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Review

Omega-3 Polyunsaturated Fatty Acids and Their Anti-Oxidant, Anti-Inflammatory and Neuroprotective Effects in Diabetic Retinopathy: A Narrative Review

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Abstract

Diabetic retinopathy (DR) is a common microvascular complication of type 2 Diabetes Mellitus (T2DM) that can have vision-threatening consequences, particularly if it advances to the proliferative stage and is left untreated. Owing to the central role of hyperglycemia-induced oxidative stress, multiple anti-oxidants have been investigated for their therapeutic value. However, there is a lack of substantial data to support the use of any of the compounds tested so far. Omega-3 polyunsaturated fatty acids (PUFAs), namely docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have received much acclaim for their positive impact on cardiovascular health outcomes. The anti-oxidative, anti-inflammatory and neuroprotective properties of PUFAs also make them promising therapeutic and preventive agents for DR. The current evidence is derived mainly from *in vitro* and animal studies and provides some insight into the underlying mechanisms involved. These fatty acids are capable of direct anti-oxidative and anti-inflammatory effects. They also concomitantly promote intrinsic defense mechanisms and recovery, particularly of photoreceptor neurons. Hence, dietary supplementation with PUFAs, mainly from marine sources, can halt and reverse the retinal damage seen in DR. Furthermore, clinical trials have reported improved vision and quality of life in DR patients after supplementation. However, a major limitation of these trials is the use of nutraceutical formulations in which omega-3 PUFAs are combined with other anti-oxidant compounds, thereby preventing the evaluation of omega-3 as standalone treatment. Although the results of experimental studies to date have been promising, more clinical trials are required to determine the full extent of benefits in patients with DR.

Keywords: diabetic retinopathy; omega-3 polyunsaturated fatty acids; docosahexaenoic acid; eicosapentaenoic acid

1. Introduction

The prevalence of diabetes mellitus in the global population has soared and it is now the most common metabolic disorder. This has spurred the development of new therapeutic options aimed at improving patients' quality of life, primarily by attenuating the complications from diabetes. Diabetic retinopathy (DR) is one of the most common microvascular complications of diabetes, with a prevalence that is projected to increase by >50% by 2045. Hence, it is imperative to investigate strategies that could prevent an escalation in the global incidence of this condition [1].

DR is the leading cause of preventable blindness in developed and developing countries alike [2]. The risk of blindness is further increased in type 2 Diabetes Mellitus (T2DM), which accounts for 90–95% of all diabetic cases in individuals over the age of 20. The increased likelihood of blindness is due to the development of macular edema, a complication that occurs more frequently in T2DM patients and contributes directly to a loss of visual acuity [1–3]. Two broad categories of DR exist: non-proliferative and proliferative, with the primary distinction being that the latter is characterized by neovascularization and vitreous hemorrhaging within the retina [4]. Laser photocoagulation and anti-vascular endothelial growth factor (VEGF) agents remain at the forefront of therapy, particularly for proliferative type DR. However, the multifaceted pathophysiology of DR warrants the investigation of other therapeutic strategies that are less invasive and have better safety profiles [3].

One of the many proposed mechanisms for DR is that accumulation of cytokines and interleukins causes oxidative stress, which induces the production of vasoactive factors such as VEGF, leading to angiogenesis in the retina [3]. Several anti-oxidants and dietary supplements have therefore been investigated in this context. Studies conducted on Vitamin C, E and selenium supplements have yielded inconsistent results [5,6]. Other anti-oxidants such as omega-3 polyunsaturated fatty acids (PUFAs) and docosahexaenoic acid (DHA) have received less attention, but could potentially yield superior results because they have additional properties aside from anti-oxidant activity, such as neuroprotection [7–9]. DHA supplementation has been shown to inhibit apoptosis and increase cell viability, not only of retinal neurons but also of neurons present in the central nervous system (CNS) [10]. Additionally, DHA in-

creases opsin expression in the photoreceptors of the retina, thereby promoting their normal differentiation [8].

The other form of omega-3 PUFAs, eicosapentaenoic acid (EPA), is also important to study. Although the only structural difference between the two omega-3 compounds is an additional double bond, DHA and EPA exert different effects on membranes. DHA accounts for 50–60% of the membrane fatty acid composition, while EPA has superior anti-oxidant effects on the membrane, primarily due to its greater stability within phospholipid membranes [11].

Omega-3 PUFAs have received much acclaim for their beneficial systemic effects in promoting cardiovascular and metabolic health, which is mainly due to their anti-inflammatory effects. While the exact mechanism remains unknown, omega-3 PUFAs can reduce the level of key inflammatory cytokines such as tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6). They can also downregulate nuclear factor kappa B (NF-κB), a central mediator in activation of the "nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3" (NLRP3) inflammasome [12–14].

These promising results have prompted the investigation of omega-3 PUFAs for ophthalmological conditions, the most notable being age-related macular degeneration (AMD). For this particular condition, dietary supplementation is usually for prevention, although omega-3 PUFAs can also cause the regression of established disease. Taking this into consideration, together with the lipid profile in the retina having the highest concentration of PUFAs in the body, it can be suggested that dietary supplementation with omega-3 PUFAs could be beneficial for the management of DR [15,16]. Hence, the aim of this narrative review is to evaluate the current literature to determine whether omega-3 PUFAs might be useful in preventing and slowing the progression of DR in a clinical setting.

1.1 Omega-3 Fatty Acids and Their Anti-Oxidative Actions

As with other conditions, the beneficial effects of omega-3 PUFAs on DR are not restricted to a single pathway. Nevertheless, the overall effect eventually culminates in increased cell viability and reduced apoptosis of retinal cells affected by oxidative stress [8,10,17]. Our understanding of these multiple pathways has grown immensely over the years, mainly due to the findings of *in vitro* studies. The increasing confidence in the anti-oxidant actions of these compounds has led to human trials that have provided more insight into their possible clinical applications.

