NUTRITION FOR EYE HEALTH

FACT OR FICTION ?

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Théa
let's open our eyes

PREFACE

Among the eye pathologies, there is one that is of great interest to researchers: **age-related macular degeneration** (usually called AMD) that is the major cause of visual loss in individuals older than 50 in developed countries. It is a progressive degenerative disease of the macula (the central part of the retina), leading consequently to disturbances or partial loss of central vision ending in legal blindness.

The AMD pathology is characterized by degeneration involving the retinal photoreceptors, retinal pigment epithelium, Bruch's membrane and, in some case, also the choroidal layers.

Moreover, oxidative stress may contribute to this condition. Indeed, the centrality of oxidative stress as a disease contributor to AMD was highlighted by the Age-Related Eye Disease Study (AREDS) between 1992 and 2001.

Actually, there is no efficient treatment to prevent or to cure AMD but an adequate supplementation may slow its progression. As a matter of fact, a wide variety of nutrients such as minerals, vitamins, omega-3 fatty acids and carotenoids have been associated with reducing the risk of AMD in population-based studies as well as in the AREDS. Thus, patients should be advised that a healthy diet rich in fruits, green leafy vegetables and fish may decrease the risk of AMD.

As practitioners and also as retina specialists, we have the duty to give to the patients the best advices and the best treatment available for their condition. This booklet gathers, with more than 250 references, the current knowledge about the impact of nutrition on age-related macular degeneration. We would keenly encourage everyone to read this leaflet with interest. It may help you to advise your patients about their diet, and it might make you consider a supplementation for patients with an early or intermediate AMD.

We believe this brochure brings basic information about the nutrition for eye health to all of us, and we hope you will enjoy reading and learning with this booklet as much as we had pleasure to undertake it.

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ABBREVIATIONS

AA: Arachidonic Acid
AAO: American Academy of Ophthalmology
Aβ: Amyloid beta protein
AGE: Advanced Glycation End product
AMD: Age-related Macular Degeneration
ALA: Alpha Linolenic Acid
APC: Amyloid P Component
ApoB: Apolipoprotein B
ApoE: Apolipoprotein E
AREDS: Age-Related Eye Disease Study
BM: Brusch' s Membrane
BMI: Body Mass Index
CAT: Catalase
CEC: Choroidal Endothelial Cells
CFB: Complement Factor B
CFH: Complement Factor H
CFI: Complement factor I
CI: Confidence Interval
CNV: Choroidal Neovascularisation
DHA: Docosahexaenoic Acid
DRI: Dietary Recommendation Intake
EAR: Estimated Average Requirements
ECM: Extracellular Matrix
EFSA: European Food Safety Authority
EMA: European Medicines Agency
EPA: Eicosapentaenoic Acid
ETDRS: Early Treatment Diabetic Retinopathy Study
EU: European Union
FAF: Fundus Autofluorescence
FAO: Food and Agriculture Organization
FDA: Food and Drug Administration
FFQ: Frequency Food Questionnaire
GA: Geographic Atrophy
GGT: Gamma Glutamyl Transferase
GPx: Glutathione peroxidase
GSH/GSSG: Glutathione (reduced/oxidized form)
HDL-C: High Density Lipoprotein Cholesterol
HR: Hazard ratio
HT: Hydroxytyrosol
ICER: Incremental Cost-Effectiveness Ratio
IL: Interleukin
IOM: Institute of Medicine
INF-γ: Interferon gamma
IR: Infrared
IU: International Unit
L: Lutein
LA: Linoleic Acid
LTB4: Leukotriene B4
L/Z: Lutein / Zeaxanthin
MMP: Matrix Metalloproteinase
MPOD: Macular Pigment Optical Density
MZ: Meso-Zeaxanthin
NHANES: National Health And Nutrition Examination Survey
NFkB: Nuclear Factor kappa B
NEI: National Eye Institute
NO: Nitric oxide
OCT: Optical Coherence Tomography
OR: Odds Ratio
PDT: Photodynamic Therapy
PEDF: Pigment-Epithelium Derived Factor
PLA2: Phospholipase A2
PGE2: Prostaglandin E2
PR: Photoreceptor
PUFA: Polyunsaturated Fatty Acid
QALY: Quality-Adjusted Life Years
RBC: Red Blood Cells
RCT: Randomised Controlled Trial
RDA: Recommended Dietary Allowance
ROS: Radical Oxygen Species
RPE: Retinal Pigmentary Epithelium
RR: Relative Risk
SD-OCT: Spectral Domain – optical Coherence Tomography
SNP: Single Nucleotide Polymorphism
SOD: Superoxide Dismutase
TGFβ: Tumor Growth Factor β
TIMP: Tissue Inhibitor of Metalloproteinase
TIMP3: Tissue Inhibitor of Metalloproteinase 3
TNFα: Tumor Necrosis Factor alpha
UK: United Kingdom
USA: United States of America
UV: Ultraviolet
UVB: Ultraviolet B
VDR: Vitamin D Receptor
VEGF: Vascular Endothelium Growth Factor
WHO: World Health Organization
Z: Zeaxanthin

1. AMD DIAGNOSIS & CLASSIFICATION

1.1 DEFINITION AND DIAGNOSIS

Age-related macular degeneration (AMD) is a progressive degenerative disease of the central part of the retina (the macula) which may result in blurred or no vision in the center of the visual field in one or both eyes. This is a painless pathology with only few symptoms in the early stages. The advanced forms are often symptomatic with loss of contrast sensitivity, visual distortion (metamorphopsia), presence of black or gray spots (scotomata), or blind spot and blurred vision *[AAO, 2015]*. The patient may complain about difficulty with performing normal activities that require good central vision, such as reading and writing, watching television, driving and recognizing faces. AMD may develop unilaterally or bilaterally at the same time or sequentially. Frequently, people are unaware that their disturbed binocular vision is caused by changes in only one eye *[Solomon et al., 2014]*.

Since the early and intermediate stages of AMD usually start without symptoms, only a comprehensive dilated eye exam can detect AMD. According to the disease stage, clinical findings may include drusen, retinal pigment epithelial (RPE) abnormalities, geographic atrophy (GA), RPE detachment, choroidal neovascularization and its consequences (e.g., serous sensory retinal detachment, often accompanied by hard exudates and subretinal hemorrhages), and disciform scar *[AREDS Report No 1, 1999]*.

Fundus photographs are usually used to determine the size and extent of drusen as well as pigmentary abnormalities, and atrophy of the RPE. Reticular pseudodrusen (also called subretinal drusenoid deposits) are often under-recognized *[AAO, 2015]*. They are best imaged using fundus autofluorescence, infrared reflectance, and/or spectral domain – optical coherence tomography (SD-OCT) *[Zweifel et al., 2010; AAO, 2015]*. Fluorescein angiography is the gold standard for detecting and confirming the presence of choroidal neovascularization *[Jia et al., 2014].* OCT angiography is a recent, non-invasive test , that is rapidly replacing fluorescein angiography in clinical practice for the diagnosis of choroidal neovascularization, as well as useful tool for the follow up of this pathology. Indocyanin angiography is still indicated in selected cases (ie. polypoidal subtype).

1.2 CLASSIFICATION

The current classification is based on the results of the Age-Related Eye Disease Study (AREDS). A simplified severity scale divided into three categories was proposed: early, intermediate, and advanced *[Ferris et al., 2013]* (Table 1). Such a simplified classification is useful to evaluate the relationship between nutritional, medical, and environmental factors and incidence rates of progression from early to intermediate disease to late stages AMD *[Seddon et al., 2006a]* and current treatment recommendations are based on these classifications *[AAO, 2015]*..

Table 1 : Simplified classification of AMD (AREDS)

Stage 1	**No AMD**	No or only few small drusen (< 63 µm)
Stage 2	**Early AMD**	**At least one of the following** • multiple small drusen • few drusen with size between 63 and 125 µm • pigmentary abnormalities
Stage 3	**Intermediate AMD**	**At least one of the following** • multiples drusen with size between 63 et 125 µm and at least one drusen > 125 µm • geographic atrophy outside the fovea.
Stage 4	**Advanced AMD**	Geographic atrophy affecting the fovea and/or exudative AMD

Adapted from AAO, 2015

Subjects with lesions associated with neovascular AMD or geographic atrophy (GA) are considered to have late or advanced late AMD *[Ferris et al., 2013].* Late AMD is classically divided into 2 categories: dry form (non-exudative) and wet form (exudative).

