong, D., Espat, N.J., 2002. Omega-3 fatty acid lipid emulsion reduces LPS-stimulated macrophage TNF-alpha production. Surg. Infect. (Larchmt). 3, 145–149.

Badawi, A.F., El-Sohemy, A., Stephen, L.L., Ghoshal, A.K., Archer, M.C., 1998. The effect of dietary n-3 and n-6 polyunsaturated fatty acids on the expression of cyclooxygenase 1 and 2 and levels of p21ras in rat mammary glands. Carcinogenesis 19, 905–910.

Balazy, M., Nigam, S., 2003. Aging, lipid modifications and phospholipases—new concepts. Age. Res. Rev. 2, 191–209.

Baldie, G., Kaimakamis, D., Rotondo, D., 1993. Fatty acid modulation of cytokine release from human monocytic cells. Biochim. Biophys. Acta 1179, 125–133.

Balsinde, J., Dennis, E.A., 1996. Distinct roles in signal transduction for each of the phospholipase A(2) enzymes present in P388D(1) macrophages. J. Biol. Chem. 271, 6758–6765.

Barber, A.J., 2003. A new view of diabetic retinopathy: a neurodegenerative disease of the eye. Prog. Neuropsychopharmacol. Biol. Psychiatry 27, 283–290.

Barber, A.J., Lieth, E., Khin, S.A., Antonetti, D.A., Buchanan, A.G., Gardner, T.W., 1998. Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. J. Clin. Invest. 102, 783–791.

Barber, A.J., Antonetti, D.A., Gardner, T.W., 2000. Altered expression of retinal occludin and glial fibrillary acidic protein in experimental diabetes. The Penn State Retina Research Group. Invest. Ophthalmol. Vis. Sci. 41, 3561–3568.

Barcelli, U., Glas-Greenwalt, P., Pollak, V.E., 1985. Enhancing effect of dietary supplementation with omega-3 fatty acids on plasma fibrinolysis in normal subjects. Thromb. Res. 39, 307–312.

Bayon, Y., Croset, M., Daveloose, D., Guerbette, F., Chirouze, V., Viret, J., Kader, J.C., Lagarde, M., 1995. Effect of specific phospholipid molecular species incorporated in human platelet membranes on thromboxane A2/prostaglandin H2 receptors. J. Lipid Res. 36, 47–56.

Bazan, N.G., 1990. Supply of n-3 polyunsaturated fatty acids and their significance in the central nervous system. In: Wurtman, R.J., Wurtman, J.J. (Eds.), Nutrition and the Brain, Vol. 8. Raven Press, New York, pp. 1–24.

Bazan, N.G., Rodriguez de Turco, E.B., 1994. Review: pharmacological manipulation of docosahexaenoic-phospholipid biosynthesis in photoreceptor cells: implications in retinal degeneration. J. Ocul. Pharmacol. 10, 591–604.

Bazan, N.G., Rodriguez de Turco, E.B., Gordon, W.C., 1993. Supply, uptake, and retention of DHA by the developing and mature retina and brain. Paper presented at: Lipids, Learning, and the Brain: Fats in Infant Formulas, 103rd Ross Conference on Pediatric Research, Adelaide, South Australia.

Bazan, H.E., Tao, Y., DeCoster, M.A., Bazan, N.G., 1997. Platelet-activating factor induces cyclooxygenase-2 gene expression in corneal epithelium. Requirement of calcium in the signal transduction pathway. Invest. Ophthalmol. Vis. Sci. 38, 2492–2501.

Bazan, N.G., Colangelo, V., Lukiw, W.J., 2002. Prostaglandins and other lipid mediators in Alzheimer's disease. Prostaglandins Other Lipid Mediat. 68–69, 197–210.

Beatty, S., Koh, H., Phil, M., Henson, D., Boulton, M., 2000. The role of oxidative stress in the pathogenesis of age-related macular degeneration. Surv. Ophthalmol. 45, 115–134.

Bechoua, S., Dubois, M., Vericel, E., Chapuy, P., Lagarde, M., Prigent, A.F., 2003. Influence of very low dietary intake of marine oil on some functional aspects of immune cells in healthy elderly people. Br. J. Nutr. 89, 523–531.

Bergmann, M., Schutt, F., Holz, F.G., Kopitz, J., 2004. Inhibition of the ATP-driven proton pump in RPE lysosomes by the major lipofuscin fluorophore A2-E may contribute to the pathogenesis of age-related macular degeneration. FASEB J. 8, 8.

Bernoud, N., Fenart, L., Benistant, C., Pageaux, J.F., Dehouck, M.P., Moliere, P., Lagarde, M., Cecchelli, R., Lecerf, J., 1998. Astrocytes are mainly responsible for the polyunsaturated fatty acid enrichment in blood-brain barrier endothelial cells in vitro. J. Lipid Res. 39, 1816–1824.

Bhathena, S.J., 2000. Dietary fatty acids and fatty acid metabolism in diabetes. In: Chow, K.C. (Ed.), Fatty Acids in Foods and their Health Implications. Marcel Dekker, Inc., New York, pp. 915–961.

Bicknell, R., Vallee, B.L., 1989. Angiogenin stimulates endothelial cell prostacyclin secretion by activation of phospholipase A2. Proc. Natl. Acad. Sci. USA 86, 1573–1577.

Billiar, T.R., Bankey, P.E., Svingen, B.A., Curran, R.D., West, M.A., Holman, R.T., Simmons, R.L., Cerra, F.B., 1988. Fatty acid intake and Kupffer cell function: fish oil alters eicosanoid and monokine production to endotoxin stimulation. Surgery 104, 343–349.

Bizik, J., Kankuri, E., Ristimaki, A., Taieb, A., Vapaatalo, H., Lubitz, W., Vaheri, A., 2004. Cell–cell contacts trigger programmed necrosis and induce cyclooxygenase-2 expression. Cell Death Differ. 11, 183–195.

Blok, W.L., Deslypere, J.P., Demacker, P.N., van der Ven-Jongekrijg, J., Hectors, M.P., van der Meer, J.W., Katan, M.B., 1997. Pro- and anti-inflammatory cytokines in healthy volunteers fed various doses of fish oil for 1 year. Eur. J. Clin. Invest. 27, 1003–1008.

Bogatcheva, N.V., Dudek, S.M., Garcia, J.G., Verin, A.D., 2003. Mitogen-activated protein kinases in endothelial pathophysiology. J. Investig. Med. 51, 341–352.

Bonanome, A., Biasia, F., De Luca, M., Munaretto, G., Biffanti, S., Pradella, M., Pagnan, A., 1996. n-3 fatty acids do not enhance LDL susceptibility to oxidation in hypertriacylglycerolemic hemodialyzed subjects. Am. J. Clin. Nutr. 63, 261–266.

Bordin, P., Bodamer, O.A., Venkatesan, S., Gray, R.M., Bannister, P.A., Halliday, D., 1998. Effects of fish oil supplementation on apolipoprotein B100 production and lipoprotein metabolism in normolipidaemic males. Eur. J. Clin. Nutr. 52, 104–109.

Boulton, M., Moriarty, P., Jarvis-Evans, J., Marcyniuk, B., 1994. Regional variation and age-related changes of lysosomal enzymes in the human retinal pigment epithelium. Br. J. Ophthalmol. 78, 125–129.

Boulton, M., Rozanowska, M., Rozanowski, B., 2001. Retinal photodamage. J. Photochem. Photobiol. B. 64, 144–161.

Broillet, M.C., Firestein, S., 1999. Cyclic nucleotide-gated channels. Molecular mechanisms of activation. Ann. NY Acad. Sci. 868, 730–740.

Brouard, C., Pascaud, M., 1993. Modulation of rat and human lymphocyte function by n-6 and n-3 polyunsaturated fatty acids and acetylsalicylic acid. Ann. Nutr. Metab. 37, 146–159.

Brown, M.F., 1994. Modulation of rhodopsin function by properties of the membrane bilayer. Chem. Phys. Lipids 73, 159–180.

Brude, I.R., Drevon, C.A., Hjermann, I., Seljeflot, I., Lund-Katz, S., Saarem, K., Sandstad, B., Solvoll, K., Halvorsen, B., Arnesen, H., Nenseter, M.S., 1997. Peroxidation of LDL from combined-hyperlipidemic male smokers supplied with omega-3 fatty acids and antioxidants. Arterioscler. Thromb. Vasc. Biol. 17, 2576–2588.

Bush, R.A., Reme, C.E., Malnoe, A., 1991. Light damage in the rat retina: the effect of dietary deprivation of N-3 fatty acids on acute structural alterations. Exp. Eye Res. 53, 741–752.

Cai, J., Boulton, M., 2002. The pathogenesis of diabetic retinopathy: old concepts and new questions. Eye 16, 242–260.

Calder, P.C., 2001. Polyunsaturated fatty acids, inflammation, and immunity. Lipids 36, 1007–1024.

Calder, P.C., Newsholme, E.A., 1992. Polyunsaturated fatty acids suppress human peripheral blood lymphocyte proliferation and interleukin-2 production. Clin. Sci. (Lond). 82, 695–700.

Calder, P.C., Bevan, S.J., Newsholme, E.A., 1992. The inhibition of T-lymphocyte proliferation by fatty acids is via an eicosanoid-independent mechanism. Immunology 75, 108–115.

Cammisotto, P.G., Gelinas, Y., Deshaies, Y., Bukowiecki, L.J., 2003. Regulation of leptin secretion from white adipocytes by free fatty acids. Am. J. Physiol. Endocrinol. Metab. 285, E521–E526.

Candelario-Jalil, E., Gonzalez-Falcon, A., Garcia-Cabrera, M., Alvarez, D., Al-Dalain, S., Martinez, G., Leon, O.S., Springer, J.E., 2003. Assessment of the relative contribution of COX-1 and COX-2 isoforms to ischemia-induced oxidative damage and neurodegeneration following transient global cerebral ischemia. J. Neurochem. 86, 545–555.

Cannon, J.G., Fiatarone, M.A., Meydani, M., Gong, J., Scott, L., Blumberg, J.B., Evans, W.J., 1995. Aging and dietary modulation of elastase and interleukin-1 beta secretion. Am. J. Physiol. 268, R208–R213.

Caughey, G.E., Mantzioris, E., Gibson, R.A., Cleland, L.G., James, M.J., 1996. The effect on human tumor necrosis factor alpha and interleukin 1 beta production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. Am. J. Clin. Nutr. 63, 116–122.

Chandrasekar, B., Fernandes, G., 1994. Decreased pro-inflammatory cytokines and increased antioxidant enzyme gene expression by

omega-3 lipids in murine lupus nephritis. Biochem. Biophys. Res. Commun. 200, 893–898.

Chemtob, S., Hardy, P., Abran, D., Li, D.Y., Peri, K., Cuzzani, O., Varma, D.R., 1995. Peroxide–cyclooxygenase interactions in postasphyxial changes in retinal and choroidal hemodynamics. J. Appl. Physiol. 78, 2039–2046.

Chen, H., Anderson, R.E., 1993. Differential incorporation of docosahexaenoic and arachidonic acids in frog retinal pigment epithelium. J. Lipid Res. 34, 1943–1955.

Chen, Y., Saari, J.C., Noy, N., 1993. Interactions of all-trans-retinol and long-chain fatty acids with interphotoreceptor retinoid-binding protein. Biochemistry 32, 11311–11318.

Chen, L.Y., Lawson, D.L., Mehta, J.L., 1994. Reduction in human neutrophil superoxide anion generation by n-3 polyunsaturated fatty acids: role of cyclooxygenase products and endothelium-derived relaxing factor. Thromb. Res. 76, 317–322.

Chen, Y., Houghton, L.A., Brenna, J.T., Noy, N., 1996. Docosahexaenoic acid modulates the interactions of the interphotoreceptor retinoid-binding protein with 11-*cis*-retinal. J. Biol. Chem. 271, 20507–20515.

Cho, E., Hung, S., Willett, W.C., Spiegelman, D., Rimm, E.B., Seddon, J.M., Colditz, G.A., Hankinson, S.E., 2001. Prospective study of dietary fat and the risk of age-related macular degeneration. Am. J. Clin. Nutr. 73, 209–218.

Choe, H.G., Anderson, R.E., 1990. Unique molecular species composition of glycerolipids of frog rod outer segments. Exp. Eye Res. 51, 159–165.

Chow, K.C., 2000. Fatty Acids in Foods and their Health Implications, 2nd ed. Marcel Dekker, Inc., New York.

Cingle, K.A., Kalski, R.S., Bruner, W.E., O'Brien, C.M., Erhard, P., Wyszynski, R.E., 1996. Age-related changes of glycosidases in human retinal pigment epithelium. Curr. Eye Res. 15, 433–438.

Clandinin, M.T., Jumpsen, J., Suh, M., 1994. Relationship between fatty acid accretion, membrane composition, and biologic functions. J. Pediatr. 125, S25–S32.

Collett, E.D., Davidson, L.A., Fan, Y.Y., Lupton, J.R., Chapkin, R.S., 2001. n-6 and n-3 polyunsaturated fatty acids differentially modulate oncogenic Ras activation in colonocytes. Am. J. Physiol. Cell Physiol. 280, C1066–C1075.