The protective effects of omega-3 PUFAs against oxidative damage in diabetic patients have been shown to involve multiple pathways. An *in vitro* study on monocyte cultures conducted by Laubertová *et al.* [17] elucidated several of these pathways. After simulating hyperglycemic conditions to induce oxidative damage, a fish oil emulsion containing omega-3 PUFAs was added to study the potential anti-oxidative mechanisms. The first apparent effect was direct scavenging of oxidative radicals within the serum. Omega-3 PUFAs also act indirectly by increasing the activity of other protective enzymes, such as superoxide dismutase. This action was attributed to the modulation of pre-existing enzyme activity and was not due to the induction of gene expression or to increased production of enzyme. Furthermore, it was also found that fish oil emulsion did not protect against DNA damage induced by hyperglycemia [17]. This *in vitro* study is valuable because it identified the site of action of fish oil emulsion, as well as the fact that it does not mediate DNA repair.

A significant proportion of the oxidative stress in DR originates within the mitochondria. Effects at this level are important when evaluating the potential therapeutic use of omega-3 PUFAs in DR [16,18]. As shown in Table 1 (Ref. [7,8,16,17,19–22]), an *in vitro* study used retinal pigment epithelium (RPE) cells to mirror the conditions found in DR. In addition to restoring the anti-oxidant balance by scavenging reactive oxidative species produced during cellular aerobic respiration, omega-3 PUFAs increased the activity of mitochondrial dehydrogenase, thereby attenuating the deleterious effects of oxidative radicals on the mitochondria. Furthermore, omega-3 PUFAs are able to prevent apoptosis in cells affected by oxidative stress by preventing pathological alternations in the mitochondrial membrane potential that cause induction of the apoptotic cascade [8,17]. A prospective randomized clinical trial (RCT) involving patients with T2DM, were supplementated with a nutraceutical formulation containing DHA as the principal ingredient. Improvements were seen in best corrected visual acuity (BCVA) and in the appearance of the retina in optical fundus examinations, as well as less lipid peroxidation products in the plasma [19]. While the mitochondria appear to be an important target for the action of the omega-3 PUFAs, the actual mitochondrial pathways still require further investigation.

In vitro studies have proven invaluable for understanding the mechanisms involving omega-3 PUFAs. However, studies in humans more closely reflect the reality of treating patients with DR. Such studies are able to take into account the presence of additional risk factors in diabetic patients, including obesity, hyperlipidemia and hypertension, all of which are potential confounders. The aim of the PREDIMED trial was to examine the effect of omega-3 PUFAs on cardiovascular health. A sub-study of this trial showed that high omega-3 PUFAs levels, which reflected the level of dietary intake, reduced the risk of developing DR in T2DM patients [20].

Another RCT confirmed these positive findings, but due to the presence of other anti-oxidants in the nutraceutical supplements it could not be confirmed that the reduced risk of DR was solely attributable to the anti-oxidative effects of DHA [19]. Nevertheless, other clinical trials in patients with T2DM have confirmed the anti-oxidant benefits of omega-3 fatty acids [7,21]. A prospective case-control

Table 1. Summary of selected findings from experimental studies performed using omega-3 fatty acids, as discussed in Section 1.1.

Study	Type of study	Omega-3 formulation	Subjects of study/Culture used	Outcome measures & Results
"Fish oil emulsion supplementation might improve quality of life of diabetic patients due to its anti-oxidant and anti-inflammatory properties" (Laubertová *et al*., 2017 [17]) *Science Direct, Nutrition Research*	*In vitro* study	45% fish oil (8.1% EPA, 5.9% DHA)	Monocyte cell cultures	♦ **Anti-oxidant capacity**: Concentration dependent increase with fish oil supplementation ♦ **Superoxide dismutase enzyme activity**: Increased in the groups supplemented with the 2 most concentrated formulations ♦ **Cytokine Secretion**: Decrease in TNF and IL-6 secretion in supplemented group. No significant effects on IL-8 release and MCP-1 release in the supplemented group
"Synthesis of docosahexaenoic acid from eicosapentaenoic acid in retina neurons protects photoreceptors from oxidative stress" (Simón *et al*., 2016 [8]) *Journal of Neurochemistry (JNC)*	*In vitro* study	EPA concentrations ranging from 1 to 6 µM	Retinal neuron cell cultures	♦ **Reduction in apoptosis**: Decrease in TUNEL positive cells and cells with pyknotic nuclei in the supplemented group ♦ **Cell viability**: Increased, however in groups supplemented with concentrations higher than 6 µM, there was increased cell death ♦ **Mitochondrial membrane potential**: EPA supplementation allowed preservation of potential in the face of oxidative stress ♦ **Photoreceptor differentiation**: Promotes opsin expression in the group supplemented with EPA ♦ **Fatty acid composition in neuronal membranes**: Increased percentage of DHA in the membrane of supplemented groups
"New approach to modulate retinal cellular toxic effects of high glucose using marine EPA and DHA" (Dutot *et al*., 2011 [16]) *BioMed Central, Nutrition and Metabolism*	*In vitro* study	Fish oil (36% EPA and 26% DHA)	Human derived RPE (ARPE-19) cells	♦ **ROS production** (detection of 2'-7'- dichlorofluorescein diacetate): Fish oil supplementation prevented hyperglycemia-induced increase in ROS ♦ **Mitochondrial dehydrogenase activity**: Fish oil supplementation prevented hyperglycemia induced decrease in enzyme activity ♦ **Cytokine release**: Prevention of TNF-α increase in groups supplemented with fish oil ♦ **Fatty acid composition**: Increased EPA levels and decrease DHA levels within the cells of groups supplemented with fish oil ♦ **Caveolin-1 expression**: Increased expression in supplemented groups

Table 1. Continued.