Geographic atrophy is characterized by the development of uni- or multifocal atrophic patches that involve the RPE, the neurosensory retina and choriocapillaris layer of the choroid without defect in the Bruch's membrane (BM). Dry AMD (i.e. non exudative AMD) is characterized by geographic atrophy of the RPE in the absence of serous or hemorrhagic leakage *[Geltzer et al., 2013]*. Patients with GA, even in the late stage, can maintain a good central vision until the disease progresses to involve the central fovea. Patients with geographic atrophy not necessarily involving the central fovea may have relatively good distance visual acuity, but manifest decreased ability to perform near visual tasks such as reading *[AAO, 2015]*. Individuals with atrophic AMD typically present with a slow and gradual deterioration in visual acuity for which treatment options remain limited. Late stage atrophic AMD involves widespread atrophic loss of retinal tissue and has poor visual outcomes, similar to those seen with untreated neovascular AMD *[Yehoshua et al., 2011, Broadhead et al., 2015, Kolar, 2013]*.

Neovascular AMD (i.e. choroidal neovascularization) occurs less frequently but is far more aggressive when compared with dry AMD *[Kolar, 2013].* Neovascular AMD is caused by abnormal growth of new blood vessels into retinal tissues. Endothelial cells from the choriocapillaris of the choroid migrate to and across the RPE monolayer and into the sensory retina. Neovascular AMD is characterized by occurrence of RPE detachment, choroidal neovascularization (CNV), leakage of blood and serum into the surrounding retina (subretinal hemorrhage). RPE detachment can be divided into 4 categories:

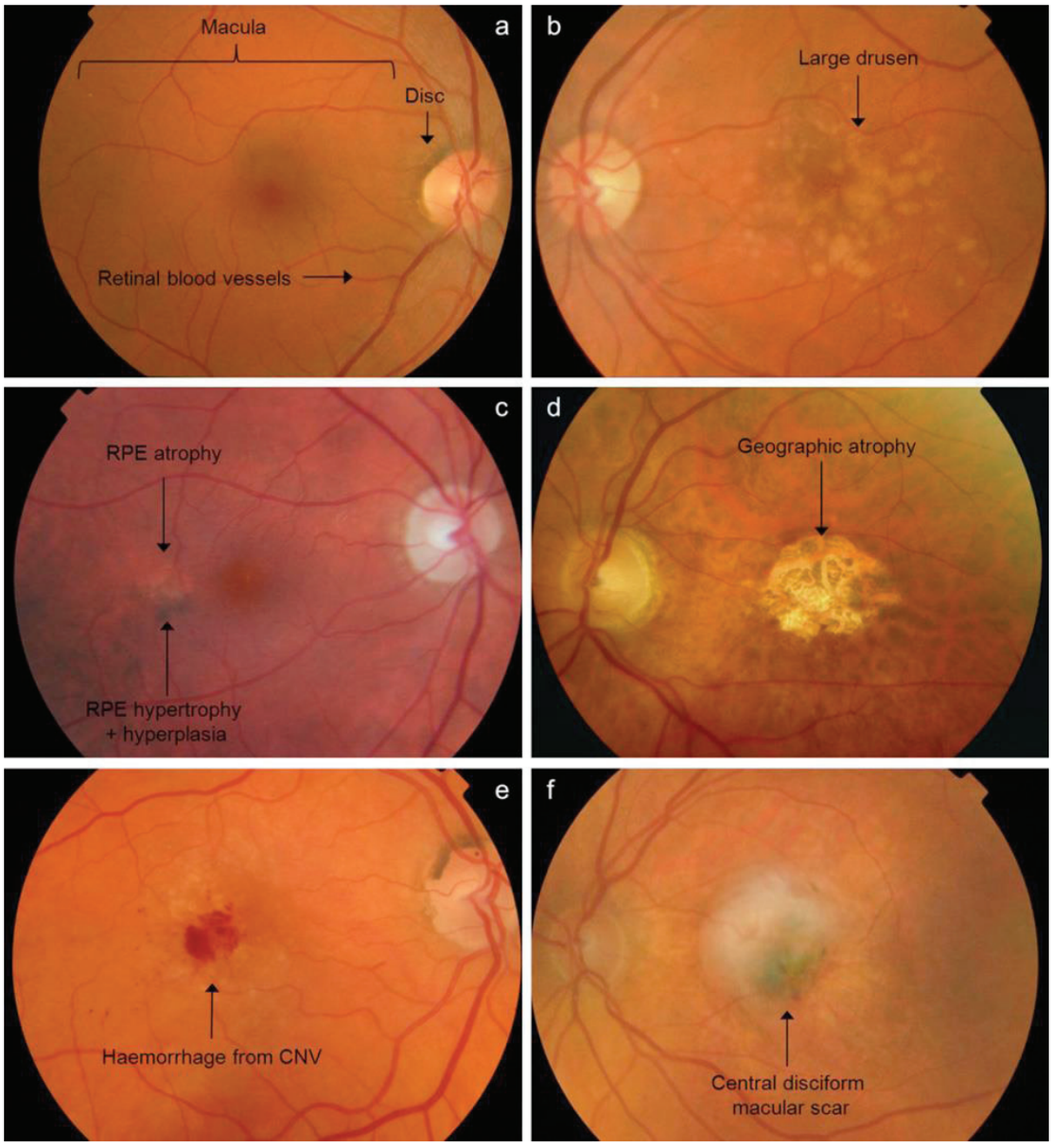
1) drusenoid RPE detachment,

2) serous RPE detachment,

3) hemorrhagic RPE detachment, and

4) fibrovascular detachment. *[AAO, 2015].* This initially leads to central blurring or visual distortion and subsequently to more significant loss of vision caused by scarring and extensive leakage.

Figure 1 : Fundus photographs of various stages of age-related macular degeneration



(a) Normal eye. Photograph of the fundus (back) of the right eye. The optic disc, where nerve fibers come out of the eye, can be seen as a white spot, due to myelinization of the nerve fibers when they leave the globe. Radiating out from the optic disc in 4 quadrants are the retinal blood vessels. The macula is the area just temporal to the optic disc. It usually appears darker due to increased pigment, xanthopill and lutein (herein the latin term "macula lutea"), and its centre is marked by an absence of visible blood vessels the foveal avascular zone (FAZ). **(b) Early AMD-drusen.** Large drusen can be seen as multiple yellowish clumps. **(c) Early AMD-pigmentary irregularities.** An area of hyperpigmentation (due to RPE cell hypertrophy and hyperplasia) and hypopigmentation (RPE atrophy) can be seen just temporal to the centre of the macula. **(d) Late AMD-GA.** This patient has an extensive area of sharply demarcated RPE atrophy. The underlying choroidal vessels are atrophic and central vision is affected. **(e) Late AMD-CNV.** Blood vessels have grown from the choroid (the vascular layer posterior to the retina) to form a choroidal neovascular membrane. This has caused a haemorrhage within the retina, causing sudden loss of central vision. **(f) Late AMD-scar.** Untreated CNV may result in a permanent macular scar. The central vision is permanently poor *[Adapted from Khandhadia et al., 2012]*.