Collie-Duguid, E.S., Wahle, K.W., 1996. Inhibitory effect of fish oil N-3 polyunsaturated fatty acids on the expression of endothelial cell adhesion molecules. Biochem. Biophys. Res. Commun. 220, 969–974.

Connor, W.E., Neuringer, M., 1988. The effects of n-3 fatty acid deficiency and repletion upon the fatty acid composition and function of the brain and retina. Prog. Clin. Biol. Res. 282, 275–294.

Conquer, J.A., Holub, B.J., 1996. Supplementation with an algae source of docosahexaenoic acid increases (n-3) fatty acid status and alters selected risk factors for heart disease in vegetarian subjects. J. Nutr. 126, 3032–3039.

Conquer, J.A., Holub, B.J., 1997. Dietary docosahexaenoic acid as a source of eicosapentaenoic acid in vegetarians and omnivores. Lipids 32, 341–345.

Cooper, A.L., Gibbons, L., Horan, M.A., Little, R.A., Rothwell, N.J., 1993. Effect of dietary fish oil supplemenation on fever and cytokine production in human volunteers. Clin. Nutr. 12, 321–328.

Corey, E.J., Shih, C., Cashman, J.R., 1983. Docosahexaenoic acid is a strong inhibitor of prostaglandin but not leukotriene biosynthesis. Proc. Natl. Acad. Sci. USA 80, 3581–3584.

Curtis, C.L., Rees, S.G., Little, C.B., Flannery, C.R., Hughes, C.E., Wilson, C., Dent, C.M., Otterness, I.G., Harwood, J.L., Caterson, B., 2002. Pathologic indicators of degradation and inflammation in human osteoarthritic cartilage are abrogated by exposure to n-3 fatty acids. Arthritis Rheum. 46, 1544–1553.

Daemen, F.J., 1973. Vertebrate rod outer segment membranes. Biochim. Biophys. Acta 300, 255–288.

D'Ambola, J.B., Aeberhard, E.E., Trang, N., Gaffar, S., Barrett, C.T., Sherman, M.P., 1991. Effect of dietary (n-3) and (n-6) fatty acids on in vivo pulmonary bacterial clearance by neonatal rabbits. J. Nutr. 121, 1262–1269.

Das, A., McGuire, P.G., 2003. Retinal and choroidal angiogenesis: pathophysiology and strategies for inhibition. Prog. Retina Eye Res. 22, 721–748.

Davies, S., Elliott, M.H., Floor, E., Truscott, T.G., Zareba, M., Sarna, T., Shamsi, F.A., Boulton, M.E., 2001. Photocytotoxicity of lipofuscin in human retinal pigment epithelial cells. Free Radic. Biol. Med. 31, 256–265.

De Caterina, R., Liao, J.K., Libby, P., 2000. Fatty acid modulation of endothelial activation. Am. J. Clin. Nutr. 71, 213S–223S.

De Caterina, R., Madonna, R., Zucchi, R., La Rovere, M.T., 2003. Antiarrhythmic effects of omega-3 fatty acids: from epidemiology to bedside. Am. Heart J. 146, 420–430.

de la Cruz, J.P., Moreno, A., Ruiz-Ruiz, M.I., Garcia-Campos, J., Sanchez de la Cuesta, F., 1998. Effect of WEB 2086-BS, an antagonist of platelet-activating factor receptors, on retinal vascularity in diabetic rats. Eur. J. Pharmacol. 360, 37–42.

de La Paz, M., Anderson, R.E., 1992. Region and age-dependent variation in susceptibility of the human retina to lipid peroxidation. Invest. Ophthalmol. Vis. Sci. 33, 3497–3499.

de Urquiza, A.M., Liu, S., Sjoberg, M., Zetterstrom, R.H., Griffiths, W., Sjovall, J., Perlmann, T., 2000. Docosahexaenoic acid, a ligand for the retinoid X receptor in mouse brain. Science 290, 2140–2144.

de Wilde, M.C., Farkas, E., Gerrits, M., Kiliaan, A.J., Luiten, P.G., 2002. The effect of n-3 polyunsaturated fatty acid-rich diets on cognitive and cerebrovascular parameters in chronic cerebral hypoperfusion. Brain Res. 947, 166–173.

DeCoster, M.A., Mukherjee, P.K., Davis, R.J., Bazan, N.G., 1998. Platelet-activating factor is a downstream messenger of kainate-induced activation of mitogen-activated protein kinases in primary hippocampal neurons. J. Neurosci. Res. 53, 297–303.

Del Priore, L.V., Kuo, Y.H., Tezel, T.H., 2002. Age-related changes in human RPE cell density and apoptosis proportion in situ. Invest. Ophthalmol. Vis. Sci. 43, 3312–3318.

Delerive, P., Oudot, F., Ponsard, B., Talpin, S., Sergiel, J.P., Cordelet, C., Athias, P., Grynberg, A., 1999. Hypoxia-reoxygenation and polyunsaturated fatty acids modulate adrenergic functions in cultured cardiomyocytes. J. Mol. Cell. Cardiol. 31, 377–386.

Delion, S., Chalon, S., Guilloteau, D., Besnard, J.C., Durand, G., 1996. Alpha-linolenic acid dietary deficiency alters age-related changes of dopaminergic and serotoninergic neurotransmission in the rat frontal cortex. J. Neurochem. 66, 1582–1591.

Delion, S., Chalon, S., Guilloteau, D., Lejeune, B., Besnard, J.C., Durand, G., 1997. Age-related changes in phospholipid fatty acid composition and monoaminergic neurotransmission in the hippocampus of rats fed a balanced or an n-3 polyunsaturated fatty acid-deficient diet. J. Lipid Res. 38, 680–689.

Delton-Vandenbroucke, I., Grammas, P., Anderson, R.E., 1997. Polyunsaturated fatty acid metabolism in retinal and cerebral microvascular endothelial cells. J. Lipid Res. 38, 147–159.

Demaison, L., Sergiel, J.P., Moreau, D., Grynberg, A., 1994. Influence of the phospholipid n-6/n-3 polyunsaturated fatty acid ratio on the mitochondrial oxidative metabolism before and after myocardial ischemia. Biochim. Biophys. Acta 1227, 53–59.

DHHS/FDA, 1997. Department of Health and Human Services, US Food and Drug Administration. Substances affirmed as generally recognized as safe: menhaden oil. *Federal Register*. June 5, 1997. Vol. 62, No. 108: pp 30751–30757. 21 CFR Part 184 [Docket No. 86G-0289]. 2003.

DHHS/FDA, 2002. Office of Nutritional Products, Labeling, and Dietary Supplements, Center for Food Safety and Applied

Nutrition, US Food and Drug Administration. Letter responding to a request to reconsider the qualified claim for a dietary supplement health claim for omega-3 fatty acids and coronary heart disease. Docket No. 91N-0103. February 8, 2002.

Diep, Q.N., Amiri, F., Touyz, R.M., Cohn, J.S., Endemann, D., Neves, M.F., Schiffrin, E.L., 2002. PPARalpha activator effects on Ang II-induced vascular oxidative stress and inflammation. Hypertension 40, 866–871.

Djamgoz, M.B., Hankins, M.W., Hirano, J., Archer, S.N., 1997. Neurobiology of retinal dopamine in relation to degenerative states of the tissue. Vision Res. 37, 3509–3529.

Dominguez, Z., Merhi-Soussi, F., MacOvschi, O., Nemoz, G., Lagarde, M., Prigent, A.F., 2001. Endothelial cell prostacyclin synthesis induced by lymphocytes is independent of the membrane fatty acid composition of both cell types and of E-selectin, VCAM-1 or ICAM-1-mediated adhesion. Br. J. Haematol. 113, 521–532.

Dorey, C.K., Wu, G., Ebenstein, D., Garsd, A., Weiter, J.J., 1989. Cell loss in the aging retina, Relationship to lipofuscin accumulation and macular degeneration. Invest. Ophthalmol. Vis. Sci. 30, 1691–1699.

Doucet, J.P., Squinto, S.P., Bazan, N.G., 1990. Fos-jun and the primary genomic response in the nervous system. Possible physiological role and pathophysiological significance. Mol. Neurobiol. 4, 27–55.

Dreyer, C., Keller, H., Mahfoudi, A., Laudet, V., Krey, G., Wahli, W., 1993. Positive regulation of the peroxisomal beta-oxidation pathway by fatty acids through activation of peroxisome proliferator-activated receptors (PPAR). Biol. Cell 77, 67–76.

Du, C., Sato, A., Watanabe, S., Wu, C.Z., Ikemoto, A., Ando, K., Kikugawa, K., Fujii, Y., Okuyama, H., 2003. Cholesterol synthesis in mice is suppressed but lipofuscin formation is not affected by long-term feeding of n-3 fatty acid-enriched oils compared with lard and n-6 fatty acid-enriched oils. Biol. Pharm. Bull. 26, 766–770.

du Toit, P.J., van Aswegen, C.H., du Plessis, D.J., 1996. The effect of essential fatty acids on growth and urokinase-type plasminogen activator production in human prostate DU-145 cells. Prostaglandins Leukot. Essent. Fatty Acids 55, 173–177.

Dubois, R.N., Abramson, S.B., Crofford, L., Gupta, R.A., Simon, L.S., Van De Putte, L.B., Lipsky, P.E., 1998. Cyclooxygenase in biology and disease. FASEB J. 12, 1063–1073.

Dubuquoy, L., Dharancy, S., Nutten, S., Pettersson, S., Auwerx, J., Desreumaux, P., 2002. Role of peroxisome proliferator-activated receptor gamma and retinoid X receptor heterodimer in hepatogastroenterological diseases. Lancet 360, 1410–1418.

Dunaief, J.L., Dentchev, T., Ying, G.S., Milam, A.H., 2002. The role of apoptosis in age-related macular degeneration. Arch. Ophthalmol. 120, 1435–1442.

Dyerberg, J., Bang, H.O., 1979. Lipid metabolism, atherogenesis, and haemostasis in Eskimos: the role of the prostaglandin-3 family. Haemostasis 8, 227–233.

Eicher, S.D., McVey, D.S., 1995. Dietary modulation of Kupffer cell and splenocyte function during a *Salmonella typhimurium* challenge in mice. J. Leukoc. Biol. 58, 32–39.

Elner, V.M., 2002. Retinal pigment epithelial acid lipase activity and lipoprotein receptors: effects of dietary omega-3 fatty acids. Trans. Am. Ophthalmol. Soc. 100, 301–338.

El-seweidy, M.M., El-swefy, S.E., Ameen, R.S., Hashem, R.M., 2002. Effect of age receptor blocker and/or anti-inflammatory coadministration in relation to glycation, oxidative stress and cytokine production in stz diabetic rats. Pharmacol. Res. 45, 391–398.

Endres, S., Ghorbani, R., Kelley, V.E., Georgilis, K., Lonnemann, G., van der Meer, J.W., Cannon, J.G., Rogers, T.S., Klempner, M.S., Weber, P.C., et al., 1989. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N. Engl. J. Med. 320, 265–271.

Farber, D.B., Flannery, J.G., Lolley, R.N., Bok, D., 1985. Distribution patterns of photoreceptors, protein, and cyclic nucleotides in the human retina. Invest. Ophthalmol. Vis. Sci. 26, 1558–1568.

Favrelere, S., Stadelmann-Ingrand, S., Huguet, F., De Javel, D., Piriou, A., Tallineau, C., Durand, G., 2000. Age-related changes in ethanolamine glycerophospholipid fatty acid levels in rat frontal cortex and hippocampus. Neurobiol. Aging 21, 653–660.

Feng, C., Keisler, D.H., Fritsche, K.L., 1999. Dietary omega-3 polyunsaturated fatty acids reduce IFN-gamma receptor expression in mice. J. Interferon. Cytokine Res. 19, 41–48.

Feng, L., Xia, Y., Garcia, G.E., Hwang, D., Wilson, C.B., 1995. Involvement of reactive oxygen intermediates in cyclooxygenase-2 expression induced by interleukin-1, tumor necrosis factor-alpha, and lipopolysaccharide. J. Clin. Invest. 95, 1669–1675.

Fernyhough, P., Gallagher, A., Averill, S.A., Priestley, J.V., Hounsom, L., Patel, J., Tomlinson, D.R., 1999. Aberrant neurofilament phosphorylation in sensory neurons of rats with diabetic neuropathy. Diabetes 48, 881–889.

Ferrara, N., Davis-Smyth, T., 1997. The biology of vascular endothelial growth factor. Endocr. Rev. 18, 4–25.

FitzGerald, G.A., 2003. COX-2 and beyond: approaches to prostaglandin inhibition in human disease. Nat. Rev. Drug Discov. 2, 879–890.

Flamand, N., Surette, M.E., Picard, S., Bourgoin, S., Borgeat, P., 2002. Cyclic AMP-mediated inhibition of 5-lipoxygenase translocation and leukotriene biosynthesis in human neutrophils. Mol. Pharmacol. 62, 250–256.

Fliesler, S.J., Anderson, R.E., 1983. Chemistry and metabolism of lipids in the vertebrate retina. Prog. Lipid Res. 22, 79–131.

Fornaro, P., Calabria, G., Corallo, G., Picotti, G.B., 2002. Pathogenesis of degenerative retinopathies induced by thioridazine and other antipsychotics: a dopamine hypothesis. Doc. Ophthalmol. 105, 41–49.