Study	Type of study	Omega-3 formulation	Subjects of study/Culture used	Outcome measures & Results
"Enhanced Oxidative Stress and Other Potential Biomarkers for Retinopathy in Type 2 Diabetics: Beneficial Effects of the Nutraceutic Supplements" (Roig-Revert *et al*., 2015 [19]) *BioMed Research International*	RCT	Nutraceutical formulation containing DHA along with other vitamins and antioxidant compounds	208 patients suffering from T2DM	♦ **Ocular Examinations** (BCVA, SD-OCT, fundus imaging): Decreased progression of DR and incidence of macular edema in groups which received the nutraceutical formulation ♦ **Blood sampling**: Decrease in lipid peroxidation product formation and increased anti-oxidant activity in groups which received the nutraceutical formulation
"Dietary Marine ω-3 Fatty Acids and Incident Sight-Threatening Retinopathy in Middle-Aged and Older Individuals with Type 2 Diabetes: Prospective Investigation from the PREDIMED Trial" (Sala Vila *et al*., 2016 [20]) *JAMA Ophthalmology*	Prospective sub-study from RCT	≥500 mg/dL of dietary omega-3 consumption	3482 patients suffering from T2DM	♦ **Incidence of DR**: Decreased in supplemented group
"Supplementation with a highly concentrated docosahexaenoic acid plus xanthophyll carotenoid multivitamin in non-proliferative diabetic retinopathy: prospective controlled study of macular function by fundus microperimetry" (González-Herrero *et al*., 2018 [7]) *Clinical Ophthalmology, Dove Medical Press*	Prospective controlled trial	Daily nutraceutical supplementation containing DHA (1050 mg triglyceride bound and 90 mg free), EPA (127 mg) along with other vitamins and antioxidant compounds	24 T2DM patients with pre-existing NPDR	♦ **Macular function by microperimetry** (primary outcome): Increased macular sensitivity in supplemented group ♦ **Vision related quality of life**: Increased in supplemented group ♦ **Blood sampling**: Increased DHA content in erythrocyte membrane and total anti-oxidant capacity. Decreased IL-6 levels in supplemented group
"Docosahexaenoic acid (DHA) has neuroprotective effects against oxidative stress in retinal ganglion cells" (Shimazawa *et al*., 2009 [21]) *Science Direct, Brain Research*	*In vitro* study	DHA	Retinal ganglion cells	♦ **Radical scavenging capacity**: Direct scavenging of oxidative radicals by DHA in supplemented group ♦ **Effects on damage induced by oxidative stress (H_2O_2) & hypoxia**: Prevented reductions in cell viability and changes in nuclear morphology induced by H_2O_2 and oxygen deprivation in supplemented group
"Clinical and Molecular-Genetic Insights into the Role of Oxidative Stress in Diabetic Retinopathy: Anti-oxidant Strategies and Future Avenues" (Sanz-González *et al*., 2020 [22]) *Molecular Diversity Preservation International (MDPI)*	Prospective case control study	Nutraceutical formulation containing DHA along with other vitamins and antioxidant compounds	480 T2DM patients	♦ **Ocular examinations** (BCVA, ocular fundus imaging, SD-OCT): Decreased progression of DR and amelioration of vision in supplemented group ♦ **Blood sampling**: Decrease in oxidative stress, production of lipid peroxidation products. Increase in total anti-oxidant capacity in supplemented group

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; BCVA, best corrected visual acuity; DR, diabetic retinopathy; T2DM, type 2 Diabetes Mellitus; RPE, retinal pigment epithelium; RCT, randomized clinical trial; NPDR, Non-Proliferative Diabetic Retinopathy; ROS, reactive oxygen species; SD-OCT, spectral-domain optical coherence tomography. Bold text indicates the type of the experimental study performed.

Table 2. Summary of selected findings from experimental studies performed using omega-3 fatty acids, as discussed in Section 1.2.

Study	Type of study	Omega-3 formulation	Subjects of study/Culture used	Outcome measures & Results
"Short communication: PPAR gamma mediates a direct antiangiogenic effect of omega 3-PUFAs in proliferative retinopathy" (Stahl *et al*., 2010 [24]) *American Heart Association, Circulation Research*	*In vivo* study	Standard diet supplemented with 2% DHA and EPA	Mouse pups	♦ **Retinal neovascularization**: Attenuates established neovascularization in supplemented group, by actions mediated by PPAR-γ ♦ **Inflammatory activity**: Reduced- TNF-α levels are also decreased in supplemented group, mediated by PPAR-γ ♦ **Endothelial cell activation**: Reduced in supplemented group
"N-3 polyunsaturated fatty acids prevent diabetic retinopathy by inhibition of retinal vascular damage and enhanced endothelial progenitor cell reparative function" (Tikhonenko *et al*., 2013 [26]) *Public Library of Science (PloS)*	*In vivo* study	Fish oil (menhaden) with DHA (10.26%) and EPA (14.16%)	Rats with T2DM	♦ **Acid sphingomyelinase enzyme activity**: Inhibited increase in activity in supplemented group when compared to control diabetic rats ♦ **IL-1β, IL-6, TNF-α and ICAM-1 levels** (qRT-PCR): Decreased levels in DHA supplemented rats ♦ **Endothelial progenitor cell migration**: Increased production and migration of endothelial cells in supplemented rats ♦ **Survival**: Increased life span in supplemented rats
"Neurotrophins enhance retinal pigment epithelial cell survival through neuroprotectin D1 signaling" (Mukherjee *et al*., 2007 [27]) *National Research Council (US), PNAS*	*In vitro* study	50 nM DHA/50 nM DHA + added neurotrophins including pigment epithelial derived factor (PEDF)	Human RPE cell culture	♦ **NPD1 synthesis**: DHA synergistically increases PEDF induced production of NPD1 from the apical side of the photoreceptor membrane in supplemented group ♦ **Cell viability**: DHA acts in synergy with PEDF to cause cryoprotection in supplemented group ♦ **Expression of apoptotic proteins**: Decreased expression of pro-apoptotic proteins (caspase3, Bid, Bax, Bad) and increased expression of antiapoptotic proteins (Bcl-2, Bfl-1) in supplemented group

Table 2. Continued.