2. EPIDEMIOLOGY & RISK FACTORS

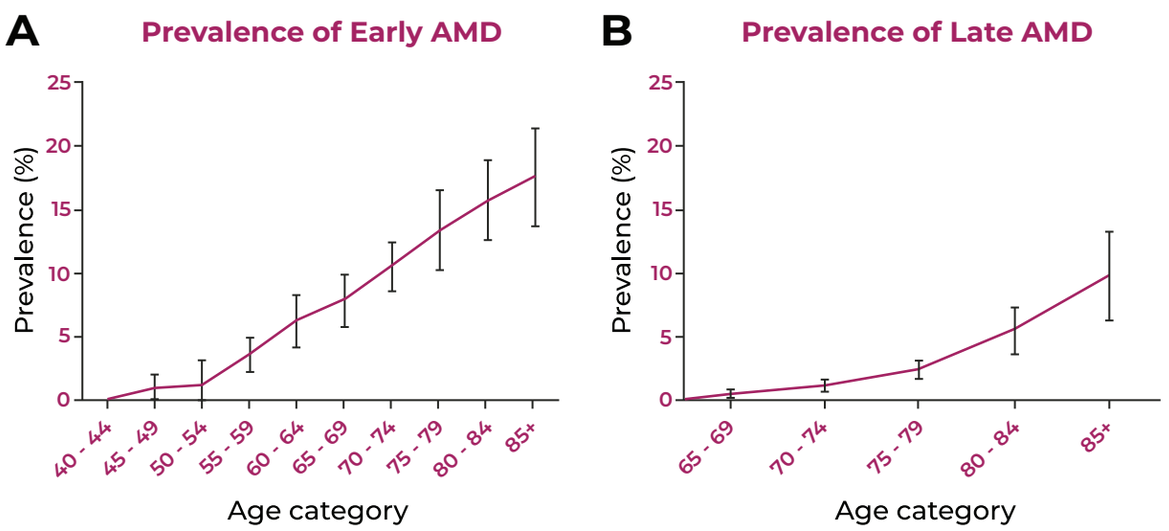
2.1 EPIDEMIOLOGY

AMD is the leading cause of blindness in developed countries and is ranked third worldwide after cataract and glaucoma by the World Health Organization (WHO) *[WHO, 2017]*. The global prevalence of any type or stage of AMD has been estimated at 8.7% with a 95% confidence interval (CI) ranging between 4.3 and 17.4 among people aged between 45 and 85 years *[Jonas et al., 2017]*.

The prevalence of AMD increases with age (Figure 2). In a recent meta-analysis of 14 population-based cohorts from 10 countries in Europe in individuals of 40 years of age or older (Table 2), the prevalence of early AMD was 3.5% (95%CI: 2.1-5.0) in subjects aged 55-59 years and 17.6% (95%CI: 13.6-21.6) in those aged ≥ 85 years. The prevalence of late AMD was 0.1% (95% CI: 0.004-0.3%) and 9.8% (95%CI: 6.3-16.3), respectively *[Colijn et al., 2017]*. In the USA, among those aged 80 years or older, the prevalence of neovascular AMD has been estimated to 8.18% (95% CI: 7.07% to 9.29%) *[Friedman et al., 2004]*.

Despite a decreasing prevalence, AMD projections show an almost doubling of affected persons: by 2040, the number of individuals in Europe with early AMD will range between 14.9 and 21.5 million, and for late AMD between 3.9 and 4.8 million.

Figure 2 : Prevalence of early and late AMD according to age in Europe



Adapted from Colijn et al., 2017

Table 2 : Prevalence of early and late AMD according to countries in Europe

Country	Period	Sample size	Median age	Prevalence of early AMD (%)	Prevalence of late AMD (%)
France	1995-1997	2196	65-69	-	8.7%
France	2006-2008	879	75-79	-	16.8%
France	2009-2013	1069	80-84	9.2%	2.2%
Germany	2007-2012	3839	50-54	2.3%	0.2%
Greece	2000-2005	2107	65-69	-	2.7%
Italy	2005-2006	853	65-69	13.5%	2.1%
Netherlands	1990-1993	6419	60-64	7.5%	1.7%
Netherlands	2000-2002	2545	55-59	6.0%	0.7%
Netherlands	2005-2008	3449	55-59	4.6%	0.4%
Norway	2007-2008	2631	65-69	-	3.5%
Portugal	2009-2011	2975	65-69	6.9%	0.7%
Portugal	2012-2013	3021	60-64	15.4%	1.3%
UK	2004-2011	5344	60-64	-	0.5%
Multiple	2000-2002	4753	65-69	12.6%	3.3%

Adapted from Colijn et al., 2017

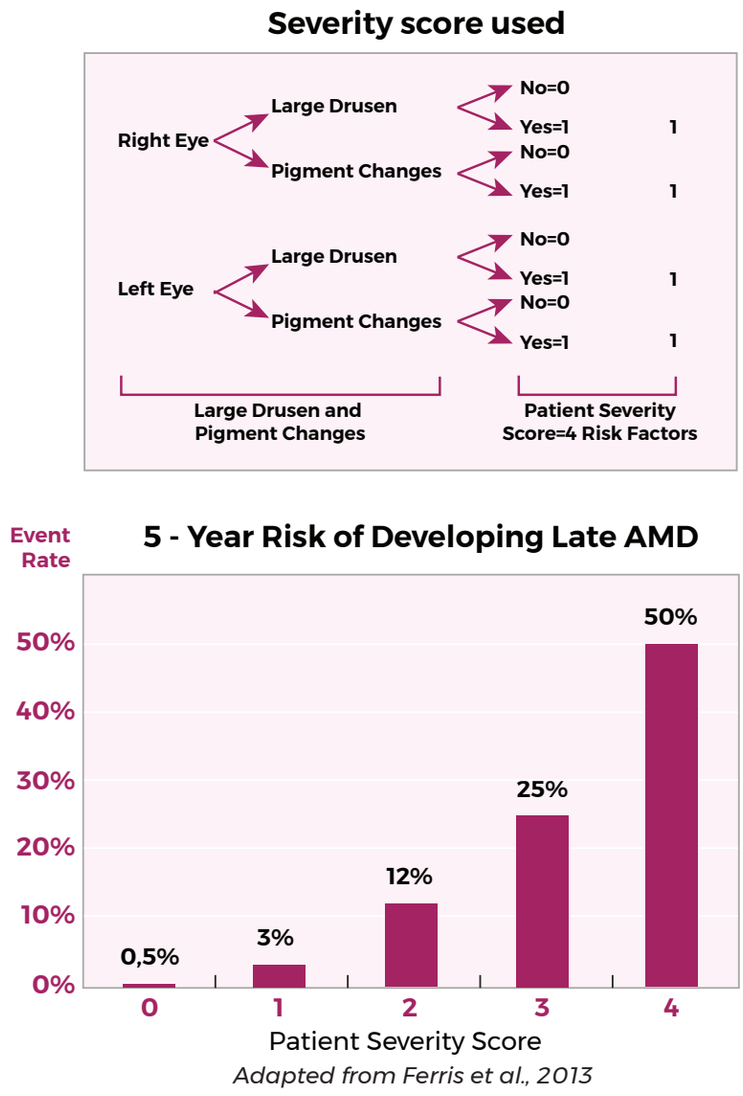
2.2 RISK FACTORS

The pathogenesis of AMD is multifactorial, resulting from a combination of genetic and environmental risk factors *[Chen et al., 2010]*. Given the high prevalence of AMD worldwide, it is important to identify those individuals with early AMD who are at greatest risk to progress to advanced, vision-threatening AMD (geographic atrophy or neovascular AMD) and the capability to predict when progression to advanced AMD might occur *[Klein et al., 2011b]*.

2.2.1 Macular phenotypes

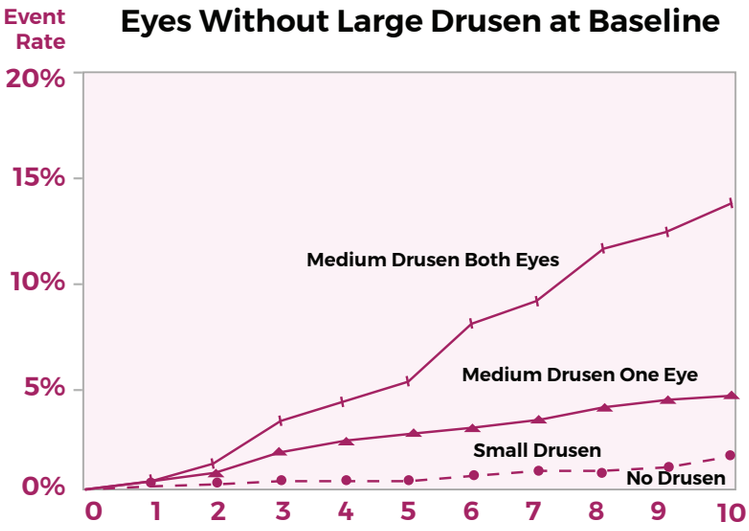
Macular phenotypes (including drusen size) and presence of unilateral advanced disease at baseline are strong predictors of the future course of the disease (Figures 3 & 4), and is the causal pathway for progression *[Seddon et al., 2015]*.