Frank, R.N., 2004. Diabetic retinopathy. N. Engl. J. Med. 350, 48–58.

Frankel, E.N., Parks, E.J., Xu, R., Schneeman, B.O., Davis, P.A., German, J.B., 1994. Effect of n-3 fatty acid-rich fish oil supplementation on the oxidation of low density lipoproteins. Lipids 29, 233–236.

Fritsche, K.L., Cassity, N.A., 1992. Dietary n-3 fatty acids reduce antibody-dependent cell cytotoxicity and alter eicosanoid release by chicken immune cells. Poult. Sci. 71, 1646–1657.

Fritsche, K.L., Cassity, N.A., Huang, S.C., 1991. Effect of dietary fat source on antibody production and lymphocyte proliferation in chickens. Poult. Sci. 70, 611–617.

Fulton, A.B., Reynaud, X., Hansen, R.M., Lemere, C.A., Parker, C., Williams, T.P., 1999. Rod photoreceptors in infant rats with a history of oxygen exposure. Invest. Ophthalmol. Vis. Sci. 40, 168–174.

Gallai, V., Sarchielli, P., Trequattrini, A., Franceschini, M., Floridi, A., Firenze, C., Alberti, A., Di Benedetto, D., Stragliotto, E., 1995. Cytokine secretion and eicosanoid production in the peripheral blood mononuclear cells of MS patients undergoing dietary supplementation with n-3 polyunsaturated fatty acids. J. Neuroimmunol. 56, 143–153.

Gao, H., Hollyfield, J.G., 1992. Aging of the human retina. Differential loss of neurons and retinal pigment epithelial cells. Invest. Ophthalmol. Vis. Sci. 33, 1–17.

Gardner, T.W., Antonetti, D.A., Barber, A.J., LaNoue, K.F., Nakamura, M., 2000. New insights into the pathophysiology of diabetic retinopathy: potential cell-specific therapeutic targets. Diabetes Technol. Ther. 2, 601–608.

Gardner, T.W., Antonetti, D.A., Barber, A.J., LaNoue, K.F., Levison, S.W., 2002. Diabetic retinopathy: more than meets the eye. Surv. Ophthalmol. 47 (Suppl 2), S253–S262.

Gariano, R.F., 2003. Cellular mechanisms in retinal vascular development. Prog. Retina Eye Res. 22, 295–306.

Geijsen, N., Dijkers, P.F., Lammers, J.J., Koenderman, L., Coffer, P.J., 2000. Cytokine-mediated cPLA(2) phosphorylation is regulated by multiple MAPK family members. FEBS Lett. 471, 83–88.

Geusens, P., Wouters, C., Nijs, J., Jiang, Y., Dequeker, J., 1994. Long-term effect of omega-3 fatty acid supplementation in active rheumatoid arthritis. A 12-month, double-blind, controlled study. Arthritis Rheum. 37, 824–829.

Goldman, R., Moshonov, S., Chen, X., Berchansky, A., Furstenberger, G., Zor, U., 1997. Crosstalk between elevation of [Ca2+]i, reactive oxygen species generation and phospholipase A2 stimulation in a human keratinocyte cell line. Adv. Exp. Med. Biol. 433, 41–45.

Gordon, W.C., Bazan, N.G., 1997. Retina. In: Harding, J.J. (Ed.), Biochemistry of the Eye, 1st ed. Chapman & Hall, London, pp. 144–275.

Gottlicher, M., Demoz, A., Svensson, D., Tollet, P., Berge, R.K., Gustafsson, J.A., 1993. Structural and metabolic requirements for activators of the peroxisome proliferator-activated receptor. Biochem. Pharmacol. 46, 2177–2184.

Grimm, H., Tibell, A., Norrlind, B., Blecher, C., Wilker, S., Schwemmle, K., 1994. Immunoregulation by parenteral lipids: impact of the n-3 to n-6 fatty acid ratio. JPEN J. Parenter Enteral. Nutr. 18, 417–421.

Grynberg, A., Demaison, L., 1996. Fatty acid oxidation in the heart. J. Cardiovasc. Pharmacol. 28, S11–S17.

Grynberg, A., Fournier, A., Sergiel, J.P., Athias, P., 1996. Membrane docosahexaenoic acid vs. eicosapentaenoic acid and the beating function of the cardiomyocyte and its regulation through the adrenergic receptors. Lipids 31 (Suppl), S205–S210.

Gu, X., Samuel, S., El-Shabrawey, M., Caldwell, R.B., Bartoli, M., Marcus, D.M., Brooks, S.E., 2002. Effects of sustained hyperoxia on revascularization in experimental retinopathy of prematurity. Invest. Ophthalmol. Vis. Sci. 43, 496–502.

Gupta, N., Brown, K.E., Milam, A.H., 2003. Activated microglia in human retinitis pigmentosa, late-onset retinal degeneration, and age-related macular degeneration. Exp. Eye Res. 76, 463–471.

Haegerstrom-Portnoy, G., 1988. Short-wavelength-sensitive-cone sensitivity loss with aging: a protective role for macular pigment? J. Opt. Soc. Am. A. 5, 2140–2144.

Hageman, G.S., Luthert, P.J., Victor Chong, N.H., Johnson, L.V., Anderson, D.H., Mullins, R.F., 2001. An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE–Bruch's membrane interface in aging and age-related macular degeneration. Prog. Retina Eye Res. 20, 705–732.

Hallaq, H., Smith, T.W., Leaf, A., 1992. Modulation of dihydropyridine-sensitive calcium channels in heart cells by fish oil fatty acids. Proc. Natl. Acad. Sci. USA 89, 1760–1764.

Hamid, R., Singh, J., Reddy, B.S., Cohen, L.A., 1999. Inhibition by dietary menhaden oil of cyclooxygenase-1 and -2 in *N*-nitrosomethylurea-induced rat mammary tumors. Int. J. Oncol. 14, 523–528.

Han, W.K., Sapirstein, A., Hung, C.C., Alessandrini, A., Bonventre, J.V., 2003. Cross-talk between cytosolic phospholipase A2{alpha} (cPLA2{alpha}) and secretory phospholipase A2 (sPLA2) in hydrogen peroxide-induced arachidonic acid release in murine mesangial cells: sPLA2 regulates cPLA2{alpha} activity that is responsible for arachidonic acid release. J. Biol. Chem. 278, 24153–24163.

Han, J.H., Roh, M.S., Park, C.H., Park, K.C., Cho, K.H., Kim, K.H., Eun, H.C., Chung, J.H., 2004. Selective COX-2 inhibitor, NS-398, inhibits the replicative senescence of cultured dermal fibroblasts. Mech. Ageing Dev. 125, 359–366.

Harman, A.M., Fleming, P.A., Hoskins, R.V., Moore, S.R., 1997. Development and aging of cell topography in the human retinal pigment epithelium. Invest. Ophthalmol. Vis. Sci. 38, 2016–2026.

Harris, W.S., 1997. n-3 fatty acids and serum lipoproteins: human studies. Am. J. Clin. Nutr. 65, 1645S–1654S.

Harris, W.S., Connor, W.E., Illingworth, D.R., Rothrock, D.W., Foster, D.M., 1990. Effects of fish oil on VLDL triglyceride kinetics in humans. J. Lipid Res. 31, 1549–1558.

Harris, M.A., Hansen, R.A., Vidsudhiphan, P., Koslo, J.L., Thomas, J.B., Watkins, B.A., Allen, K.G., 2001. Effects of conjugated linoleic acids and docosahexaenoic acid on rat liver and reproductive tissue fatty acids, prostaglandins and matrix metalloproteinase production. Prostaglandins Leukot Essent. Fatty Acids 65, 23–29.

Hawrami, K., Hitman, G.A., Rema, M., Snehalatha, C., Viswanathan, M., Ramachandran, A., Mohan, V., 1996. An association in non-insulin-dependent diabetes mellitus subjects between susceptibility to retinopathy and tumor necrosis factor polymorphism. Hum. Immunol. 46, 49–54.

Hayakawa, M., Jayadev, S., Tsujimoto, M., Hannun, Y.A., Ito, F., 1996. Role of ceramide in stimulation of the transcription of cytosolic phospholipase A2 and cyclooxygenase 2*1. Biochem. Biophys. Res. Commun. 220, 681–686.

Hellstrom, A., Perruzzi, C., Ju, M., Engstrom, E., Hard, A.L., Liu, J.L., Albertsson-Wikland, K., Carlsson, B., Niklasson, A., Sjodell, L., LeRoith, D., Senger, D.R., Smith, L.E., 2001. Low IGF-I suppresses VEGF-survival signaling in retinal endothelial cells: direct correlation with clinical retinopathy of prematurity. Proc. Natl. Acad. Sci. USA 98, 5804–5808.

Hendrickx, N., Volanti, C., Moens, U., Seternes, O.M., de Witte, P., Vandenheede, J.R., Piette, J., Agostinis, P., 2003. Up-regulation of cyclooxygenase-2 and apoptosis resistance by p38 MAPK in hypericin-mediated photodynamic therapy of human cancer cells. J. Biol. Chem. 278, 52231–52239 Epub 52003 Oct 52213.

Hernandez, M., Burillo, S.L., Crespo, M.S., Nieto, M.L., 1998. Secretory phospholipase A2 activates the cascade of mitogen-activated protein kinases and cytosolic phospholipase A2 in the human astrocytoma cell line 1321N1. J. Biol. Chem. 273, 606–612.

Heuberger, R.A., Mares-Perlman, J.A., Klein, R., Klein, B.E., Millen, A.E., Palta, M., 2001. Relationship of dietary fat to age-related maculopathy in the Third National Health and Nutrition Examination Survey. Arch. Ophthalmol. 119, 1833–1838.

Hida, M., Fujita, H., Ishikura, K., Omori, S., Hoshiya, M., Awazu, M., 2003. Eicosapentaenoic acid inhibits PDGF-induced mitogenesis and cyclin D1 expression via TGF-beta in mesangial cells. J. Cell Physiol. 196, 293–300.

Higdon, J.V., Liu, J., Du, S.H., Morrow, J.D., Ames, B.N., Wander, R.C., 2000. Supplementation of postmenopausal women with fish oil rich in eicosapentaenoic acid and docosahexaenoic acid is not associated with greater in vivo lipid peroxidation compared with oils rich in oleate and linoleate as assessed by plasma malondialdehyde and F(2)-isoprostanes. Am. J. Clin. Nutr. 72, 714–722.

Higgins, S., Carroll, Y.L., McCarthy, S.N., Corridan, B.M., Roche, H.M., Wallace, J.M., O'Brien, N.M., Morrissey, P.A., 2001. Susceptibility of LDL to oxidative modification in healthy volunteers supplemented with low doses of n-3 polyunsaturated fatty acids. Br. J. Nutr. 85, 23–31.

Hirafuji, M., Machida, T., Tsunoda, M., Miyamoto, A., Minami, M., 2002. Docosahexaenoic acid potentiates interleukin-1beta induction of nitric oxide synthase through mechanism involving p44/42 MAPK activation in rat vascular smooth muscle cells. Br. J. Pharmacol. 136, 613–619.

Hirafuji, M., Machida, T., Hamaue, N., Minami, M., 2003. Cardiovascular protective effects of n-3 polyunsaturated fatty acids with special emphasis on docosahexaenoic acid. J. Pharmacol. Sci. 92, 308–316.

Hirschi, K.K., D'Amore, 1997. Control of angiogenesis by the pericyte: molecular mechanisms and significance. Inhibition of

capillary endothelial cell growth by pericytes and smooth muscle cells. Exs. 79, 419–428.

Hoffman, D.R., Birch, D.G., 1995. Docosahexaenoic acid in red blood cells of patients with X-linked retinitis pigmentosa. Invest. Ophthalmol. Vis. Sci. 36, 1009–1018.

Hoffman, D.R., DeMar, J.C., Heird, W.C., Birch, D.G., Anderson, R.E., 2001. Impaired synthesis of DHA in patients with X-linked retinitis pigmentosa. J. Lipid Res. 42, 1395–1401.

Hogan, M.J., Alvarado, J.A., Weddel, J.E., 1971. Histology of the Human Eye: An Atlas Textbook. W.B. Saunders, Philadelphia.

Holz, F.G., Schutt, F., Kopitz, J., Eldred, G.E., Kruse, F.E., Volcker, H.E., Cantz, M., 1999. Inhibition of lysosomal degradative functions in RPE cells by a retinoid component of lipofuscin. Invest. Ophthalmol. Vis. Sci. 40, 737–743.

Hong, S., Gronert, K., Devchand, P.R., Moussignac, R.L., Serhan, C.N., 2003. Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood and glial cells: autacoids in anti-inflammation. J. Biol. Chem. 278 (17), 14677–14687.

Horton, J.D., Goldstein, J.L., Brown, M.S., 2002. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J. Clin. Invest. 109, 1125–1131.

Huang, S.C., Misfeldt, M.L., Fritsche, K.L., 1992. Dietary fat influences Ia antigen expression and immune cell populations in the murine peritoneum and spleen. J. Nutr. 122, 1219–1231.

Hubbard, N.E., Somers, S.D., Erickson, K.L., 1991. Effect of dietary fish oil on development and selected functions of murine inflammatory macrophages. J. Leukoc. Biol. 49, 592–598.