Study	Type of study	Omega-3 formulation	Subjects of study/Culture used	Outcome measures & Results
"Omega-3 from flaxseed oil protects obese mice against diabetic retinopathy through GPR120 receptor" (Dátilo *et al*., 2018 [25]) *Nature Publishing Group*	*In vivo* study	Flaxseed oil 10% of total fat composition in supplemented diet	Swiss male mice with induced obesity	♦ **Activation of the GPR120 receptor-** activation by omega-3 PUFAs is mediated by the b-arrestin2 protein ♦ **Glucose control:** Improves fasting blood glucose and glucose tolerance ♦ **Retinal changes**: Supplemented groups were protected from retinal damage induced by the high fat diet ♦ **GPR expression**: upregulated the expression of GPR120 receptors but not GPR40 ♦ **Retinal inflammation**: decrease in levels of multiple inflammatory cytokines such as IL-Ib, TNF-a and VEGF. Expression of IL-10, however, remains unchanged
"New approach to modulate retinal cellular toxic effects of high glucose using marine EPA and DHA" (Dutot *et al*., 2011 [16]) *BioMed Central, Nutrition and Metabolism*			Refer to Table 1	
"Fish oil emulsion supplementation might improve quality of life of diabetic patients due to its anti-oxidant and anti-inflammatory properties" Laubertová *et al*., 2017 [17]) - *Science Direct, Nutrition Research*				
"Synthesis of docosahexaenoic acid from eicosapentaenoic acid in retina neurons protects photoreceptors from oxidative stress" (Simón *et al*., 2016 [8]) *Journal of Neurochemistry (JNC)*				

VEGF, vascular endothelial growth factor; PPAR-γ, peroxisome proliferator-activated receptor-gamma; TNF-α, tumour necrosis factor-α; IL, interleukin; ICAM-1, intercellular adhesion molecule 1; PEDF, pigment epithelium-derived factor; NPD1, neuroprotectin D1; GPR, G protein-coupled receptor. Bold text indicates the type of the experimental study performed.

study by Sanz-González *et al*. [22] enrolled 575 participants, 287 of whom had T2DM and 129 of whom had pre-existing DR. These were randomized and assigned to an intervention group that received an omega-3-containing supplement in combination with other vitamins, or to a control group. Importantly, this study applied rigorous inclusion and exclusion criteria to limit the recall bias usually associated with conventional retrospective case-control studies, as well as the effects of confounding. Optical coherence tomography (OCT) imaging revealed that retinas of patients who received supplementation with omega-3 PUFAs had fewer pathological changes, such as thickening of the membranes. Furthermore, a considerable proportion of the patients with diabetes who developed DR or who had worsening of pre-existing DR during the course of the study were in the control group, indicating that omega-3 conferred protection against DR. This supports the use of omega-3 PUFAs in patients who suffer from DR, as well as their use as a prophylactic measure in individuals who have not already developed DR [22]. Although these findings are in accordance with those of previous studies, more clinical trials are needed to determine the benefits of a supplement that contains only omega-3 PUFAs in patients with DR.

1.2 Omega-3 Fatty Acids and Their Anti-Inflammatory Actions

There is a considerable overlap between the anti-oxidant and anti-inflammatory actions of omega-3 PUFAs [16,23]. In addition to altering the membrane composition and reducing the levels of arachidonic acid within membranes, Laubertová *et al.* [17] demonstrated that omega-3 PUFAs can also reduce oxidative stress-induced damage by activating the peroxisome proliferator-activated receptor-gamma (PPAR-γ) receptor. The effect of omega-3 PUFAs on PPAR-γ receptors was explored in an animal model of oxygen-induced retinopathy which may represent the neovascular stage of DR. The ability of omega-3 PUFAs to reduce neovascularization and thus reverse proliferative DR was mediated by their activation of PPAR-γ receptors. This activation was as strong as the effects produced by thiazolidinediones, thereby reducing inflammation and the expression of various integrins and selectins involved in the angiogenic process [24].

With regard to their anti-inflammatory activity, omega-3 fatty acids appear to attenuate the glucose-induced increase in multiple inflammatory agents, including TNF-α and IL-6 [7,16,24]. Based on results from *in vitro* studies using human retinal cells, Dutot *et al*. [16] and Laubertová *et al.* [17] both hypothesized that the anti-inflammatory actions of EPA in particular, were a result of the competitive antagonism of cyclooxygenase (COX) enzymes against arachidonic acid (Table 2, Ref. [8,16,17,24–27]). Together with the modulation of inflammatory signaling via PPAR-γ receptors, this is the most substantial explanation to date for the anti-inflammatory effects of omega-3 PUFAs.

However, competitive antagonism of pro-inflammatory enzymes is not the only mode of action for omega-3 PUFAs. In a study conducted by Dátilo *et al*. [25], another pivotal mechanism is discussed the activation and upregulation of the GPR120 receptor in the RPE, which responds to PUFAs. In this study, a group of Swiss male mice, were first made to undergo a high fat diet for 8 weeks to induce obesity. Following this period, 10% of the fat composition was substituted with flaxseed oil, a natural source rich in omega-3 PUFAs. When the flaxseed oil reached a sufficient bioavailability, the retinas of the mice were extracted and studied showing that it was able to reduce the inflammatory effects caused by the previous diet. Furthermore, on a systemic level, omega-3 PUFAs also improve the fasting blood glucose and the glucose tolerance. These actions where primarily mediated by the ability of omega-3 to modulate G protein-coupled receptor 120 (GPR120) expression which prevents phosphorylation of the transforming growth factor beta-activated kinase-1 (TAK1) cascade. This results in the mitigation of multiple pro-inflammatory cytokines, IL-1β and TNF-α [25].