Figure 3 : 5-year risk of advanced AMD according to macular phenotype



Adapted from Ferris et al., 2013

Figure 4 : 10-year risk of advanced AMD according to macular phenotype



Adapted from Ferris et al., 2013

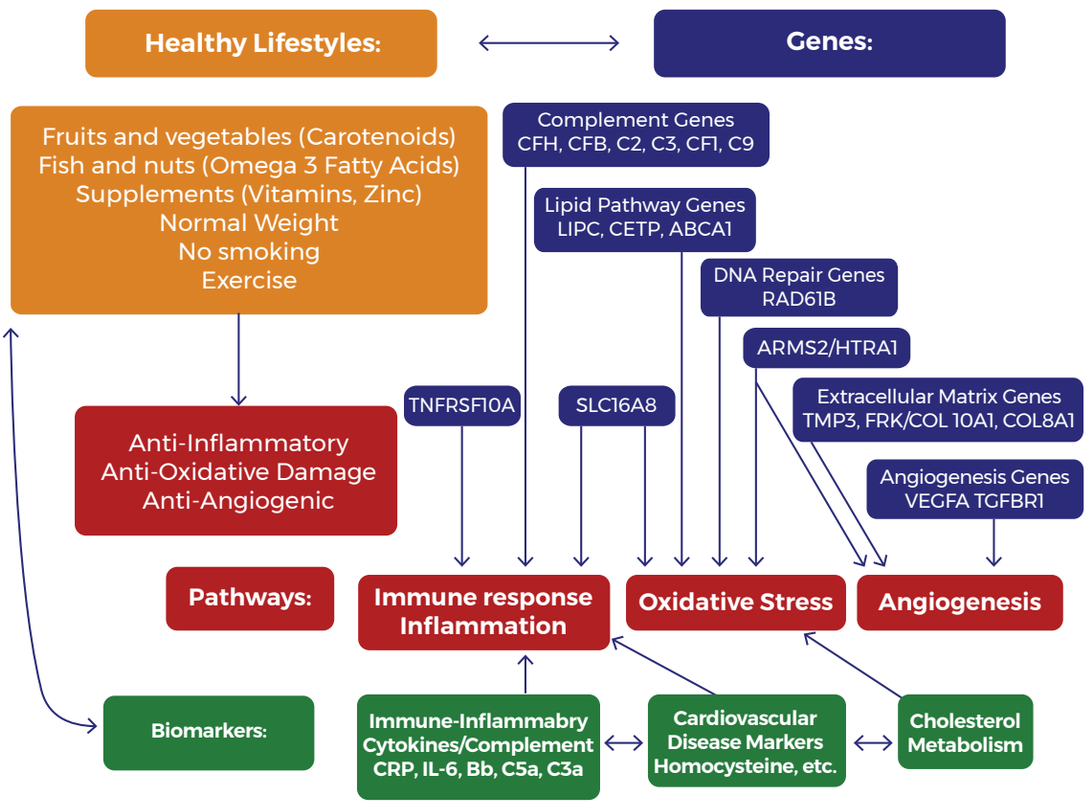
2.2.2 Genetics

AMD is a highly heritable disease as confirmed by familial aggregation studies from general populations or tertiary eye care clinical centers *[Seddon et al., 1997]*.

In a population-based familial aggregation study, Klaver et al. showed that the prevalence of early (OR = 4.8, 95% CI: 1.8; 12.2) and late (OR = 19.8, 95% CI: 3.1; 126.0) AMD was significantly higher in relatives of patients with late AMD independently of other risk factors *[Klaver et al., 1998]*. The lifetime risk estimate of late AMD was 50% (95% CI: 26%; 73%) for relatives of patients vs 12% (95% CI: 2%; 16%) for relatives of controls (P < 0.001), yielding a risk ratio of 4.2 (95% CI: 2.6; 6.8). Relatives of patients expressed the various features of AMD at a younger age. The population-attributable risk related to genetic factors was 23%.

In a large US population-based twin survey of all stages of AMD, it was estimated that genetic factors may explain 46% to 71% of overall and advanced AMD variation, respectively *[Seddon et al., 2005]*. In addition, genome-wide association studies have revealed numerous common or several rare genetic variants strongly associated with AMD *[Seddon, 2017]*. Most of these genes are linked to various mechanisms involved in AMD pathogenesis including genes involved in the alternative complement cascade, lipid pathway, extracellular matrix, and angiogenesis, and DNA repair. Evidence for interactions between environmental, therapeutic and genetic factors is emerging (Figure 5) and elucidating the mechanisms of this interplay remains a major challenge in the field of AMD *[Sobrin & Seddon, 2014]*.

Figure 5 : Diagram of interplay between environmental and genetic risk factors of AMD*



** according to Sobrin & Seddon 2014*

2.2.3 Environmental factors

2.2.3.1 Smoking

Smoking is the most consistently reported modifiable risk factor for the development of early or advanced AMD *[Klein et al., 2010; Lawrenson & Evans, 2013; Velilla et al., 2013].* Smoking significantly increases the risk of AMD with a dose response relationship, and smoking cessation seems associated with a reduced risk of AMD progression and the risk of developing AMD. Smoking cessation is thus strongly recommended when advising patients who have AMD or at risk for AMD *[AAO, 2015]*. Possible mechanisms by which smoking mediates increased AMD risk include the impairment in the generation of antioxidant (e.g. plasma vitamin C and carotenoids), induction of hypoxia, generation of ROS, alteration of choroidal blood flow, and effect on the immune system *[Ding et al., 2009; Tsoumakidou et al., 2008].*

2.2.3.2 Dietary habits

Glycaemic index: A high glycaemic index has been associated with a higher risk of large drusen, geographic atrophy, and neovascularization *[Chiu et al., 2007]*. In the Blue Mountains Eye Study, among 3654 participants 10 years after baseline, those with a dietary glycaemic index in the highest quartile were at an increased risk for early AMD compared to those in the lowest quartile, after adjusting for age, sex, body mass index (BMI), smoking, blood pressure, history of cardiovascular disease and vegetable, fruit and fat intake (RR = 1.67; 95%CI: 1.06-2.64; P=0.04, for trend). A significant trend of decreasing risk for early AMD with increased consumption of cereal fibres (P=0.05) and breads and grains (P=0.03) was found. Those consuming the highest amounts of cereal fibres, breads and grains had a reduced risk of soft drusen (RR = 0.61; 95%CI: 0.39-0.96; P=0.01, for trend) and pigment abnormalities (RR = 0.61; 95%CI: 0.43-0.85; P=0.04, for trend). Comparison of the highest to lowest quartile of glycaemic index also showed an increased risk for soft drusen over 10 years (RR = 1.68; 95%CI: 1.03-2.74; P=0.04, for trend) *[Kaushik et al., 2008]*. As pathologic mechanisms, it was hypothesised that diets with high-glycaemic index could induce higher post-prandial glycoxidative stress through the formation of advanced glycation end products, glycoxidation, accumulation and precipitation of glycated protein aggregates, and subsequent inflammatory and angiogenic responses *[Chiu et al., 2009]*. Altogether, these data suggest that a reduction in the dietary glycaemic index may provide a means of diminishing the risk of AMD *[Chiu et al., 2007].*

Dietary fats:

High total fat intake was shown to significantly increase the risk of progression to advanced AMD with a RR of 2.90 (95%CI: 1.15-7.32) for the highest fat-intake quartile relative to the lowest fat-intake quartile after controlling for other factors. Saturated, mono-unsaturated, poly-unsaturated, and trans-unsaturated fats increased the likelihood of progression with relative risk of respectively 2.09 (P=0.08), 2.21 (P=0.04), 2.28 (P=0.04) and 2.39 (P=0.008) *[Souied et al., 2015]*. Proposed mechanisms for this increased risk include progressive accumulation of lipids in Bruch's membrane, atherosclerosis causing hemodynamic changes in the retinal and choroidal blood supply, and the depletion of omega-3 fatty acids and high serum levels of polyunsaturated fatty acids (PUFAs) that cause oxidative damage to the retina *[Coleman, 2011]*.