Hughes, D.A., Southon, S., Pinder, A.C., 1996. (n-3) Polyunsaturated fatty acids modulate the expression of functionally associated molecules on human monocytes in vitro. J. Nutr. 126, 603–610.

Hurst, J.S., Bazan, H.E., 1995. Platelet-activating factor preferentially stimulates the phospholipase A2/cyclooxygenase cascade in the rabbit cornea. Curr. Eye Res. 14, 769–775.

Hutcheson, K.A., 2003. Retinopathy of prematurity. Curr. Opin. Ophthalmol. 14, 286–290.

Innis, S.M., de La Presa Owens, S., 2001. Dietary fatty acid composition in pregnancy alters neurite membrane fatty acids and dopamine in newborn rat brain. J. Nutr. 131, 118–122.

Jackson, G.R., Owsley, C., Curcio, C.A., 2002. Photoreceptor degeneration and dysfunction in aging and age-related maculopathy. Ageing Res. Rev. 1, 381–396.

James, M.J., Cleland, L.G., 1997. Dietary n-3 fatty acids and therapy for rheumatoid arthritis. Semin. Arthritis. Rheum. 27, 85–97.

Jeffrey, B.G., Weisingerb, H.S., Neuringer, M., Mitcheli, D.C., 2001. The role of docosahexaenoic acid in retinal function. Lipids 36, 859–871.

Joe, B., Lokesh, B.R., 1994. Role of capsaicin, curcumin and dietary n-3 fatty acids in lowering the generation of reactive oxygen species in rat peritoneal macrophages. Biochim. Biophys. Acta 1224, 255–263.

Johansen, O., Seljeflot, I., Hostmark, A.T., Arnesen, H., 1999. The effect of supplementation with omega-3 fatty acids on soluble markers of endothelial function in patients with coronary heart disease. Arterioscler. Thromb. Vasc. Biol. 19, 1681–1686.

Johnson, E.I., Dunlop, M.E., Larkins, R.G., 1999. Increased vasodilatory prostaglandin production in the diabetic rat retinal vasculature. Curr. Eye Res. 18, 79–82.

Jolly, C.A., Jiang, Y.H., Chapkin, R.S., McMurray, D.N., 1997. Dietary (n-3) polyunsaturated fatty acids suppress murine lymphoproliferation, interleukin-2 secretion, and the formation of diacylglycerol and ceramide. J. Nutr. 127, 37–43.

Joussen, A.M., Poulaki, V., Mitsiades, N., Kirchhof, B., Koizumi, K., Dohmen, S., Adamis, A.P., 2002. Nonsteroidal anti-inflammatory drugs prevent early diabetic retinopathy via TNF-alpha suppression. FASEB J. 16, 438–440.

Ju, W.-K., Kim, K.-Y., Neufeld, A.H., 2003. Increased activity of cyclooxygenase-2 signals early neurodegenerative events in the rat retina following transient ischemia. Exp. Eye Res. 77, 137–145.

Jump, D.B., 2002. Dietary polyunsaturated fatty acids and regulation of gene transcription. Curr. Opin. Lipidol. 13, 155–164.

Jump, D.B., 2004. Fatty acid regulation of gene transcription. Crit. Rev. Clin. Lab. Sci. 41, 41–78.

Jumpsen, J., Clandinin, M.T., 1997. Brain Development: Relationship to Dietary Lipid and Lipid Metabolism. AOCS Press, Champaign, IL.

Jung, H., Reme, C., 1994. Light-evoked arachidonic acid release in the retina: illuminance/duration dependence and the effects of quinacrine, mellitin and lithium. Light-evoked arachidonic acid release. Graefes Arch. Clin. Exp. Ophthalmol. 232, 167–175.

Kanayasu, T., Morita, I., Nakao-Hayashi, J., Asuwa, N., Fujisawa, C., Ishii, T., Ito, H., Murota, S., 1991a. Eicosapentaenoic acid inhibits tube formation of vascular endothelial cells in vitro. Lipids 26, 271–276.

Kanayasu, T., Morita, I., Nakao-Hayashi, J., Ito, H., Murota, S., 1991b. Enhancement of migration in bovine endothelial cells by eicosapentaenoic acid pretreatment. Atherosclerosis 87, 57–64.

Kang, J.X., Leaf, A., 1996. Evidence that free polyunsaturated fatty acids modify Na+ channels by directly binding to the channel proteins. Proc. Natl. Acad. Sci. USA 93, 3542–3546.

Kaur, M., Kaur, J., Ojha, S., Mahmood, A., 1996. Dietary fat effects on brush border membrane composition and enzyme activities in rat intestine. Ann. Nutr. Metab. 40, 269–276.

Kawamura, H., Sugiyama, T., Wu, D.M., Kobayashi, M., Yamanishi, S., Katsumura, K., Puro, D.G., 2003. ATP: a vasoactive signal in the pericyte-containing microvasculature of the rat retina. J. Physiol. 551, 787–799.

Kelley, C., D'Amore, P., Hechtman, H.B., Shepro, D., 1987. Microvascular pericyte contractility in vitro: comparison with other cells of the vascular wall. J. Cell Biol. 104, 483–490.

Kelley, D.S., Nelson, G.J., Serrato, C.M., Schmidt, P.C., Branch, L.B., 1988. Effects of type of dietary fat on indices of immune status of rabbits. J. Nutr. 118, 1376–1384.

Khalfoun, B., Thibault, G., Lacord, M., Gruel, Y., Bardos, P., Lebranchu, Y., 1996. Docosahexaenoic and eicosapentaenoic acids inhibit human lymphoproliferative responses in vitro but not the expression of T cell surface activation markers. Scand. J. Immunol. 43, 248–256.

Kielar, M.L., Jeyarajah, D.R., Zhou, X.J., Lu, C.Y., 2003. Docosahexaenoic acid ameliorates murine ischemic acute renal failure and prevents increases in mRNA abundance for both TNF-alpha and inducible nitric oxide synthase. J. Am. Soc. Nephrol. 14, 389–396.

Kim, H.Y., Edsall, L., 1999. The role of docosahexaenoic acid (22:6n-3) in neuronal signaling. Lipids 34 (Suppl), S249–S250.

Kim, H.Y., Akbar, M., Kim, K.Y., 2001. Inhibition of neuronal apoptosis by polyunsaturated fatty acids. J. Mol. Neurosci. 16, 223–227 discussion 279–284.

Kinoshita, I., Itoh, K., Nishida-Nakai, M., Hirota, H., Otsuji, S., Shibata, N., 1994. Antiarrhythmic effects of eicosapentaenoic acid during myocardial infarction—enhanced cardiac microsomal (Ca(2+)-Mg2+)-ATPase activity. Jpn. Circ. J. 58, 903–912.

Kiritoshi, S., Nishikawa, T., Sonoda, K., Kukidome, D., Senokuchi, T., Matsuo, T., Matsumura, T., Tokunaga, H., Brownlee, M., Araki, E., 2003. Reactive oxygen species from mitochondria induce cyclooxygenase-2 gene expression in human mesangial cells: potential role in diabetic nephropathy. Diabetes 52, 2570–2577.

Klein, R., Davis, M.D., Magli, Y.L., Segal, P., Klein, B.E., Hubbard, L., 1991. The Wisconsin age-related maculopathy grading system. Ophthalmology 98, 1128–1134.

Klein, R., Meuer, S.M., Moss, S.E., Klein, B.E., 1992. Detection of drusen and early signs of age-related maculopathy using a

nonmydriatic camera and a standard fundus camera. Ophthalmology 99, 1686–1692.

Klein, R., Klein, B.E., Moss, S.E., Cruickshanks, K.J., 1995. The Wisconsin Epidemiologic Study of Diabetic Retinopathy XV. The long term incidence of macular edema. Ophthalmology 102 (1), 7–16.

Knapp, H.R., 1997. Dietary fatty acids in human thrombosis and hemostasis. Am. J. Clin. Nutr. 65, 1687S–1698S.

Kohner, E.M., Chibber, R., 2001. Retinal Ischemia. In: Schachat, A.P. (Ed.), Retina, 3rd ed. Vol. 2, St. Mosby, Louis, pp. 982–994.

Kohno, M., Yasunari, K., Minami, M., Kano, H., Maeda, K., Yoshikawa, J., 2000. Suppression by eicosapentaenoic acid of oxidized low-density lipoprotein and lysophosphatidylcholine-induced migration in cultured rat vascular smooth muscle cells. Metabolism 49, 560–562.

Kolb, H., Fernandez, E., and Nelson, R., 2004. WEBVISION: The Organization of the Retina and Visual System. Available at: http://www.webvision.med.utah.edu. Accessed March 2004.

Kolko, M., de Turco, E.B., Diemer, N.H., Bazan, N.G., 2002. Secretory phospholipase A2-mediated neuronal cell death involves glutamate ionotropic receptors. Neuroreport 13, 1963–1966.

Komatsu, W., Ishihara, K., Murata, M., Saito, H., Shinohara, K., 2003. Docosahexaenoic acid suppresses nitric oxide production and inducible nitric oxide synthase expression in interferon-gamma plus lipopolysaccharide-stimulated murine macrophages by inhibiting the oxidative stress. Free Radic. Biol. Med. 34, 1006–1016.

Kramer, R.M., Roberts, E.F., Um, S.L., Borsch-Haubold, A.G., Watson, S.P., Fisher, M.J., Jakubowski, J.A., 1996. p38 Mitogen-activated protein kinase phosphorylates cytosolic phospholipase A2 (cPLA2) in thrombin-stimulated platelets. Evidence that proline-directed phosphorylation is not required for mobilization of arachidonic acid by cPLA2. J. Biol. Chem. 271, 27723–27729.

Kris-Etherton, P.M., Taylor, D.S., Yu-Poth, S., Huth, P., Moriarty, K., Fishell, V., Hargrove, R.L., Zhao, G., Etherton, T.D., 2000. Polyunsaturated fatty acids in the food chain in the United States. Am. J. Clin. Nutr. 71, 179S–188S.

Kris-Etherton, P.M., Harris, W.S., Appel, L.J., 2002. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 106, 2747–2757.

Kulkarni, P., Cai, J., Hurst, H.E., 2002. Lipids and nitric oxide in porcine retinal and choroidal blood vessels. J. Ocul. Pharmacol. Ther. 18, 265–275.

Kumar, S., Boehm, J., Lee, J.C., 2003. p38 MAP kinases: key signalling molecules as therapeutic targets for inflammatory diseases. Nat. Rev. Drug Discov. 2, 717–726.

Kuratko, C.N., 2000. Proliferation of colonic lymphocytes in response to inflammatory cytokines is lower in mice fed fish oil than in mice fed corn oil. Cancer Lett. 148, 27–32.

Kuroki, T., Inoguchi, T., Umeda, F., Nawata, H., 1998. Effect of eicosapentaenoic acid on glucose-induced diacylglycerol synthesis in cultured bovine aortic endothelial cells. Biochem. Biophys. Res. Commun. 247, 473–477.

Leaf, A., Kang, J.X., Xiao, Y.F., Billman, G.E., Voskuyl, R.A., 1999. The antiarrhythmic and anticonvulsant effects of dietary N-3 fatty acids. J. Membr. Biol. 172, 1–11.

Lecomte, M., Paget, C., Ruggiero, D., Wiernsperger, N., Lagarde, M., 1996. Docosahexaenoic acid is a major n-3 polyunsaturated fatty acid in bovine retinal microvessels. J. Neurochem. 66, 2160–2167.

Levine, P.H., Fisher, M., Schneider, P.B., Whitten, R.H., Weiner, B.H., Ockene, I.S., Johnson, B.F., Johnson, M.H., Doyle, E.M., Riendeau, P.A., et al., 1989. Dietary supplementation with omega-3 fatty acids prolongs platelet survival in hyperlipidemic patients with atherosclerosis. Arch. Int. Med. 149, 1113–1116.

Li, X.L., Steiner, M., 1991. Dose response of dietary fish oil supplementations on platelet adhesion. Arterioscler Thromb. 11, 39–46.

Li, F., Chen, H., Anderson, R.E., 2001. Biosynthesis of docosahexaenoate-containing glycerolipid molecular species in the retina. J. Mol. Neurosci. 16, 205–214 discussion 215–221.

Li, Q., Zemel, E., Miller, B., Perlman, I., 2002. Early retinal damage in experimental diabetes: electroretinographical and morphological observations. Exp. Eye Res. 74, 615–625.

Lieth, E., Barber, A.J., Xu, B., Dice, C., Ratz, M.J., Tanase, D., Strother, J.M., 1998. Glial reactivity and impaired glutamate metabolism in short-term experimental diabetic retinopathy. Penn State Retina Research Group. Diabetes 47, 815–820.

Limb, G.A., Chignell, A.H., Green, W., LeRoy, F., Dumonde, D.C., 1996. Distribution of TNF alpha and its reactive vascular adhesion molecules in fibrovascular membranes of proliferative diabetic retinopathy. Br. J. Ophthalmol. 80, 168–173.

Limb, G.A., Soomro, H., Janikoun, S., Hollifield, R.D., Shilling, J., 1999. Evidence for control of tumour necrosis factor-alpha (TNF-alpha) activity by TNF receptors in patients with proliferative diabetic retinopathy. Clin. Exp. Immunol. 115, 409–414.