As well as preventing neovascularization in the retina by reducing the dysfunction of endothelial cells, omega-3 PUFAs are also able to promote vascular repair by modulating endothelial cells. A study conducted on rats with type 2 diabetes found that the effect of omega-3 PUFAs on vascular repair was mediated by the enzyme acid sphingomyelinase. By downregulating the conversion of sphingomyelin to ceramide within the membrane, DHA corrected the disordered chemotactic migration of endothelial cells, thus impairing vascular repair in the retina of diabetic rats. The dampening effect of DHA on the activity of this enzyme also led to reductions in the levels of cytokines and adhesion molecules due to interruption of the inflammatory signaling pathways caused by increased ceramide levels within the membrane [17,26].

In addition to generalized anti-inflammatory effects, omega-3 PUFAs may also confer other beneficial effects. The EPA and DHA derivatives neuroprotectin D1 (NPD1) and resolvin E1 (RvE1), respectively, were able to reverse abnormal glucose receptor expression in the retina of diabetic patients [8,16,27]. Upon supplementation with these PUFAs, increased expression of caveolin-1 scaffolding protein was found in the caveolar membrane, signifying improved glucose uptake within caveolar membranes and alleviation of the hyperglycemia [16,17]. This suggests that omega-3 PUFAs can target the underlying oxidative and inflammatory stressor, thus potentially increasing their value in the therapy of DR.

1.3 Neuroprotective Effects of Omega-3 Fatty Acids

The neuroprotective effects of DHA and EPA are unique to the omega-3 PUFAs. This sets them apart from other anti-oxidants that have been studied for their thera-

Table 3. Summary of selected findings from experimental studies performed using omega-3 fatty acids, as discussed in Section 1.3.

Study	Type of study	Omega-3 formulation	Subjects of study/Culture used	Outcome measures & Results
"Mfsd2a Is a Transporter for the Essential ω-3 Fatty Acid Docosahexaenoic Acid (DHA) in Eye and Is Important for Photoreceptor Cell Development" (Wong *et al*., 2016 [28]) *The Journal of Biological Chemistry (J Biol Chem)*	*In vivo* study	Radiolabeled lyso-phosphatidylcholine [^{14}C] DHA	Mfsd2a transporter deficient mice	♦ **DHA levels**: Reduced by 40% in RPE of mice lacking the Mfsd2a compared to control mice ♦ **Localization of Mfsd2a transporter**: In the RPE, not retinal vasculature ♦ **Morphological changes**: Shortened photoreceptor membrane, edematous RPE in the Mfsd2a deficient mice
"Protective effect of docosahexaenoic acid on oxidative stress-induced apoptosis of retina photoreceptors" (Rotstein *et al*., 2003 [29]) *Investigative Ophthalmology and Visual Science (IOVS)*	*In vitro* study	DHA and other fatty acids (oleic, palmitic and arachidonic acid)	Rat retinal neuron cultures	♦ **Protection from oxidative stress**: Selective reduction of apoptosis in photoreceptor cells and not amacrine ones (effects of other fatty acids was excluded) in supplemented group ♦ **Mitochondrial membrane depolarization**: Protection of mitochondrial membrane integrity in the face of oxidative stress exhibited by DHA in supplemented group ♦ **Expression of apoptotic proteins**: Decreased expression of pro-apoptotic Bax and increased expression of anti-apoptotic Bcl-2 in supplemented group
"Docosahexaenoic acid aggravates photooxidative damage in retinal pigment epithelial cells via lipid peroxidation" (Liu *et al*., 2014 [30]) *Science Direct, Journal of Photochemistry and Photobiology*	*In vitro* study	DHA	Human RPE cell cultures	♦ **Effects of RPE:** Aggravation of light induced damage on the retina by DHA in supplemented group ♦ **VEGF secretion:** Increase of light induced secretions of VEGF in the presence of DHAin supplemented group ♦ **Effect on RPE phagocytes**: DHA promotes light induced dysfunction of RPE phagocytic cells in supplemented group
"A Higher Proportion of Eicosapentaenoic Acid (EPA) When Combined with Docosahexaenoic Acid (DHA) in Omega-3 Dietary Supplements Provides Higher Anti-oxidant Effects in Human Retinal Cells" (de Viteri *et al*., 2020 [31]) *Molecular Diversity Preservation International (MDPI)*	*In vitro* study	10 different combinations of DHA and EPA, containing different proportions of the two	RPE (ARPE-19) cell culture	♦ **Cell viability**: Both individual and combined formulations of DHA and EPA increased cell viability under oxidative and inflammatory stressors in supplemented group ♦ **ROS production**: Both individual and combined formulations of DHA and EPA reduced ROS productionin supplemented group

Table 3. Continued.

Study	Type of study	Omega-3 formulation	Subjects of study/Culture used	Outcome measures & Results
"Synthesis of docosahexaenoic acid from eicosapentaenoic acid in retina neurons protects photoreceptors from oxidative stress" (Simón *et al*., 2016 [8]) *Journal of Neurochemistry (JNC)*			Refer to Table 1	
"Docosahexaenoic acid (DHA) has neuroprotective effects against oxidative stress in retinal ganglion cells" (Shimazawa *et al*., 2009 [21]) *Science Direct, Brain Research*				
"Neurotrophins enhance retinal pigment epithelial cell survival through neuroprotectin D1 signaling" (Mukherjee *et al*., 2007 [27]) National Research Council (US), PNAS			Refer to Table 2	

Bold text indicates the type of the experimental study performed.