Information from AREDS suggests that enhanced intake of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are protective against AMD *[Chiu et al., 2009]*. Higher intakes of DHA (≥64.0 mg/day vs <26.0 mg/day) and EPA (≥42.3 vs <12.7 mg/day) were associated with a lower risk for progression to advanced AMD with hazard ratio of 0.73 (95%CI: 0.57-0.94) and 0.74 (95% CI: 0.59-0.94). The omega-6/omega-3 fatty acid ratio also appears to be an important parameter and is much harder to practically manipulate given the typical Western diet *[Kishan et al., 2011]*.

Fish intake:

There is strong evidence that fish intake has protective effect on the development of AMD.

In a cross-sectional study involving subjects of the National Health and Nutrition Examination Survey (NHANES) III, fish intake was inversely associated with advanced AMD (OR=0.41; 95% CI: 0.2-0.9) *[Parekh et al., 2007]*.

In 2454 participants of an elderly Australian cohort, weekly consumption of fish was associated with a significant risk reduction in the development of early AMD (RR=0.69, 95% CI: 0.49-0.98). Thus, protection against early AMD may be obtained from regularly eating fish *[Tan et al., 2009]*.

In EUREYE (European Eye Study) involving 2277 participants aged ≥ 65 years, it was found that eating oily fish more than once weekly decreased the odds of neovascular AMD by two (OR=0.50; 95%CI: 0.28-0.88; P=0.02). A further reduction was seen with greater weekly consumption, but results were not statistically significant *[Augood et al., 2008]*.

In a large prospective cohort of 38,022 female health professionals (Women's Health Study), women who consumed one or more servings of fish per week, compared to those who consumed less than 1 serving per month had reduced relative risk of AMD of 0.58 (CI, 0.38–0.87) *[Christen et al., 2011]*.

The beneficial effect of fish is believed to be related to the high content of omega-3 fatty acid such as DHA and EPA and other protective nutrients (e.g. vitamin D) *[Schleicher et al., 2013; McCusker et al., 2016, Holick, 2007]*.

Fruits and vegetables:

Additional risk factors may include low systemic levels of antioxidants, and individuals with a diet higher in fruit and vegetable may have a lower risk of AMD. A recent literature review looking for association between food components and ocular diseases indicates that dietary intake of fruit and vegetables, and vitamin C probably lowered incidence of AMD although high supplemental doses of vitamin C increases macular degeneration risk *[Raman et al., 2017].* For example, in a prospective follow-up cohort study, in 77,562 women and 40,866 men who were at least 50 years of age with no diagnosis of AMD at baseline followed for 18 years (for women) and up to 12 years (for men), individuals who consumed ≥ 3 servings of fruit per day had a reduced risk of AMD (OR=0.64, 95%CI: 0.44-0.93; P for trend = 0.004) *[Cho et al., 2004].* A strong, inverse association was shown for dietary intake of spinach or collard greens, with a statistically significant trend for a lower risk for AMD with a greater frequency of intake of these vegetables (P<0.001) *[Seddon et al., 1994]*. Compared with those consuming these greens less than once per month in the multivariate model, the OR for those eating them two to four times per week was 0.54 (95% CI: 0.3-0.9); for those eating the greens five or more times per week, the OR was 0.14 (95% CI: 0.01-1.2).

Nuts:

Seddon et al. reported that nuts which are a dietary source of various protective nutrients including vitamin E, copper, magnesium, and dietary fiber, and other nutrients such as polyphenols (resveratrol) with antioxidant, antithrombotic and anti-inflammatory properties *[Kris-Etherton et al., 2002]* were associated with a lower rate of AMD progression, with a multivariate relative risk of 0.60 (95% CI: 0.32-1.02) for 1 or more servings per week, compared with no intake *[Seddon et al., 2003].*

Mediterranean diet:

The Mediterranean diet is characterized by high consumption of fruits, vegetables, legumes, cereals, fish and olive oil, a low-to-moderate consumption of dairy products, a low consumption of meat and a regular but moderate consumption of alcohol, namely wine *[Willett et al., 1995; Raimundo, 2018]*. In a recent nested case-control study (n=883 subjects) within the Coimbra Eye Study, adherence to the Mediterranean diet seems to be associated with a decreased prevalence of AMD with an odd ratio of 0.62 (95%CI: 0.38-0.97) compared to low adhesion to the Mediterranean diet. Analyses showed a protective role for increased consumption of fruits, caffeines, fibres, β-carotene, vitamin C and vitamin E (p<0.05). In another case-control study (n=1992 subjects) within the Coimbra Eye Study, high adherence to a Mediterranean diet and regular physical activity seem to be protective factors for AMD. The effect of the diet is likely driven by the increased consumption of vegetables, fruits, and nuts *[Nunes et al., 2018].*

2.2.4 Systemic diseases

The potential link between AMD and vascular diseases is consistent among several studies *[van Leeuwen et al., 2003; Duan et al., 2007; Hogg et al., 2008].* According to current concept, age-related macular degeneration is a local manifestation of systemic vascular disease *[Fischer, 2015].* AMD, stroke and cardiovascular disease may share a common pathogenesis including oxidative stress, and systemic inflammation *[McLeod et al., 2009; Snow & Seddon, 1999].* The vascular model proposes that the progressive deposition of lipid, seen in atherosclerosis, is the underlying cause of AMD. This deposition of lipid leads to its accumulation in the sclera and in Bruch membrane, with a consequential increase in vascular resistance. This process would then interfere with the metabolism of the RPE and lead to pigmentary abnormalities, drusen formation, and, ultimately, the changes that are represented by the clinical manifestations of AMD. Choroidal blood flow in subjects with AMD was consistently shown to be lower than in age-matched controls. Furthermore, accumulation of extracellular cholesterol in Bruch membrane resembles that found in the walls of large systemic arteries *(Connell et al., 2009).*

2.3 PREDICTIVE RISK ASSESSMENT MODELS

As reviewed by Sobrin & Seddon, the knowledge of non-genetic, modifiable risk factors along with information about heritability and genetic risk variants for this disease acquired over the past 25 years have greatly improved patient management and our ability to predict which patients will develop or progress to advanced forms of AMD. Personalized medicine and individualized prevention and treatment strategies may become a reality in the near future *[Sobrin & Seddon, 2014].* Risk assessment can be of potential value in clinical practice and could help to determine the frequency of follow-up examinations, the use of home-monitoring of central vision, and the advisability of initiating preventive measures including beneficial lifestyle changes such as dietary alterations and nutritional supplement use *[Klein et al., 2011b].*

Early detection is essential for a better management of AMD. In recent years, several risk assessment models based on AMD phenotype, environmental and genetic factors have been proposed to predict the risk of advanced AMD over 5-10 years *[Klein et al., 2011b; Sobrin & Seddon, 2014; Seddon et al., 2015]* and online applications have been developed to assist in clinical decision making.

Klein et al. developed a risk assessment model based on the longitudinal data derived from the AREDS population *[Klein et al., 2011b].* The proposed model is composed of 3 risk factor components (i.e. demographic/environmental, phenotypic, and genetic) and was shown to meet acceptable performance standards allowing application in

clinical practice. This model can be used with or without the genetic component i.e. CFH Y204H and ARMS2 A69S polymorphisms). Other factors including age, family history, smoking history, BMI, and drusen phenotype, and unilateral AMD can be readily obtained at a routine eye examination, and provided most of the predictive value while genetic testing may be not available, especially at screening.