Limb, G.A., Hollifield, R.D., Webster, L., Charteris, D.G., Chignell, A.H., 2001. Soluble TNF receptors in vitreoretinal proliferative disease. Invest. Ophthalmol. Vis. Sci. 42, 1586–1591.

Lin, L.L., Wartmann, M., Lin, A.Y., Knopf, J.L., Seth, A., Davis, R.J., 1993. cPLA2 is phosphorylated and activated by MAP kinase. Cell 72, 269–278.

Lin, Q., Ruuska, S.E., Shaw, N.S., Dong, D., Noy, N., 1999. Ligand selectivity of the peroxisome proliferator-activated receptor alpha. Biochemistry 38, 185–190.

Litman, B.J., Mitchell, D.C., 1996. A role for phospholipid polyunsaturation in modulating membrane protein function. Lipids 31 (Suppl), S193–S197.

Litman, B.J., Niu, S.L., Polozova, A., Mitchell, D.C., 2001. The role of docosahexaenoic acid containing phospholipids in modulating G protein-coupled signaling pathways: visual transduction. J. Mol. Neurosci. 16, 237–242 discussion 279–284.

Liu, X.H., Rose, D.P., 1995. Suppression of type IV collagenase in MDA-MB-435 human breast cancer cells by eicosapentaenoic acid in vitro and in vivo. Cancer Lett. 92, 21–26.

Liu, B., Timar, J., Howlett, J., Diglio, C.A., Honn, K.V., 1991. Lipoxygenase metabolites of arachidonic and linoleic acids modulate the adhesion of tumor cells to endothelium via regulation of protein kinase C. Cell Regul. 2, 1045–1055.

Liu, J., Itagaki, Y., Ben-Shabat, S., Nakanishi, K., Sparrow, J.R., 2000. The biosynthesis of A2E, a fluorophore of aging retina, involves the formation of the precursor, A2-PE, in the photoreceptor outer segment membrane. J. Biol. Chem. 275, 29354–29360.

Liu, J., Vanttinen, T., Hyden-Granskog, C., Voutilainen, R., 2002. Regulation of follistatin-related gene (FLRG) expression by protein kinase C and prostaglandin E(2) in cultured granulosa-luteal cells. Mol. Hum. Reprod. 8, 992–997.

Lopes de Faria, J.M., Russ, H., Costa, V.P., 2002. Retinal nerve fibre layer loss in patients with type 1 diabetes mellitus without retinopathy. Br. J. Ophthalmol. 86, 725–728.

Lu, M., Adamis, A.P., 2002. Vascular endothelial growth factor gene regulation and action in diabetic retinopathy. Ophthalmol. Clin. North Am. 15, 69–79.

Lucius, R., Sievers, J., 1996. Postnatal retinal ganglion cells in vitro: protection against reactive oxygen species (ROS)-induced axonal degeneration by cocultured astrocytes. Brain Res. 743, 56–62.

Luostarinen, R., Saldeen, T., 1996. Dietary fish oil decreases superoxide generation by human neutrophils: relation to cyclooxygenase pathway and lysosomal enzyme release. Prostaglandins Leukot Essent. Fatty Acids 55, 167–172.

Mabile, L., Piolot, A., Boulet, L., Fortin, L.J., Doyle, N., Rodriguez, C., Davignon, J., Blache, D., Lussier-Cacan, S., 2001. Moderate intake of n-3 fatty acids is associated with stable erythrocyte

resistance to oxidative stress in hypertriglyceridemic subjects. Am. J. Clin. Nutr. 74, 449–456.

Majka, S., McGuire, P., Colombo, S., Das, A., 2001. The balance between proteinases and inhibitors in a murine model of proliferative retinopathy. Invest Ophthalmol. Vis. Sci. 42, 210–215.

Majka, S., McGuire, P.G., Das, A., 2002. Regulation of matrix metalloproteinase expression by tumor necrosis factor in a murine model of retinal neovascularization. Invest. Ophthalmol. Vis. Sci. 43, 260–266.

Mancini, M.A., Frank, R.N., Keirn, R.J., Kennedy, A., Khoury, J.K., 1986. Does the retinal pigment epithelium polarize the choriocapillaris? Invest. Ophthalmol. Vis. Sci. 27, 336–345.

Marckmann, P., Bladbjerg, E.M., Jespersen, J., 1997. Dietary fish oil (4 g daily) and cardiovascular risk markers in healthy men. Arterioscler Thromb. Vasc. Biol. 17, 3384–3391.

Mares-Perlman, J.A., Brady, W.E., Klein, R., VandenLangenberg, G.M., Klein, B.E., Palta, M., 1995. Dietary fat and age-related maculopathy. Arch. Ophthalmol. 113, 743–748.

Martin, R.E., 1998. Docosahexaenoic acid decreases phospholipase A2 activity in the neurites/nerve growth cones of PC12 cells. J. Neurosci. Res. 54, 805–813.

Martinez, M., 1989. Polyunsaturated fatty acid changes suggesting a new enzymatic defect in Zellweger syndrome. Lipids 24, 261–265.

Martinez, M., 1990. Severe deficiency of docosahexaenoic acid in peroxisomal disorders: a defect of delta 4 desaturation? Neurology 40, 1292–1298.

Martinez, M., 1992. Abnormal profiles of polyunsaturated fatty acids in the brain, liver, kidney and retina of patients with peroxisomal disorders. Brain Res. 583, 171–182.

Martinez, J., Moreno, J.J., 2001. Role of Ca2+-independent phospholipase A2 on arachidonic acid release induced by reactive oxygen species. Arch. Biochem. Biophys. 392, 257–262.

Martinez, M., Vazquez, E., Garcia-Silva, M.T., Manzanares, J., Bertran, J.M., Castello, F., Mougan, I., 2000. Therapeutic effects of docosahexaenoic acid ethyl ester in patients with generalized peroxisomal disorders. Am. J. Clin. Nutr. 71, 376S–385S.

Masferrer, J.L., Leahy, K.M., Koki, A.T., Zweifel, B.S., Settle, S.L., Woerner, B.M., Edwards, D.A., Flickinger, A.G., Moore, R.J., Seibert, K., 2000. Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. Cancer Res. 60, 1306–1311.

Mata, N.L., Weng, J., Travis, G.H., 2000. Biosynthesis of a major lipofuscin fluorophore in mice and humans with ABCR-mediated retinal and macular degeneration. Proc. Natl. Acad. Sci. USA 97, 7154–7159.

Matsugi, T., Chen, Q., Anderson, D.R., 1997. Suppression of CO2-induced relaxation of bovine retinal pericytes by angiotensin II. Invest. Ophthalmol. Vis. Sci. 38, 652–657.

Mayer, K., Merfels, M., Muhly Reinholz, M., Gokorsch, S., Rosseau, S., Lohmeyer, J., Schwarzer, N., Krull, M., Suttorp, N., Grimminger, F., Seeger, W., 2002. Omega-3 fatty acids suppress monocyte adhesion to human endothelial cells: role of endothelial PAF generation. Am. J. Physiol. Heart Circ. Physiol. 283, H811–H818.

McGuire, P.G., Jones, T.R., Talarico, N., Warren, E., Das, A., 2003. The urokinase/urokinase receptor system in retinal neovascularization: inhibition by A6 suggests a new therapeutic target. Invest. Ophthalmol. Vis. Sci. 44, 2736–2742.

McLean, W.G., 1997. The role of axonal cytoskeleton in diabetic neuropathy. Neurochem. Res. 22, 951–956.

Meydani, S.N., Lichtenstein, A.H., Cornwall, S., Meydani, M., Goldin, B.R., Rasmussen, H., Dinarello, C.A., Schaefer, E.J., 1993. Immunologic effects of national cholesterol education panel step-2 diets with and without fish-derived N-3 fatty acid enrichment. J. Clin. Invest. 92, 105–113.

Meydani, S.N., Endres, S., Woods, M.M., Goldin, B.R., Soo, C., Morrill-Labrode, A., Dinarello, C.A., Gorbach, S.L., 1991. Oral (n-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women. J. Nutr. 121, 547–555.

Meydani, S.N., Yogeeswaran, G., Liu, S., Baskar, S., Meydani, M., 1988. Fish oil and tocopherol-induced changes in natural killer cell-mediated cytotoxicity and PGE2 synthesis in young and old mice. J. Nutr. 118, 1245–1252.

Mickleborough, T.D., Murray, R.L., Ionescu, A.A., Lindley, M.R., 2003. Fish oil supplementation reduces severity of exercise-induced bronchoconstriction in elite athletes. Am. J. Respir. Crit. Care Med. 168, 1181–1189.

Miles, E.A., Calder, P.C., 1998. Modulation of immune function by dietary fatty acids. Proc. Nutr. Soc. 57, 277–292.

Mitchell, D.C., Niu, S.L., Litman, B.J., 2001. Optimization of receptor-G protein coupling by bilayer lipid composition I: kinetics of rhodopsin-transducin binding. J. Biol. Chem. 276, 42801–42806.

Mitchell, D.C., Niu, S.L., Litman, B.J., 2003. Enhancement of G protein-coupled signaling by DHA phospholipids. Lipids 38, 437–443.

Mizutani, M., Kern, T.S., Lorenzi, M., 1996. Accelerated death of retinal microvascular cells in human and experimental diabetic retinopathy. J. Clin. Invest. 97, 2883–2890.

Mizutani, M., Gerhardinger, C., Lorenzi, M., 1998. Muller cell changes in human diabetic retinopathy. Diabetes 47, 445–449.

Molvig, J., Pociot, F., Worsaae, H., Wogensen, L.D., Baek, L., Christensen, P., Mandrup-Poulsen, T., Andersen, K., Madsen, P., Dyerberg, J., et al., 1991. Dietary supplementation with omega-3-polyunsaturated fatty acids decreases mononuclear cell proliferation and interleukin-1 beta content but not monokine secretion in healthy and insulin-dependent diabetic individuals. Scand. J. Immunol. 34, 399–410.

Moore, S.A., 2001. Polyunsaturated fatty acid synthesis and release by brain-derived cells in vitro. J. Mol. Neurosci. 16, 195–200 discussion 215–121.

Mori, T.A., Beilin, L.J., Burke, V., Morris, J., Ritchie, J., 1997. Interactions between dietary fat, fish, and fish oils and their effects on platelet function in men at risk of cardiovascular disease. Arterioscler Thromb. Vasc. Biol. 17, 279–286.

Mori, T.A., Puddey, I.B., Burke, V., Croft, K.D., Dunstan, D.W., Rivera, J.H., Beilin, L.J., 2000. Effect of omega 3 fatty acids on oxidative stress in humans: GC-MS measurement of urinary F2-isoprostane excretion. Redox. Rep. 5, 45–46.

Mori, T.A., Woodman, R.J., Burke, V., Puddey, I.B., Croft, K.D., Beilin, L.J., 2003. Effect of eicosapentaenoic acid and docosahexaenoic acid on oxidative stress and inflammatory markers in treated-hypertensive type 2 diabetic subjects. Free Radic. Biol. Med. 35, 772–781.

Morita, I., Zhang, Y.W., Murota, S.I., 2001. Eicosapentaenoic acid protects endothelial cell function injured by hypoxia/reoxygenation. Ann. NY Acad. Sci. 947, 394–397.

Morris, M.C., Sacks, F., Rosner, B., 1993. Does fish oil lower blood pressure? A meta-analysis of controlled trials. Circulation 88, 523–533.

Morrow, J.D., Roberts, L.J., 1997. The isoprostanes: unique bioactive products of lipid peroxidation. Prog. Lipid. Res. 36, 1–21.

Muggli, R., 2003. n-3 Long-chain chain polyunsaturated fatty acids vitamin, E in cardiovascular disease prevention: epiphenomenon or causal dependency? In: Huang, Y.S., Lin, S.J., Huang, P.C. (Eds.), Essential Fatty Acids and Eicosanoids—Invited Papers from the Fifth International Congress. AOCS Press, Champaign, IL, pp. 65–70.

Mukutmoni, M., Hubbard, N.E., Erickson, K.L., 2001. Prostaglandin E(2) modulation of vascular endothelial growth factor production in murine macrophages. Prostaglandins Leukot Essent. Fatty Acids 65, 123–131.

Mukutmoni-Norris, M., Hubbard, N.E., Erickson, K.L., 2000. Modulation of murine mammary tumor vasculature by dietary n-3 fatty acids in fish oil. Cancer Lett. 150, 101–109.

Murota, S.I., Onodera, M., Morita, I., 2000. Regulation of angiogenesis by controlling VEGF receptor. Ann. NY Acad. Sci. 902, 208–212 discussion 212–203.

Nakamichi, N., Chidlow, G., Osborne, N.N., 2003. Effects of intraocular injection of a low concentration of zinc on the rat retina. Neuropharmacology 45, 637–648.

Nakata, H., Uemura, Y., Kobayashi, M., Harada, R., Taguchi, H., 2003. Cyclooxygenase-2 inhibitor NS-398 suppresses cell growth and constitutive production of granulocyte-colony stimulating factor and granulocyte macrophage-colony stimulating factor in lung cancer cells. Cancer Sci. 94, 173–180.