Table 4. Summary of selected findings from experimental studies performed using omega-3 fatty acids, as discussed in Section 2.

Study	Type of study	Omega-3 formulation	Subjects of study/Culture used	Outcome measures & Results
"Omega-3 polyunsaturated fatty acids preserve retinal function in type 2 diabetic mice" (Sapieha *et al*., 2012 [37]) *Nature, Nutrition and Diabetes*	*In vivo* study	Standard diet supplemented with 2% DHA and EPA	Mouse leptin receptor deficient mice	♦ **Visual function**: Prevented hyperglycemia induced degeneration of retinal function and loss of sensitivity in diabetic mice ♦ **Glucose homeostasis**: Improved tolerance and response to systemic spikes of glucose in supplemented group
"*Serum levels of plasmalogens and fatty acid metabolite* associated with retinal microangiopathy in participants from the Finnish Diabetes Prevention Study" (de Mello *et al*., 2021 [38]) Molecular Diversity Preservation International (MDPI)	Randomized controlled trial	Lifestyle intervention following which serum lipid analysis was performed	522 individuals with impaired glucose tolerance	♦ **Presence of retinal microaneurysms**: Decreased in patients with higher levels of omega-3 PUFAs in the serum ♦ **Overall risk of T2DM**: Decreased in patients with higher levels of omega-3 PUFAs in the serum
"N-3 polyunsaturated fatty acids prevent diabetic retinopathy by inhibition of retinal vascular damage and enhanced endothelial progenitor cell reparative function" (Tikhonenko *et al*., 2013 [26]) *Public Library of Science (PloS)*			Refer to Table 2	
"Docosahexaenoic acid aggravates photooxidative damage in retinal pigment epithelial cells via lipid peroxidation" (Liu *et al*., 2014 [30]) Science Direct, Journal of Photochemistry and Photobiology			Refer to Table 3	

Bold text indicates the type of the experimental study performed.

peutic potential in the context of DR; the relevant studies are summarized in Table 3 (Ref. [8,21,27–31]).

A study on retinal neurons found the neuroprotective effects were primarily exerted by DHA, with EPA acting simply as a precursor for DHA synthesis within the cell. Retinal neuron cell cultures extracted from albino Wistar rats were exposed to increasing concentrations of EPA ranging from 1–6 µm. These were compared to controls not exposed to EPA, but to other fatty acids such as palmitic, oleic and arachidonic acid. The primary finding of this study was that treatment with EPA increased the intra-neuronal level of DHA. This attenuated the oxidative stress, but also led to the progression of photoreceptors by increasing opsin expression. *De novo* synthesis of DHA was attributed to the enzyme Δ6 desaturase (FADS2) within the neurons, since inhibition of this enzyme resulted in decreased levels of DHA within the retina. The presence of FADS2 within retinal neurons could indicate a significant role for neurons in maintaining DHA levels in the retina for protection from oxidative damage [8].

Another pathway for the maintenance of DHA levels in the retina is the presence of transporters, specifically the sodium-dependent major facilitator superfamily domain containing protein 2a (Msfd2a). This transporter is responsible for movement of DHA from the circulation across the blood retinal barrier and into the retinal pigment epithelium (RPE) membrane. In Msdf2a-deficient mice, the resulting decrease in DHA led to pathological changes in the RPE, including thickening of the photoreceptor membrane, blunting of the apical villous processes, and edema within the basal membrane. This could be attributed to the upregulation of physiological phagocytosis within the RPE in order to induce new membrane formation, but which instead leads to pathological changes in the RPE [10,28]. Shimazawa *et al.* [21] also found that administration of DHA to retinal ganglion cells attenuated the damage and apoptosis caused by oxidative radicals in a dose-dependent manner. This reinforces the critical role of DHA within the retina [21].

Furthermore, DHA appears to exert more potent cytoprotective effects on photoreceptor neurons than on amacrine neurons. Rotstein *et al.* [29] demonstrated that upon induction of oxidative stress in photoreceptor neurons, DHA prevented apoptosis by two main mechanisms. The first was by acting upstream of the mitochondrial apoptotic pathway to decrease the level of BAX (a pro-apoptotic protein whose levels increase in the presence of induced oxidative stress), while also increasing the level of the anti-apoptotic protein Bcl-2 [29]. Mukherjee *et al.* [27] further expanded on this by demonstrating the anti-apoptotic effects were mediated by the DHA-derived molecule NPD1. This molecule shows polarity within the retinal photoreceptor membrane, being more abundant on the apical side. Treatment with DHA can restore polarization within the photoreceptor membrane, which is also disrupted in many other retinal degenerative conditions [27].

DHA can also exert anti-apoptotic effects at the mitochondrial level. In addition to directly decreasing apoptosis by restoring mitochondrial membrane function and permeability, DHA can act through NPD1 to reduce the cleavage and subsequent activation of caspase-3 [27,29]. Both the pre-mitochondrial and mitochondrial pathways demonstrate that DHA can act as a survival factor by reversing the changes induced by oxidative stress, thereby exerting neuroprotective effects on retinal photoreceptor neurons.

In addition to preventing photoreceptors' apoptosis, DHA can also induce the differentiation of these cells. With EPA supplementation, conversion to DHA leads to increased expression of opsin and the progression of previously halted photoreceptor differentiation. This suggests that DHA not only prevents further oxidative damage, but also promotes the recovery from oxidative damage [8].

A noteworthy discovery has been the decreased level of VEGF in patients suffering from dry eye disorder. This is somewhat paradoxical considering the inflammatory origin of this condition. However, it was hypothesized that neurodegeneration could lead to a decrease in VEGF, which is an important mediator of neural interactions in the eye [32].