Seddon et al. proposed another highly predictive progression model with 10 genes variants which has been validated for different risk threshold *[Seddon et al., 2015]*. The risk prediction model has been disseminated to clinicians as an online browser-based calculator available in the public domain (seddonamdriskscore.org). This tool is designed to be used on mobile device platform such as smartphones and tablets. These models require genetic testing of several variants including rare variants, and thus are still of limited value for medical management of patients with AMD.

The Simplified Théa Risk-Assessment Scale (STARS®)

The STARS® questionnaire is a validated tool to assess the risk of AMD in routine clinical practice *[Delcourt et al., 2017]*. It does not require any biological sampling by contrast to the predictive questionnaire described above. This is a simple and quick 13-item self-completed questionnaire including demographic data (age, sex, BMI, and ethnicity), family history of AMD, personal medical history (smoking, BMI, hypertension, myocardial infarction, hypercholesterolemia), and eye-related parameters (iris color, cataract, refraction) (Figure 6). The total score leads to three groups of patients with low (score 0-9), moderate (score 10-19) or high risk (score >20). The questionnaire showed a good discrimination of patients with and without risk of AMD in two cross-sectional studies of 12639 and 6897 patients, respectively. The sensitivity was high in both studies (91.9% and 79.6%, respectively). The specificity was 43.6% and 44.7%, respectively), suggesting that some patients may be misclassified at risk (false positive). Nevertheless, the negative predictive value was high in both studies (75.7% and 84.5%, respectively) showing that among those classified as low risk, the vast majority would actually be free of AMD *[Delcourt et al., 2017]*.

Figure 6: The STARS® Questionnaire to assess the risk of AMD

STARS®

Simplified Théa AMD Risk assesment Scale

		Marks
Gender	Male	☐ 0
	Female	☐ 1
Age (years)	< 65	☐ 0
	65-74	☐ 2
	75-85	☐ 4
	>85	☐ 9
Ethnic origin	Caucasian	☐ 0
	North-African	☐ 5
Family history of AMD (brothers, parents)	Yes	☐ 7
	No	☐ 0
BMI (kg/m²)	BMI < 25	☐ 0
	BMI between 25 and 30	☐ 1
	BMI > 30	☐ 2
Smoker	Never smoked	☐ 0
	Current smoker	☐ 2
	Former smoker (interrupted by less than 10 years)	☐ 3
	Former smoker (interrupted for over 10 years)	☐ 2
History of Arterial hypertension		☐ 3
History of Myocardial infarction		☐ 3
History of Hypercholesterolemia		☐ 2
History of atherosclerosis		☐ 4
Cataract surgery	Yes	☐ 5
	No	☐ 0
Refractive errors	Myopia	☐ 2
	Hyperopia	☐ 5
Iris colour	Dark	☐ 0
	Light	☐ 0

Your score : ☐

When you have your score, get closer to your ophthalmologist

Score 0-9: Low risk for AMD

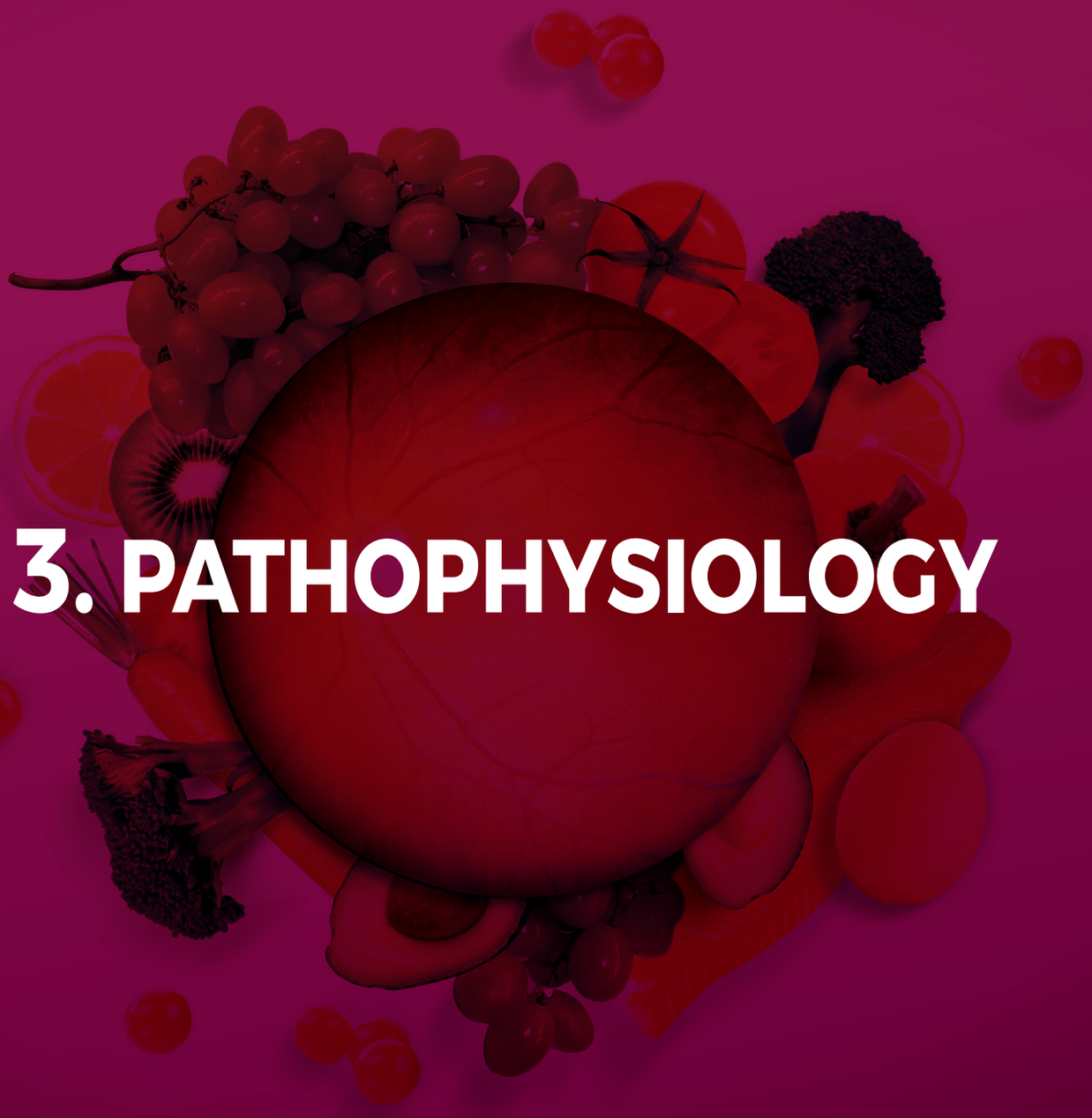
Score 10-19: Moderate risk for AMD

Score > 20: High risk for AMD

*Development and Validation of a Risk Score for Age-Related Macular Degeneration: The STARS Questionnaire. Delcourt C, Souied E, Sanchez A, Bandello F; STARS Survey Group. Invest Ophthalmol Vis Sci. 2017 Dec 1;58(14):6399-6407.

Key messages

- AMD is the leading cause of irreversible vision loss in developed countries.
- AMD prevalence increases with age.
- AMD is a multifactorial disease resulting from a combination of genetic and environmental risk factors.
- Dietary habits (including glycemic index and high fat intake) have been associated with increased risk of early and/or progression to advanced.
- Food rich in antioxidants and minerals (fruits, vegetables, and nuts) have been associated with a lower risk of AMD.
- Smoking and genetics are the most consistent risk factors.
- Predictive simple risk score questionnaires for AMD are useful to identify high risk subjects and to offer a personalized medicine approach.
- A simple and fast questionnaire (STARS®) is now available for early detection of patients at risk of AMD in routine clinical practice.