Narayanan, B.A., Narayanan, N.K., Simi, B., Reddy, B.S., 2003. Modulation of inducible nitric oxide synthase and related proinflammatory genes by the omega-3 fatty acid docosahexaenoic acid in human colon cancer cells. Cancer Res. 63, 972–979.

Naveh, N., Weissman, C., Marshall, J., 2000. Lipoxygenase metabolism following laser induced retinal injury in rabbits. Curr. Eye Res. 21, 554–559.

Nelson, G.J., 2000. Effects of dietary fatty acids on lipid metabolism. In: Chow, K.C. (Ed.), Fatty Acids in Foods and their Health Implications, 2nd ed. Marcel Dekker, Inc., New York, pp. 481–516.

Nemenoff, R., Winitz, S., Qian, N., Van Putten, V., Johnson, G., Heasley, L., 1993. Phosphorylation and activation of a high molecular weight form of phospholipase A2 by p42 microtubule-associated protein 2 kinase and protein kinase C. J. Biol. Chem. 268, 1960–1964.

Nenseter, M.S., Rustan, A.C., Lund-Katz, S., Soyland, E., Maelandsmo, G., Phillips, M.C., Drevon, C.A., 1992. Effect of dietary supplementation with n-3 polyunsaturated fatty acids on physical properties and metabolism of low density lipoprotein in humans. Arterioscler Thromb. 12, 369–379.

Nestel, P.J., 2000. Fish oil and cardiovascular disease: lipids and arterial function. Am. J. Clin. Nutr. 71, 228S–231S.

Nettleton, J.A., 1995. Omega-3 Fatty Acids and Health. Chapman & Hall, New York.

Neuringer, M., 1993. The relationship of fatty acid composition to fuction in the retina and visual system. Paper presented at: Lipids, Learning, and the Brain: Fats in Infant Formulas, 103rd Ross Conference on Pediatric Research, 1993; Adelaide, South Australia.

Neuringer, M., Jeffrey, B.G., 2003. Visual development: neural basis and new assessment methods. J. Pediatr. 143, S87–S95.

Nie, D., Hillman, G.G., Geddes, T., Tang, K., Pierson, C., Grignon, D.J., Honn, K.V., 1998. Platelet-type 12-lipoxygenase in a human prostate carcinoma stimulates angiogenesis and tumor growth. Cancer Res. 58, 4047–4051.

Nir, I., Haque, R., Iuvone, P.M., 2001. Regulation of cAMP by light and dopamine receptors is dysfunctional in photoreceptors of dystrophic retinal degeneration slow(rds) mice. Exp. Eye Res. 73, 265–272.

Nir, I., Harrison, J.M., Haque, R., Low, M.J., Grandy, D.K., Rubinstein, M., Iuvone, P.M., 2002. Dysfunctional light-evoked regulation of cAMP in photoreceptors and abnormal retinal adaptation in mice lacking dopamine D4 receptors. J. Neurosci. 22, 2063–2073.

Nitta, K., Uchida, K., Tsutsui, T., Honda, K., Kawashima, A., Yumura, W., Nihei, H., 1998. Eicosapentaenoic acid inhibits mitogen-induced endothelin-1 production and DNA synthesis in cultured bovine mesangial cells. Am. J. Nephrol. 18, 164–170.

Niu, S.L., Mitchell, D.C., Litman, B.J., 2001. Optimization of receptor-G protein coupling by bilayer lipid composition II: formation of metarhodopsin II–transducin complex. J. Biol. Chem. 276, 42807–42811.

Niu, S.L., Mitchell, D.C., Litman, B.J., 2002. Manipulation of cholesterol levels in rod disk membranes by methyl-beta-cyclodextrin: effects on receptor activation. J. Biol. Chem. 277, 20139–20145.

Ogilvie, J.M., Speck, J.D., 2002. Dopamine has a critical role in photoreceptor degeneration in the rd mouse. Neurobiol. Dis. 10, 33–40.

Onuki, Y., Morishita, M., Takayama, K., Tokiwa, S., Chiba, Y., Isowa, K., Nagai, T., 2000. In vivo effects of highly purified docosahexaenoic acid on rectal insulin absorption. Int. J. Pharm. 198, 147–156.

Organisciak, D.T., Darrow, R.M., Jiang, Y.L., Blanks, J.C., 1996. Retinal light damage in rats with altered levels of rod outer segment docosahexaenoate. Invest. Ophthalmol. Vis. Sci. 37, 2243–2257.

Organisciak, D.T., Darrow, R.M., Barsalou, L., Darrow, R.A., Kutty, R.K., Kutty, G., Wiggert, B., 1998. Light history and age-related changes in retinal light damage. Invest. Ophthalmol. Vis. Sci. 39, 1107–1116.

Orlidge, A., D'Amore, P.A., 1987. Inhibition of capillary endothelial cell growth by pericytes and smooth muscle cells. J. Cell. Biol. 105, 1455–1462.

Osborne, N.N., Casson, R.J., Wood, J.P., Chidlow, G., Graham, M., Melena, J., 2004. Retinal ischemia: mechanisms of damage and potential therapeutic strategies. Prog. Retina Eye Res. 23, 91–147.

Ozaki, N.K., Beharry, K.D., Nishihara, K.C., Akmal, Y., Ang, J.G., Sheikh, R., Modanlou, H.D., 2002. Regulation of retinal vascular endothelial growth factor and receptors in rabbits exposed to hyperoxia. Invest. Ophthalmol. Vis. Sci. 43, 1546–1557.

Pakala, R., Sheng, W.L., Benedict, C.R., 1999. Serotonin fails to induce proliferation of endothelial cells preloaded with eicosapentaenoic acid and docosahexaenoic acid. Atherosclerosis 145, 137–146.

Panda-Jonas, S., Jonas, J.B., Jakobczyk-Zmija, M., 1996. Retinal pigment epithelial cell count, distribution, and correlations in normal human eyes. Am. J. Ophthalmol. 121, 181–189.

Pawlosky, R.J., Denkins, Y., Ward, G., Salem Jr., N., 1997. Retinal and brain accretion of long-chain polyunsaturated fatty acids in developing felines: the effects of corn oil-based maternal diets. Am. J. Clin. Nutr. 65, 465–472.

Penfold, P.L., Madigan, M.C., Gillies, M.C., Provis, J.M., 2001. Immunological and aetiological aspects of macular degeneration. Prog. Retina Eye Res. 20, 385–414.

Peterson, L.D., Jeffery, N.M., Thies, F., Sanderson, P., Newsholme, E.A., Calder, P.C., 1998. Eicosapentaenoic and docosahexaenoic acids alter rat spleen leukocyte fatty acid composition and prostaglandin E2 production but have different effects on lymphocyte functions and cell-mediated immunity. Lipids 33, 171–180.

Phillis, J.W., O'Regan, M.H., 2003. The role of phospholipases, cyclooxygenases, and lipoxygenases in cerebral ischemic/traumatic injuries. Crit. Rev. Neurobiol. 15, 61–90.

Pierce, E.A., Avery, R.L., Foley, E.D., Aiello, L.P., Smith, L.E., 1995. Vascular endothelial growth factor/vascular permeability factor expression in a mouse model of retinal neovascularization. Proc. Natl. Acad. Sci. USA 92, 905–909.

Pierce, E.A., Foley, E.D., Smith, L.E., 1996. Regulation of vascular endothelial growth factor by oxygen in a model of retinopathy of prematurity. Arch. Ophthalmol. 114, 1219–1228.

Pieri, C., 2000. Fatty acids and aging. In: Chow, K.C. (Ed.), Fatty Acids in Foods and their Health Implications, 2nd ed. Marcel Dekker, Inc., New York, pp. 763–776.

Politi, L.E., Rotstein, N.P., Carri, N.G., 2001. Effect of GDNF on neuroblast proliferation and photoreceptor survival: additive protection with docosahexaenoic acid. Invest. Ophthalmol. Vis. Sci. 42, 3008–3015.

Price, S.A., Tisdale, M.J., 1998. Mechanism of inhibition of a tumor lipid-mobilizing factor by eicosapentaenoic acid. Cancer Res. 58, 4827–4831.

Price, P.T., Nelson, C.M., Clarke, S.D., 2000. Omega-3 polyunsaturated fatty acid regulation of gene expression. Curr. Opin. Lipidol. 11, 3–7.

Purasiri, P., McKechnie, A., Heys, S.D., Eremin, O., 1997. Modulation in vitro of human natural cytotoxicity, lymphocyte proliferative response to mitogens and cytokine production by essential fatty acids. Immunology 92, 166–172.

Qiu, Z., Leslie, C., 1994. Protein kinase C-dependent and -independent pathways of mitogen-activated protein kinase activation in macrophages by stimuli that activate phospholipase A2. J. Biol. Chem. 269, 19480–19487.

Rakoczy, P.E., Sarks, S.H., Daw, N., Constable, I.J., 1999. Distribution of cathepsin D in human eyes with or without age-related maculopathy. Exp. Eye Res. 69, 367–374.

Ramirez, J.M., Ramirez, A.I., Salazar, J.J., de Hoz, R., Trivino, A., 2001. Changes of astrocytes in retinal ageing and age-related macular degeneration. Exp. Eye Res. 73, 601–615.

Reinboth, J.J., Clausen, M., Reme, C.E., 1996. Light elicits the release of docosahexaenoic acid from membrane phospholipids in the rat retina in vitro. Exp. Eye Res. 63, 277–284.

Reme, C., Wei, Q., Munz, K., Jung, H., Doly, M., Droy-Lefaix, M.T., 1992. Light and lithium effects in the rat retina: modification by the PAF antagonist BN 52021. Graefes Arch. Clin. Exp. Ophthalmol. 230, 580–588.

Renier, G., Skamene, E., DeSanctis, J., Radzioch, D., 1993. Dietary n-3 polyunsaturated fatty acids prevent the development of atherosclerotic lesions in mice. Modulation of macrophage secretory activities. Arterioscler Thromb. 13, 1515–1524.

Reseland, J.E., Haugen, F., Hollung, K., Solvoll, K., Halvorsen, B., Brude, I.R., Nenseter, M.S., Christiansen, E.N., Drevon, C.A., 2001. Reduction of leptin gene expression by dietary polyunsaturated fatty acids. J. Lipid Res. 42, 743–750.

Rinaldi, B., Di Pierro, P., Vitelli, M.R., D'Amico, M., Berrino, L., Rossi, F., Filippelli, A., 2002. Effects of docosahexaenoic acid on calcium pathway in adult rat cardiomyocytes. Life Sci. 71, 993–1004.

Ringbom, T., Huss, U., Stenholm, A., Flock, S., Skattebol, L., Perera, P., Bohlin, L., 2001. COX-2 inhibitory effects of naturally occurring and modified fatty acids. J. Nat. Prod. 64, 745–749.

Roche, H.M., Gibney, M.J., 1996. Postprandial triacylglycerolaemia: the effect of low-fat dietary treatment with and without fish oil supplementation. Eur. J. Clin. Nutr. 50, 617–624.

Roche, H.M., Gibney, M.J., 2000. Effect of long-chain n-3 polyunsaturated fatty acids on fasting and postprandial triacylglycerol metabolism. Am. J. Clin. Nutr. 71, 232S–237S.

Rodriguez de Turco, E.B., Parkins, N., Ershov, A.V., Bazan, N.G., 1999. Selective retinal pigment epithelial cell lipid metabolism and remodeling conserves photoreceptor docosahexaenoic acid following phagocytosis. J. Neurosci. Res. 57, 479–486.

Romano, M., Claria, J., 2003. Cyclooxygenase-2 and 5-lipoxygenase converging functions on cell proliferation and tumor angiogenesis: implications for cancer therapy. FASEB J. 17, 1986–1995.

Rose, D.P., Connolly, J.M., 1999a. Antiangiogenicity of docosahexaenoic acid and its role in the suppression of breast cancer cell growth in nude mice. Int. J. Oncol. 15, 1011–1015.

Rose, D.P., Connolly, J.M., 1999b. Omega-3 fatty acids as cancer chemopreventive agents. Pharmacol. Ther. 83, 217–244.

Rose, D.P., Connolly, J.M., 2000. Regulation of tumor angiogenesis by dietary fatty acids and eicosanoids. Nutr. Cancer 37, 119–127.

Rose, D.P., Connolly, J.M., Rayburn, J., Coleman, M., 1995. Influence of diets containing eicosapentaenoic or docosahexaenoic acid on growth and metastasis of breast cancer cells in nude mice. J. Natl. Cancer Inst. 87, 587–592.

Rotstein, N.P., Ilincheta de Boschero, M.G., Giusto, N.M., Aveldano, M.I., 1987. Effects of aging on the composition and metabolism of docosahexaenoate-containing lipids of retina. Lipids 22, 253–260.

Rotstein, N.P., Aveldano, M.I., Barrantes, F.J., Politi, L.E., 1996. Docosahexaenoic acid is required for the survival of rat retinal photoreceptors in vitro. J. Neurochem. 66, 1851–1859.

Rotstein, N.P., Aveldano, M.I., Barrantes, F.J., Roccamo, A.M., Politi, L.E., 1997. Apoptosis of retinal photoreceptors during development in vitro: protective effect of docosahexaenoic acid. J. Neurochem. 69, 504–513.