The role of VEGF in DR is currently the subject of debate. Liu *et al.* [30] found that DHA supplementation increased VEGF levels inside RPE cells under the conditions used in their study, whereas other studies have concluded that omega-3 supplementation decreases VEGF levels within the retina [15,31]. Liu *et al.* [30] concluded the increased VEGF level would lead to accelerated retinal damage upon light exposure. However, an increase in VEGF might also provide neuroprotective benefits. In other conditions, particularly those of a neurodegenerative nature, VEGF has been investigated for its therapeutic potential, with the most widely researched target being the hippocampus [30,33]. Within this region of the brain, increased levels of VEGF promote neurogenesis and microvascular remodeling, amongst other beneficial effects. This could have implications for a variety of conditions including status epilepticus and Alzheimer's disease [34–36]. Within the eye, it was shown that VEGF conferred some degree of neuroprotection against dry eye disorders. In DR however, the effect of DHA on VEGF levels warrants further investigation due to the inconsistent results between studies. These could be due to different cell culture conditions, or to the use of different doses and formulations. Future studies should therefore aim to clarify the effects of different doses or ratios of DHA and EPA, so that an optimal formulation can be devised for clinical use. Confirmation that DHA has neuroprotective benefits would greatly increase its value as a potential therapeutic target for DR.

2. Omega-3 Fatty Acids as Preventive Therapy

Omega-3 PUFAs have proven useful for the prevention of DR. The evidence suggests that omega-3 PUFAs

have protective effects in the retina long before the microvascular changes become visible, mainly because of their beneficial effects on glucose uptake by neurons (studies summarized in Table 4, Ref. [26,30,37,38]). Animal studies have shown that elevated extracellular glucose levels, a hallmark feature of diabetes, were toxic to neurons within the retina [37,39]. This was explained by a lack of GLUT-1 transporter within these neurons, rendering them unable to utilize the extracellular glucose. However, supplementation with omega-3 PUFAs improved their glucose tolerance and reduced the hyperglycemic spikes, thereby conferring neuroprotective benefits and reducing their loss of sensitivity. These systemic changes preceded the microvascular abnormalities. From these observations, Sapieha *et al.* [37] concluded that the beneficial effects of omega-3 PUFAs derived primarily from their ability to improve glucose tolerance and hence retard cellular damage at an early stage, rather than by reducing inflammation and oxidative stress at later stages.

The utility of omega-3 PUFAs is also exhibited by their ability to mitigate one of the earliest visible changes in diabetic retinopathy- retinal microaneurysms. De Mello *et al*. [38], demonstrated as part of the Finnish Diabetes Prevention Study that an increased level of omega-3 PUFAs was linked to decreased occurrences of microaneurysm, which could be linked to their ability to dampen oxidative stress in retinal tissue. Furthermore, from a broader perspective, omega-3 PUFAs are also able to reduce the overall risk of T2DM, which in turn reduces the risk of developing DR as well [38].

The prophylactic use of omega-3 PUFAs to prevent complications in patients with T2DM should be encouraged because their systemic effects also promote cardiovascular wellbeing, thereby reducing the risk of macrovascular complications. The animal study by Tikhonenko *et al.* [26] found that the beneficial effects of omega-3 PUFAs were mediated by their inhibitory effect on the enzyme acid sphingomyelinase in the retinal membrane. However, these authors also reported that improved vascular regeneration abilities in diabetic rats were not restricted to the retina but were also seen throughout the body. This resulted in a longer lifespan for rats supplemented with DHA when compared to controls. In keeping with this observation, omega-3 PUFAs are particularly useful in humans because they can modulate obesity-induced inflammatory processes. This is very relevant in the context of T2DM, where dyslipidemia and obesity play major roles. By modulating mostly toll-like receptor 4 (TLR4), omega-3 PUFAs can reduce the systemic inflammation caused by increased lipid levels in obese patients with T2DM. However, their actions are not limited to this pathway alone. Omega-3 PUFAs also lower the systemic release of inflammatory cytokines by activating the PPAR-γ family of receptors and reducing NF-κB-induced upregulation of pro-inflammatory cytokine production. These effects, along with their ability to attenuate thrombosis and decrease triacylglycerol levels, results in cardiovascular benefits and also reduces the risk of macrovascular complications, such as recurrence of myocardial infarction (MI) [26,40,41].

There is however some risk reported with the preventive use of DHA supplementation. A study that investigated the effect of DHA on light-induced damage also found increased degeneration, oxidative stress, and senescence within the retina compared to controls without DHA. This study highlighted the implications for regular computer screen users with direct light exposure [30]. Since this now includes a major proportion of the population, the possible risks associated with DHA could prevent it from being used in people who do not already have an established diagnosis of DR.

3. Omega-3 Fatty Acids and Their Therapeutic Uses

In addition to elucidating the possible mechanisms of action of omega-3 PUFAs, *in vitro* studies have also revealed possible insights into how omega-3 formulations could be optimized for therapeutic use. When various doses and formulations of DHA and EPA were tested on human-derived ARPE-19 cells, the highest anti-oxidant activity was observed when EPA was present at higher ratios than DHA [31]. Furthermore, marine-derived EPA and DHA were the most effective, especially when present as triglyceride and phospholipids rather than ethyl esters. These results suggest that fish oil sources with high bioavailability are the most suitable for supplementation [16,31]. However, the ratios used in this study did not result in significantly reduced VEGF/PEDF levels. As mentioned previously, future studies should therefore aim to clarify the pharmacodynamics of omega-3 PUFAs with respect to VEGF levels.