3. PATHOPHYSIOLOGY

The AMD pathology is characterized by degeneration involving the retinal photoreceptors (PR), RPE, and BM, as well as, in some cases, alterations in choroidal capillaries *[Ding et al., 2009]*. Alterations of the RPE and BM is a characteristic of early changes of AMD *[Miller, 2013]* and RPE dysfunction and atrophy is believed to precede the later stages of AMD (GA or CNV AMD) *[Ambati & Fowler, 2012]*. Although the different forms of advanced AMD have distinct pathologic mechanisms, they converge on cellular pathways that lead to photoreceptor death *[Miller, 2013]*.

3.1 RETINAL PIGMENT EPITHELIUM

The RPE is a monolayer of pigmented cuboidal epithelial cells which are located behind the photoreceptors. This is a polarized monolayer with microvilli extending from the apical surface to envelop the outer segments of both rods and cone photoreceptors. The basal surface of the RPE stands in contact with the BM. Light filtration by RPE pigments functions as a preventive mechanism against photo-oxidation. Light-sensitive PR outer segments are renewed constantly and one function of the RPE is to phagocytose PR outer segment discs for lysosomal degradation. Accumulation of photo-oxidized products (lipofuscin) in the RPE occurs with aging, and this has been linked to a number of retinal diseases. In addition to its role as a protective barrier, and phagocytosis of shed PR outer segment membrane, RPE cells carry out other functions including the conversion and storage of retinoids, ion and fluid transport, ion balance in the subretinal space and RPE-PR apposition *[Sparrow et al., 2010]*.

An early sign of AMD is the formation of sub-RPE extracellular deposits of lipids, cellular debris and proteins, termed drusen, which can accumulate over time leading to RPE dysfunction, cell death and loss of central vision *[Rabin et al., 2013]*. The cell types responsible for the formation of drusen remain unclear but there is cumulative evidence that they are in part the products of RPE cells *[Rabin et al., 2013]*. Deposits can be located between the RPE plasma membrane and the RPE (basal linear deposits) and/or between the RPE basal lamina and the inner collagenous layer (basal laminar deposits). Drusen in AMD are most frequently found as clusters within the macular region. They vary in size, shape, color, consistency, and distribution, and they tend to increase in number with advancing age. The cause of drusen may be linked to one or more of key processes summarized in Table 3 *[Chen et al., 2010]*.

Table 3 : Cause of drusen formation and accumulation

• Increased outer segment turnover
• Impaired activity/function of the RPE
• Free radical/oxidative damage
• Aging and degeneration of elements of Bruch's membrane (e.g., collagen and elastin);reduced clearance of material from Bruch's membrane into choriocapillaries
• Deleterious immune system activation.

Ref: Chen et al., 2010

3.2 BRUCH'S MEMBRANE

Typical age-related changes in the BM are summarized in Table 4. The BM is an extracellular matrix (ECM) and consists of an elastin core flanked on both sides by a collagenous layer and a basal lamina. This membrane undergoes constant turnover, mediated by the matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). It thickens progressively with age, partly because of increased levels of TIMPs and a resulting reduction in ECM turnover (Miller, 2013). Age-related abnormalities in BM eventually lead to photoreceptor degeneration as a result of increased hydrophobicity, reduced permeability, and impaired nutrient exchange between the choroid and the RPE *[Gehrs et al., 2006, Miller, 2013]*. In addition, the barrier properties of the RPE and BM limit cellular migration, especially the invasion of neovascular tissue from the choroid into the subretinal space *[Gehrs et al., 2006]*.

Early AMD is characterized by the thickening of the BM due to lipid and protein accumulation. Elastin fiber destruction in the macula could also play a key role in the initiation of neovascular events because elastin degradation peptides are highly angiogenic and possess macrophage-recruiting activity *[Gehrs et al., 2006]*. It has been proposed that topographic variations in BM may render the macula more susceptible to the ingrowth of new blood vessels from the choroidal vasculature that characterizes neovascular AMD *(Gehrs et al., 2006)*.

Table 4 : Typical age-related changes in Bruch's membrane

- Progressive thickening of the two collagenous layers;
- Modification and degeneration of collagen and elastin;
- Increased levels of advanced glycation end products, noncollagenous proteins and lipids;
- Accumulation of several types of sub-RPE deposits.

3.3 CHOROIDAL CAPILLARIES

It has been suggested that AMD may be a vascular disorder characterized by impairment of choroidal perfusion mainly based on two observations: 1) RPE atrophy causes secondary choriocapillaris loss and photoreceptor degeneration; and 2) Choroidal vascular insufficiency results in dysfunction of the RPE and photoreceptor degeneration. There is also evidence for choriocapillaris degeneration in GA and exudative AMD, but the etiology of the two types of AMD may differ: RPE atrophy appears to be the initial insult in GA whereas choriocapillaris degeneration precedes RPE atrophy in wet AMD *[McLeod et al., 2009].*

Key messages

- AMD primarily affects the macular region of the retina that may cause vision loss in the aging population.
- It is a slowly progressive disease involving degeneration of the retinal photoreceptors, RPE, and BM, as well as, in some cases, alterations in choroidal capillaries.

3.4 OXIDATIVE STRESS

3.4.1 Biology of Reactive Oxygen Species

Oxidants are generated as a result of normal intracellular metabolism in mitochondria and peroxisomes, as well as from a variety of cytosolic enzyme systems. The generation of radical oxygen species (ROS) per se is a physiological event that contributes to the regulation of general metabolic health, such as mediating phagocyte killing of bacteria. Another physiological function is to modulate transcription of genes, such as nuclear factor kappa-B (NFκ-B), a well-known redox-sensitive transcription factor *[Haddad, 2002].* However, the host-defense mechanisms against ROS provided by antioxidant enzymes and glutathione (GSH) synthesis imply that ROS levels are finely regulated to keep good radicals from going bad and thereby avoid oxidative damage to cellular processes *[Schieber & Chandel, 2014].*

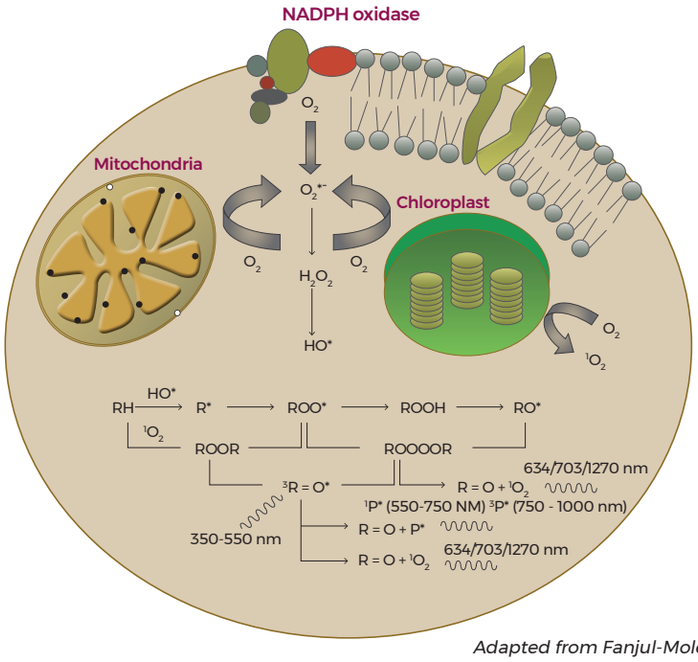
ROS encompass a variety of diverse chemical species detailed in Table 5 and Figure 7. These various radical species can be generated exogenously or produced intracellularly from several different sources such as mitochondria and phagosomes *[Finkel & Holbrook, 2000].* Some of these species, such as superoxide anion or hydroxyl radicals, are extremely unstable, whereas others, like hydrogen peroxide, are freely diffusible and relatively long-lived *[Finkel & Holbrook, 2000; Fanjul-Moles & Lopez-Riquelme, 2016].*