Rotstein, N.P., Politi, L.E., Aveldano, M.I., 1998. Docosahexaenoic acid promotes differentiation of developing photoreceptors in culture. Invest. Ophthalmol. Vis. Sci. 39, 2750–2758.

Rotstein, N.P., Politi, L.E., German, O.L., Girotti, R., 2003. Protective effect of docosahexaenoic acid on oxidative stress-induced apoptosis of retina photoreceptors. Invest. Ophthalmol. Vis. Sci. 44, 2252–2259.

Rozanowska, M., Wessels, J., Boulton, M., Burke, J.M., Rodgers, M.A., Truscott, T.G., Sarna, T., 1998. Blue light-induced singlet oxygen generation by retinal lipofuscin in non-polar media. Free Radic. Biol. Med. 24, 1107–1112.

Rungger-Brandle, E., Dosso, A.A., Leuenberger, P.M., 2000. Glial reactivity, an early feature of diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. 41, 1971–1980.

Salem Jr., N., Litman, B., Kim, H.Y., Gawrisch, K., 2001. Mechanisms of action of docosahexaenoic acid in the nervous system. Lipids 36, 945–959.

Sanderson, P., Yaqoob, P., Calder, P.C., 1995. Dietary lipid modulation of cell mediated immunity in the rat. Biochem. Soc. Trans. 23, 273S.

Sanderson, P., MacPherson, G.G., Jenkins, C.H., Calder, P.C., 1997. Dietary fish oil diminishes the antigen presentation activity of rat dendritic cells. J. Leukoc. Biol. 62, 771–777.

Sanderson, P., Thies, F., Calder, P.C., 2000. Extracellular release of free fatty acids by rat T lymphocytes is stimulus-dependent and is affected by dietary lipid manipulation. Cell Biochem. Funct. 18, 47–58.

SanGiovanni, J.P., Berkey, C.S., Dwyer, J.T., Colditz, G.A., 2000a. Dietary essential fatty acids, long-chain polyunsaturated fatty acids, and visual resolution acuity in healthy fullterm infants: a systematic review. Early Hum. Dev. 57, 165–188.

SanGiovanni, J.P., Parra-Cabrera, S., Colditz, G.A., Berkey, C.S., Dwyer, J.T., 2000b. Meta-analysis of dietary essential fatty acids and long-chain polyunsaturated fatty acids as they relate to visual resolution acuity in healthy preterm infants. Pediatrics 105, 1292–1298.

Santoli, D., Phillips, P.D., Colt, T.L., Zurier, R.B., 1990. Suppression of interleukin 2-dependent human T cell growth in vitro by prostaglandin E (PGE) and their precursor fatty acids. Evidence for a PGE-independent mechanism of inhibition by the fatty acids. J. Clin. Invest. 85, 424–432.

Sanyal, S., Fletcher, R., Liu, Y.P., Aguirre, G., Chader, G., 1984. Cyclic nucleotide content and phosphodiesterase activity in the rds mouse (020/A) retina. Exp. Eye Res. 38, 247–256.

Schaefer, E.J., Robins, S.J., Patton, G.M., Sandberg, M.A., Weigel-DiFranco, C.A., Rosner, B., Berson, E.L., 1995. Red blood cell membrane phosphatidylethanolamine fatty acid content in various forms of retinitis pigmentosa. J. Lipid Res. 36, 1427–1433.

Schalkwijk, C.G., van der Heijden, M.A., Bunt, G., Maas, R., Tertoolen, L.G., van Bergen Bergen Henegouwen, P.M., Verkleij, A.J., van den Bosch, H., Boonstra, J., 1996. Maximal epidermal growth-factor-induced cytosolic phospholipase A2 activation in vivo requires phosphorylation followed by an increased intracellular calcium concentration. Biochem. J. 313, 91–96.

Schmidt, E.B., Pedersen, J.O., Ekelund, S., Grunnet, N., Jersild, C., Dyerberg, J., 1989. Cod liver oil inhibits neutrophil and monocyte chemotaxis in healthy males. Atherosclerosis 77, 53–57.

Schmidt, E.B., Varming, K., Pedersen, J.O., Lervang, H.H., Grunnet, N., Jersild, C., Dyerberg, J., 1992. Long-term supplementation with n-3 fatty acids, II: effect on neutrophil and monocyte chemotaxis. Scand. J. Clin. Lab. Invest. 52, 229–236.

Schmidt, E.B., Varming, K., Moller, J.M., Bulow Pedersen, I., Madsen, P., Dyerberg, J., 1996. No effect of a very low dose of n-3 fatty acids on monocyte function in healthy humans. Scand. J. Clin. Lab. Invest. 56, 87–92.

Scott, B.L., Bazan, N.G., 1989. Membrane docosahexaenoate is supplied to the developing brain and retina by the liver. Proc. Natl. Acad. Sci. USA 86, 2903–2907.

Seddon, J.M., Rosner, B., Sperduto, R.D., Yannuzzi, L., Haller, J.A., Blair, N.P., Willett, W., 2001. Dietary fat and risk for advanced age-related macular degeneration. Arch. Ophthalmol. 119, 1191–1199.

Seddon, J.M., Cote, J., Rosner, B., 2003. Progression of age-related macular degeneration: association with dietary fat, transunsaturated fat, nuts, and fish intake. Arch. Ophthalmol. 121, 1728–1737.

Self, D.W., 1999. Anandamide: a candidate neurotransmitter heads for the big leagues. Nat. Neurosci. 2, 303–304.

Sennlaub, F., Valamanesh, F., Vazquez-Tello, A., El-Asrar, A.M., Checchin, D., Brault, S., Gobeil, F., Beauchamp, M.H., Mwaikambo, B., Courtois, Y., Geboes, K., Varma, D.R., Lachapelle, P., Ong, H., Behar-Cohen, F., Chemtob, S., 2003. Cyclooxygenase-2 in human and experimental ischemic proliferative retinopathy. Circulation 108, 198–204 Epub 2003 Jun 2023.

Serhan, C.N., Oliw, E., 2001. Unorthodox routes to prostanoid formation: new twists in cyclooxygenase-initiated pathways. J. Clin. Invest. 107, 1481–1489.

Serhan, C.N., Clish, C.B., Brannon, J., Colgan, S.P., Chiang, N., Gronert, K., 2000a. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. J. Exp. Med. 192, 1197–1204.

Serhan, C.N., Clish, C.B., Brannon, J., Colgan, S.P., Gronert, K., Chiang, N., 2000b. Anti-microinflammatory lipid signals generated from dietary N-3 fatty acids via cyclooxygenase-2 and transcellular processing: a novel mechanism for NSAID and N-3 PUFA therapeutic actions. J. Physiol. Pharmacol. 51, 643–654.

Shahar, E., Folsom, A.R., Wu, K.K., Dennis, B.H., Shimakawa, T., Conlan, M.G., Davis, C.E., Williams, O.D., 1993. Associations of fish intake and dietary n-3 polyunsaturated fatty acids with a hypocoagulable profile. The Atherosclerosis Risk in Communities (ARIC) Study. Arterioscler Thromb. 13, 1205–1212.

Singer, P., Melzer, S., Goschel, M., Augustin, S., 1990. Fish oil amplifies the effect of propranolol in mild essential hypertension. Hypertension 16, 682–691.

Sivak, J.M., Fini, M.E., 2002. MMPs in the eye: emerging roles for matrix metalloproteinases in ocular physiology. Prog. Retina Eye Res. 21, 1–14.

Smith, L.E., 2002. Pathogenesis of retinopathy of prematurity. Acta Paediatr. Suppl. 91, 26–28.

Smith, L.E., Kopchick, J.J., Chen, W., Knapp, J., Kinose, F., Daley, D., Foley, E., Smith, R.G., Schaeffer, J.M., 1997. Essential role of growth hormone in ischemia-induced retinal neovascularization. Science 276, 1706–1709.

Smith, L.E., Shen, W., Perruzzi, C., Soker, S., Kinose, F., Xu, X., Robinson, G., Driver, S., Bischoff, J., Zhang, B., Schaeffer, J.M., Senger, D.R., 1999. Regulation of vascular endothelial growth factor-dependent retinal neovascularization by insulin-like growth factor-1 receptor. Nat. Med. 5, 1390–1395.

Smith, W., Mitchell, P., Leeder, S.R., 2000. Dietary fat and fish intake and age-related maculopathy. Arch. Ophthalmol. 118, 401–404.

Sone, H., Okuda, Y., Kawakami, Y., Yamashita, K., 1996. Effects of high glucose concentration and a thromboxane synthase inhibitor on the production of thromboxane A2 and prostaglandin I2 and E2 by retinal endothelial cells. Life Sci. 58, 239–243.

Stone, J., Chan-Ling, T., Pe'er, J., Itin, A., Gnessin, H., Keshet, E., 1996. Roles of vascular endothelial growth factor and astrocyte degeneration in the genesis of retinopathy of prematurity. Invest. Ophthalmol. Vis. Sci. 37, 290–299.

Straiker, A., Stella, N., Piomelli, D., Mackie, K., Karten, H.J., Maguire, G., 1999a. Cannabinoid CB1 receptors and ligands in vertebrate retina: localization and function of an endogenous signaling system. Proc. Natl. Acad. Sci. USA 96, 14565–14570.

Straiker, A.J., Maguire, G., Mackie, K., Lindsey, J., 1999b. Localization of cannabinoid CB1 receptors in the human anterior eye and retina. Invest. Ophthalmol. Vis. Sci. 40, 2442–2448.

Straiker, A., Sullivan, J.M., 2003. Cannabinoid receptor activation differentially modulates ion channels in photoreceptors of the tiger salamander. J. Neurophysiol. 89 (5), 2647–2654.

Su, H.M., Bernardo, L., Mirmiran, M., Ma, X.H., Corso, T.N., Nathanielsz, P.W., Brenna, J.T., 1999. Bioequivalence of dietary alpha-linolenic and docosahexaenoic acids as sources of docosahexaenoate accretion in brain and associated organs of neonatal baboons. Pediatr. Res. 45, 87–93.

Sugawara, T., Sieving, P.A., Iuvone, P.M., Bush, R.A., 1998. The melatonin antagonist luzindole protects retinal photoreceptors from light damage in the rat. Invest. Ophthalmol. Vis. Sci. 39, 2458–2465.

Suzuki, I., Iigo, M., Ishikawa, C., Kuhara, T., Asamoto, M., Kunimoto, T., Moore, M.A., Yazawa, K., Araki, E., Tsuda, H., 1997. Inhibitory effects of oleic and docosahexaenoic acids on lung metastasis by colon-carcinoma-26 cells are associated with reduced matrix metalloproteinase-2 and -9 activities. Int. J. Cancer 73, 607–612.

Takeuchi, T., Fukumoto, Y., Harada, E., 2002. Influence of a dietary n-3 fatty acid deficiency on the cerebral catecholamine contents, EEG and learning ability in rat. Behav. Brain Res. 131, 193–203.

Talvani, A., Machado, F.S., Santana, G.C., Klein, A., Barcelos, L., Silva, J.S., Teixeira, M.M., 2002. Leukotriene B(4) induces nitric oxide synthesis in Trypanosoma cruzi-infected murine macrophages and mediates resistance to infection. Infect. Immun. 70, 4247–4253.

Tang, D.G., Diglio, C.A., Bazaz, R., Honn, K.V., 1995a. Transcriptional activation of endothelial cell integrin alpha v by protein kinase C activator 12(S)-HETE. J. Cell Sci. 108 (Part 7), 2629–2644.

Tang, D.G., Renaud, C., Stojakovic, S., Diglio, C.A., Porter, A., Honn, K.V., 1995b. 12(S)-HETE is a mitogenic factor for microvascular endothelial cells: its potential role in angiogenesis. Biochem. Biophys. Res. Commun. 211, 462–468.

Tappia, P.S., Man, W.J., Grimble, R.F., 1995. Influence of unsaturated fatty acids on the production of tumour necrosis factor and interleukin-6 by rat peritoneal macrophages. Mol. Cell Biochem. 143, 89–98.

Tevar, R., Jho, D.H., Babcock, T., Helton, W.S., Espat, N.J., 2002. Omega-3 fatty acid supplementation reduces tumor growth and vascular endothelial growth factor expression in a model of progressive non-metastasizing malignancy. JPEN J. Parenter Enteral. Nutr. 26, 285–289.

Thies, F., Nebe-von-Caron, G., Powell, J.R., Yaqoob, P., Newsholme, E.A., Calder, P.C., 2001. Dietary supplementation with eicosapentaenoic acid, but not with other long-chain n-3 or n-6 polyunsaturated fatty acids, decreases natural killer cell activity in healthy subjects aged >55 y. Am. J. Clin. Nutr. 73, 539–548.

Traverso, V., Bush, R.A., Sieving, P.A., Deretic, D., 2002. Retinal cAMP levels during the progression of retinal degeneration in

rhodopsin P23H and S334ter transgenic rats. Invest Ophthalmol. Vis. Sci. 43, 1655–1661.

Trebble, T., Arden, N.K., Stroud, M.A., Wootton, S.A., Burdge, G.C., Miles, E.A., Ballinger, A.B., Thompson, R.L., Calder, P.C., 2003. Inhibition of tumour necrosis factor-alpha and interleukin 6 production by mononuclear cells following dietary fish-oil supplementation in healthy men and response to antioxidant co-supplementation. Br. J. Nutr. 90, 405–412.