The use of combined nutraceutical formulations in clinical studies indicates a need for future trials to study omega-3 as standalone treatments. Nevertheless, such studies are valuable for demonstrating the ability of omega-3 PUFAs to work in synergy with other anti-oxidants. The current regimens include an array of antioxidants that allow targeting of multiple mechanisms, while still having a good safety profile for patients [7,19]. A study performed to investigate the anti-oxidant effects of DHA, EPA and Astaxanthin (AST) on human hepatoma cell cultures found that each of these compounds reduced oxidative stress when used alone. Moreover, they acted synergistically to induce the transcription of anti-oxidant response elements (ARE) and nuclear factor-like 2 (Nrf-2)-related genes. Hence, they exhibit superiority for increasing glutathione levels and for anti-inflammatory modulation, even when used at low concentrations. The use of combined formulations has also been studied in the context of ophthalmic disorders other than DR.

Dry eye disorders are increasingly common and share inflammatory- and oxidative stress-related etiology with DR. Nutraceutical supplementations containing DHA and EPA as their principal components have been investigated for the treatment of this disorder, as well as other antioxidant compounds, such as Vitamin C and Vitamin E. Although these studies did not show improvement in the BCVA, major improvements in the overall symptoms of dry eye disorder were observed, together with a marked reduction in the release of pro-inflammatory cytokines such as IL-1β, IL-6 and IL-10 [32]. Similar observations were also made in patients suffering from primary glaucoma, where daily oral supplementation with omega-3 PUFAs resulted in decreased levels of IL-6 and TNF-α, as well as preventing the progression of superficial ocular dysfunction. In accordance with the evidence pertaining to DR, NPD1 and RvE1 were found to be responsible for the anti-inflammatory action of omega-3 PUFAs [32,42].

In the clinical context, omega-3 PUFAs are ideally used in addition to pre-existing treatments, such as anti-VEGF therapies. Currently, there is a lack of firm evidence to show that omega-3 PUFAs can effectively reduce VEGF levels and hence impact this pathway, which is central to the pathogenesis of DR. Although Laubertová *et al.* [17] reported a decrease in the sensitivity of endothelial cells to angiogenic stimulation, omega-3 PUFAs did not reduce the level of VEGF. In a randomized clinical trial conducted by Lafuente *et al*. [43], the use of DHA-containing nutraceutical supplements in addition to anti-VEGF therapies, such as ranibizumab, produced more beneficial outcomes than the use of ranibizumab alone. While BCVA did improve with the use of ranibizumab, addition of DHA to these regimens led to an increase in the total antioxidant capacity (TAC) and to a larger reduction in the level of inflammatory cytokines, particularly IL-6. The reduction in IL-6 level is notable because there is evidence that pathways mediated by this cytokine are linked to vascular degenerative changes within the retina of patients with DR [44]. Furthermore, in terms of the clinical outcomes, a greater improvement of membrane thickness in patients with macular edema was observed with supplementary use of DHA-containing nutraceutical supplements, in addition to ranibizumab [43].

4. Conclusions

The evaluation of omega-3 PUFAs as therapy for DR is limited by the lack of clinical studies to date. Our understanding of the mechanisms involved in protection against the oxidative and inflammatory stress induced by hyperglycemic conditions in the retina has come mainly from *in vitro* studies. Protection against both of these stressors appears to be linked, although not through a single pathway or a single site of action. Omega-3 PUFAs directly scavenge oxidative radicals, but also upregulate existing defense mechanisms, such as the activity of the key anti-oxidant enzyme, superoxide dismutase. This is also mirrored in their anti-inflammatory actions. They have the ability to directly reduce the levels of inflammatory cytokines, particularly TNF-α and IL-6, while simultaneously increasing activation of the intrinsic PPAR-γ receptor, thereby enhancing the efficacy of their protective actions. Furthermore, omega-3 PUFAs also act at a deeper level in the mitochondria to mitigate cellular damage and subsequently to prevent apoptosis.

However, it is the additional feature of neuroprotection that sets omega-3 PUFAs apart from other anti-oxidants investigated for the potential therapy of DR. The level of DHA in neurons is maintained both through *de novo* synthesis from EPA and by transporters which incorporate it into membranes, resulting in the selective cytoprotection of photoreceptor neurons. As well as halting further damage, omega-3 PUFAs promote the recovery of retinal neurons by increasing cellular differentiation and opsin expression within the photoreceptors.

The *in vitro* studies conducted to date have greatly increased our understanding of the three primary protective mechanisms conferred by omega-3 PUFAs, namely their anti-oxidative actions, anti-inflammatory actions, and neuroprotection. However, some areas are still disputed and require further clarification, such as the effect of omega-3 PUFAs on the key growth factor VEGF. Results between studies remain inconsistent and hence more work is required to discern what effect, if any, omega-3 PUFAs have on VEGF levels and whether this is relevant to their therapeutic potential. Furthermore, the blood-retina barrier is a point to overpass and consider for future drug delivery systems; the fact that omega-3 PUFAs have the ability to get transported across this barrier, is a great advantage over larger impermeable therapeutic molecules.

Finally, some *in vitro* studies have shed light on how clinical formulations could be optimized for therapeutic use by demonstrating that higher ratios of EPA and marine sources of omega-3 PUFAs have the most anti-oxidative potential. Current clinical trials in patients with established disease show promising results with regard to the ability of omega-3 PUFAs to prevent DR and to work in synergy with other supplements and anti-VEGF agents. However, before they can be used clinically, more clinical trials are required to confirm the benefits of omega-3 PUFAs as standalone treatment rather than in combination with other compounds. These should establish that any of the protective benefits are indeed attributable to omega-3 PUFAs.

Author Contributions

All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work. SF has contributed to the literature search and data collection, and has prepared and formatted the manuscript. EP has contributed to conceptualization, discussions of content and has

guided the writing of the manuscript and critically revised the content. TG has provided support for the publication of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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