Treen, M., Uauy, R.D., Jameson, D.M., Thomas, V.L., Hoffman, D.R., 1992. Effect of docosahexaenoic acid on membrane fluidity and function in intact cultured Y-79 retinoblastoma cells. Arch. Biochem. Biophys. 294, 564–570.

Tsuji, M., Murota, S.I., Morita, I., 2003. Docosapentaenoic acid (22:5, n-3) suppressed tube-forming activity in endothelial cells induced by vascular endothelial growth factor. Prostaglandins Leukot Essent. Fatty Acids 68, 337–342.

Uauy, R., Mena, P., Rojas, C., 2000. Essential fatty acids in early life: structural and functional role. Proc. Nutr. Soc. 59, 3–15.

Uauy, R., Hoffman, D.R., Peirano, P., Birch, D.G., Birch, E.E., 2001. Essential fatty acids in visual and brain development. Lipids 36, 885–895.

van Kuijk, F.J., Buck, P., 1992. Fatty acid composition of the human macula and peripheral retina. Invest. Ophthalmol. Vis. Sci. 33, 3493–3496.

Vanderkooi, J.M., Erecinska, M., Silver, I.A., 1991. Oxygen in mammalian tissue: methods of measurement and affinities of various reactions. Am. J. Physiol. 260, C1131–C1150.

Vasandani, C., Kafrouni, A.I., Caronna, A., Bashmakov, Y., Gotthardt, M., Horton, J.D., Spady, D.K., 2002. Upregulation of hepatic LDL transport by n-3 fatty acids in LDL receptor knockout mice. J. Lipid Res. 43, 772–784.

Vellani, V., Reynolds, A.M., McNaughton, P.A., 2000. Modulation of the synaptic Ca2+ current in salamander photoreceptors by polyunsaturated fatty acids and retinoids. J. Physiol. 529, 333–344.

Venkatraman, J.T., Chu, W.C., 1999. Effects of dietary omega-3 and omega-6 lipids and vitamin E on serum cytokines, lipid mediators and anti-DNA antibodies in a mouse model for rheumatoid arthritis. J. Am. Coll. Nutr. 18, 602–613.

Vericel, E., Calzada, C., Chapuy, P., Lagarde, M., 1999. The influence of low intake of n-3 fatty acids on platelets in elderly people. Atherosclerosis 147, 187–192.

Vericel, E., Polette, A., Bacot, S., Calzada, C., Lagarde, M., 2003. Pro- and antioxidant activities of docosahexaenoic acid on human blood platelets. J. Thromb. Haemost. 1, 566–572.

Virella, G., Fourspring, K., Hyman, B., Haskill-Stroud, R., Long, L., Virella, I., La Via, M., Gross, A.J., Lopes-Virella, M., 1991. Immunosuppressive effects of fish oil in normal human volunteers: correlation with the in vitro effects of eicosapentanoic acid on human lymphocytes. Clin. Immunol. Immunopathol. 61, 161–176.

Volker, D., Fitzgerald, P., Major, G., Garg, M., 2000. Efficacy of fish oil concentrate in the treatment of rheumatoid arthritis. J. Rheumatol. 27, 2343–2346.

von Knethen, A., Callsen, D., Brune, B., 1999. Superoxide attenuates macrophage apoptosis by NF-kappa B and AP-1 activation that promotes cyclooxygenase-2 expression. J. Immunol. 163, 2858–2866.

von Schacky, C., Fischer, S., Weber, P.C., 1985. Long-term effects of dietary marine omega-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans. J. Clin. Invest. 76, 1626–1631.

Wainwright, P.E., 2002. Dietary essential fatty acids and brain function: a developmental perspective on mechanisms. Proc. Nutr. Soc. 61, 61–69.

Wallace, F.A., 2001. Dietary fatty acids influence the production of Th1- but not Th2-type cytokines. J. Leukoc. Biol. 69, 449–457.

Wallace, F.A., Miles, E.A., Calder, P.C., 2000. Activation state alters the effect of dietary fatty acids on pro-inflammatory mediator production by murine macrophages. Cytokine 12, 1374–1379.

Wallace, F.A., Miles, E.A., Evans, C., Stock, T.E., Yaqoob, P., Calder, P.C., 2001. Dietary fatty acids influence the production of Th1- but not Th2-type cytokines. J. Leukoc. Biol. 69, 449–457.

Wander, R.C., Du, S.H., Thomas, D.R., 1998. Influence of long-chain polyunsaturated fatty acids on oxidation of low density lipoprotein. Prostaglandins Leukot Essent. Fatty Acids 59, 143–151.

Wang, N., Anderson, R.E., 1992. Enrichment of polyunsaturated fatty acids from rat retinal pigment epithelium to rod outer segments. Curr. Eye Res. 11, 783–791.

Wang, N., Anderson, R.E., 1993. Synthesis of docosahexaenoic acid by retina and retinal pigment epithelium. Biochemistry 32, 13703–13709.

Wangsa-Wirawan, N.D., Linsenmeier, R.A., 2003. Retinal oxygen: fundamental and clinical aspects. Arch. Ophthalmol. 121, 547–557.

Watzke, R.C., Soldevilla, J.D., Trune, D.R., 1993. Morphometric analysis of human retinal pigment epithelium: correlation with age and location. Curr. Eye Res. 12, 133–142.

Weisinger, H.S., Vingrys, A.J., Sinclair, A.J., 1996. The effect of docosahexaenoic acid on the electroretinogram of the guinea pig. Lipids 31, 65–70.

Weiss, E.R., Hao, Y., Dickerson, C.D., Osawa, S., Shi, W., Zhang, L., Wong, F., 1995. Altered cAMP levels in retinas from transgenic mice expressing a rhodopsin mutant. Biochem. Biophys. Res. Commun. 216, 755–761.

Werner, J.S., Steele, V.G., Pfoff, D.S., 1989. Loss of human photoreceptor sensitivity associated with chronic exposure to ultraviolet radiation. Ophthalmology 96, 1552–1558.

Werz, O., Szellas, D., Steinhilber, D., 2000. Reactive oxygen species released from granulocytes stimulate 5-lipoxygenase activity in a B-lymphocytic cell line. Eur. J. Biochem. 267, 1263–1269.

Wetzel, M.G., Li, J., Alvarez, R.A., Anderson, R.E., O'Brien, P.J., 1991. Metabolism of linolenic acid and docosahexaenoic acid in rat retinas and rod outer segments. Exp. Eye Res. 53, 437–446.

Wiechmann, A.F., O'Steen, W.K., 1992. Melatonin increases photoreceptor susceptibility to light-induced damage. Invest. Ophthalmol. Vis. Sci. 33, 1894–1902.

Wiegand, R.D., Koutz, C.A., Stinson, A.M., Anderson, R.E., 1991. Conservation of docosahexaenoic acid in rod outer segments of rat retina during n-3 and n-6 fatty acid deficiency. J. Neurochem. 57, 1690–1699.

Wilkinson-Berka, J.L., Alousis, N.S., Kelly, D.J., Gilbert, R.E., 2003. COX-2 inhibition and retinal angiogenesis in a mouse model of retinopathy of prematurity. Invest. Ophthalmol. Vis. Sci. 44, 974–979.

Williams, B., Schrier, R.W., 1993. Glucose-induced protein kinase C activity regulates arachidonic acid release and eicosanoid production by cultured glomerular mesangial cells. J. Clin. Invest. 92, 2889–2896.

Williard, D.E., Harmon, S.D., Kaduce, T.L., Preuss, M., Moore, S.A., Robbins, M.E., Spector, A.A., 2001. Docosahexaenoic acid synthesis from n-3 polyunsaturated fatty acids in differentiated rat brain astrocytes. J. Lipid Res. 42, 1368–1376.

Wilson, J.X., 1997. Antioxidant defense of the brain: a role for astrocytes. Can. J. Physiol. Pharmacol. 75, 1149–1163.

Winkler, B.S., Boulton, M.E., Gottsch, J.D., Sternberg, P., 1999. Oxidative damage and age-related macular degeneration. Mol. Vis. 5, 32.

Wolf, G., 2003. Lipofuscin and macular degeneration. Nutr. Rev. 61, 342–346.

Woodman, R.J., Mori, T.A., Burke, V., Puddey, I.B., Barden, A., Watts, G.F., Beilin, L.J., 2003. Effects of purified eicosapentaenoic acid and docosahexaenoic acid on platelet, fibrinolytic and vascular

function in hypertensive type 2 diabetic patients. Atherosclerosis 166, 85–93.

Wu, D.M., Kawamura, H., Li, Q., Puro, D.G., 2001. Dopamine activates ATP-sensitive K+ currents in rat retinal pericytes. Vis. Neurosci. 18, 935–940.

Wu, D.M., Kawamura, H., Sakagami, K., Kobayashi, M., Puro, D.G., 2003. Cholinergic regulation of pericyte-containing retinal microvessels. Am. J. Physiol. Heart Circ. Physiol. 284, H2083–H2090.

Yamashita, N., Yokoyama, A., Hamazaki, T., Yano, S., 1986. Inhibition of natural killer cell activity of human lymphocytes by eicosapentaenoic acid. Biochem. Biophys. Res. Commun. 138, 1058–1067.

Yang, S.P., Morita, I., Murota, S.I., 1998. Eicosapentaenoic acid attenuates vascular endothelial growth factor-induced proliferation via inhibiting Flk-1 receptor expression in bovine carotid artery endothelial cells. J. Cell Physiol. 176, 342–349.

Yaqoob, P., 1998. Lipids and the immune response. Curr. Opin. Clin. Nutr. Metab. Care 1, 153–161.

Yaqoob, P., Calder, P., 1995a. Effects of dietary lipid manipulation upon inflammatory mediator production by murine macrophages. Cell Immunol. 163, 120–128.

Yaqoob, P., Calder, P.C., 1995b. The effects of dietary lipid manipulation on the production of murine T cell-derived cytokines. Cytokine 7, 548–553.

Yaqoob, P., Newsholme, E.A., Calder, P.C., 1994a. The effect of dietary lipid manipulation on rat lymphocyte subsets and proliferation. Immunology 82, 603–610.

Yaqoob, P., Newsholme, E.A., Calder, P.C., 1994b. Fatty acid oxidation by lymphocytes. Biochem. Soc. Trans. 22, 116S.

Yaqoob, P., Newsholme, E.A., Calder, P.C., 1994c. Inhibition of natural killer cell activity by dietary lipids. Immunol. Lett. 41, 241–247.

Yaqoob, P., Pala, H.S., Cortina-Borja, M., Newsholme, E.A., Calder, P.C., 2000. Encapsulated fish oil enriched in alpha-tocopherol alters plasma phospholipid and mononuclear cell fatty acid compositions but not mononuclear cell functions. Eur. J. Clin. Invest. 30, 260–274.

Yossuck, P., Yan, Y., Tadesse, M., Higgins, R.D., 2001. Dexamethasone alters TNF-alpha expression in retinopathy. Mol. Genet Metab. 72, 164–167.

Yu, B.P., Yang, R., 1996. Critical evaluation of the free radical theory of aging. A proposal for the oxidative stress hypothesis. Ann. NY Acad. Sci. 786, 1–11.

Yu, K., Bayona, W., Kallen, C.B., Harding, H.P., Ravera, C.P., McMahon, G., Brown, M., Lazar, M.A., 1995. Differential activation of peroxisome proliferator-activated receptors by eicosanoids. J. Biol. Chem. 270, 23975–23983.

Yu, W., Murray, N.R., Weems, C., Chen, L., Guo, H., Ethridge, R., Ceci, J.D., Evers, B.M., Thompson, E.A., Fields, A.P., 2003. Role of cyclooxygenase 2 in protein kinase C beta II-mediated colon carcinogenesis. J. Biol. Chem. 278, 11167–11174.

Yusufi, A.N., Cheng, J., Thompson, M.A., Walker, H.J., Gray, C.E., Warner, G.M., Grande, J.P., 2003. Differential effects of low-dose docosahexaenoic acid and eicosapentaenoic acid on the regulation of mitogenic signaling pathways in mesangial cells. J. Lab. Clin. Med. 141, 318–329.

Zeng, X.X., Ng, Y.K., Ling, E.A., 2000. Neuronal and microglial response in the retina of streptozotocin-induced diabetic rats. Vis. Neurosci. 17, 463–471.

Zhang, K., Kniazeva, M., Han, M., Li, W., Yu, Z., Yang, Z., Li, Y., Metzker, M.L., Allikmets, R., Zack, D.J., Kakuk, L.E., Lagali, P.S., Wong, P.W., MacDonald, I.M., Sieving, P.A., Figueroa, D.J., Austin, C.P., Gould, R.J., Ayyagari, R., Petrukhin, K., 2001. A 5-bp deletion in ELOVL4 is associated with two related forms of autosomal dominant macular dystrophy. Nat. Genet. 27, 89–93.

Zhang, Y.W., Yao, X.S., Murota, S., Morita, I., 2002. Inhibitory effects of eicosapentaenoic acid (EPA) on the hypoxia/reoxygenation-induced tyrosine kinase activation in cultured human umbilical vein endothelial cells. Prostaglandins Leukot Essent. Fatty Acids 67, 253–261.