- Superoxide anion radical ($O_2^{\bullet -}$) is produced via the membrane-bound enzyme complex NADPH oxidase which is found embedded within the plasma membranes and membranes of various organelles including mitochondria and phagosomes. The dismutation of $O_2^{\bullet -}$ is accompanied by the formation of hydrogen peroxide (H_2O_2) and then the hydroxyl radical ($HO^{\bullet}$) via the Fenton reaction. $HO^{\bullet}$ is highly reactive and has the capability to oxidize all types of biomolecules such as lipids, proteins, and nucleic acids. The oxidation of biomolecules is accompanied by the formation of high-energy intermediates such as dioxetane (ROOR) and tetroxide (ROOOOR), which upon further decomposition, generate electronically excited species such as triplet excited carbonyl, singlet and triplet excited pigments, and singlet oxygen ($^1O_2$) *[Fanjul-Moles & Lopez-Riquelme, 2016].* The triplet state is believed to be the pathway of most photochemical reactions, because its lifetime is sufficiently prolonged to interact with other molecules *[Young, 1988].*

Table 5 : Main reactive oxygen species

Radicals	Neutral / Anion / Cation
Superoxide $O_2^{\bullet -}$	Hydrogen peroxide H_2O_2
Hydroxyl radical $HO^{\bullet}$	Hydroperoxide R-O-O-H
Peroxyl radical $RO_2^{\bullet}$	Hypochlorous acid HClO
Alkoxyl radical $RO^{\bullet}$	Singlet oxygen $^1O_2$
Hydroperoxyl radical $HO_2^{\bullet}$	Peroxynitrite $ONOO^-$
Nitric oxide $NO^{\bullet}$	Nitronium NO_2^+

Figure 7 : Formation of reactive oxygen species



Adapted from Fanjul-Moles & Lopez-Riquelme, 2016

- Singlet oxygen is a particularly destructive oxygen metabolite. It can be generated by photosensitization reactions wherein a sensitizer (such as such as porphyrins, bilirubins, melanins, and pterins) absorbs light of a given wavelength, exciting the molecule. The increased energy of the sensitizer, in a triplet state, can be transferred to molecular oxygen creating singlet oxygen $^1O_2$, which can attack a membrane or other cell components *[Winkler, 1999]*. Targets for oxidation by $^1O_2$ would be lipids, amino acids and nucleic acids that have double bonds as well as sulfur-containing amino acids *[Buettner, 2011]*..

A sophisticated enzymatic and non-enzymatic antioxidant defense system including catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) counteracts and regulates overall ROS levels to maintain physiological homeostasis. Oxidative stress is considered as the biochemical endpoint of the imbalance between ROS production and the ability of antioxidant biological systems to fight against oxidative injury *[Pinazo-Duran et al., 2014]*. The balance between ROS production and antioxidant defenses determines the degree of oxidative stress *[Finkel & Holbrook, 2000]*.

Regardless of how or where they are generated, a rise in intracellular oxidant levels has two potentially important effects: damage to various cell components and triggering of the activation of specific signaling pathways. Both of these effects can influence numerous cellular processes linked to ageing and the development of age-related diseases *[Finkel & Holbrook, 2000]*. One of the most sensitive sites of free radical damage are cell membranes, which are rich PUFA. Lipid peroxidation (autoxidation), a process that leads to the oxidation of PUFAs due to the presence of several double bonds in their structure, involves the production of peroxides and reactive organic free radicals. The latter can then react with other fatty acids, initiating a free radical reaction cascade. ROS can also react with nucleic acids by attacking nitrogenous bases and the sugar phosphate backbone, leading to DNA damages. The inability of cells to repair the damage incurred in this process may lead to mutation or cell death *[Fanjul-Moles & Lopez-Riquelme, 2016]*.

3.4.2 Oxidative Stress in the Retina

The retina resides in an environment that is primed for the generation of ROS and resultant oxidative damage. The retina is one of the highest oxygen consuming tissues in the human body. The highest levels of oxygen are found in the choroid, and this falls dramatically across the outermost retina. The photoreceptor outer segment is composed of a dense stack of 2000 layers of phospholipids which are uniquely rich in double bonds (PUFAs). Oxygen is concentrated in the fatty acid layers in which it is highly soluble *[Young, 1988]*.

This micro-environment together with abundant photosensitizers, visible light exposure and a high energy demand supports a highly oxidative milieu *[Jarrett & Boulton, 2012]*. PUFAs in membranes of the outer segments of photoreceptors are readily oxidized by radicals produced during photonic activation. The endogenous oxygen species that are generated through this process can induce ROS-related acute or chronic retinal damage *[Fanjul-Moles & Lopez-Riquelme, 2016; Winkler et al., 1999]*. Other endogenous sources of ROS include mitochondrial metabolism, rod outer segment phagocytosis, lipofuscin phototoxicity and protoporphyrin photosensitization *[Winkler et al., 1999]*.

There is a high density of mitochondria and a high rate of respiration in the inner segments of rod and cone photoreceptor cells and outer segment tips. Thus, mitochondria represent a major source of endogenous ROS in the photoreceptors and underlying RPE *[Jarrett & Boulton, 2012]*. Mitochondrial oxidative stress is further enhanced by phagocytosis of photoreceptor outer segments, presumably through the burst of ROS generated during ingestion, and by exposure to blue light *[Jarrett & Boulton, 2012]*.

The retina also contains a large number of chromophores which become photosensitizers when excited by the appropriate wavelength of light. The two major photosensitizers in the retina are the visual pigments in photoreceptor cells and lipofuscin which accumulates with age in the RPE. Lipofuscin is the generic name given to a heterogeneous group of complex and autofluorescent bis-retinoids, lipid peroxides, and proteins and to various fluorescent compounds that are formed from modified lipids or that are derived from vitamin A *[Fanjul-Moles & Lopez-Riquelme, 2016]*. Lipofuscin is derived from the inability of the RPE to convert all all-trans-retinol into 11-cis-retinal during the visual cycle and is produced when phagocytosed material is not entirely degraded within the RPE lysosomes, resulting in the accumulation of this complex over time *[Fanjul-Moles & Lopez-Riquelme, 2016].* When near-UV photons are absorbed by lipofuscin, radiations are re-emitted into the cytoplasm of RPE cells, leading to uncontrolled side-effects including damage to cellular proteins and lipid membranes *[Young, 1988].*

3.4.3 Defense against Oxidative Stress

The health of the RPE and visual cells is dependent on their ability to metabolize free radicals, lipid hydroperoxides and other potentially toxic compounds *[Winckler et al., 1999]*. Several antioxidant defense mechanisms have evolved to protect cell components from the attack of oxidative stress and associated oxidative damage (Table 6).

Table 6 : Protection/defense against oxidative stress

	Antioxidants	Roles
Enzymes	Superoxide dismutase (SOD)	Dismutation of $O_2^{\bullet-}$ and H_2O_2
	Catalase	Dismutation of H_2O_2 to H_2O
	Glutathione peroxidase (GPx)	Removes H_2O_2 and peroxides
Multiple	Alpha-tocopherol	Breaks lipid peroxidation: lipid peroxide and $O_2^{\bullet-}$ and $HO^{\bullet}$ scavenger
	β-carotene	$HO^{\bullet}$ and $O_2^{\bullet-}$ scavenger; Prevent oxidation of vitamin A; Binds to transition metals
	Ascorbic acid	$O_2^{\bullet-}$, $HO^{\bullet}$ and H_2O_2 scavenger; Contribute to regeneration of vitamin E

Antioxidant enzymes

Antioxidant enzymes convert reactive and toxic oxidants and electrophiles into stable and less toxic or neutral molecules, and are the main first-line mechanism of maintaining redox homeostasis and defending against oxidative damage *[Zhang et al., 2015].* These mechanisms include SOD, superoxide reductases, catalase, GPx, and many heat-shock proteins. The enzymes catalase and SOD are the major defenses against ROS. SOD converts superoxide anions into H_2O_2, and catalase converts H_2O_2 to molecular oxygen and water. SOD exists in two forms: with copper and zinc (Cu/ZnSOD, or SOD-1) primarily present in the cytoplasm and with manganese (MnSOD or SOD-2), present in mitochondria.

Progress has been made in the recent decades concerning the signaling pathways of the oxidative stress response. The Nuclear factor erythroid-2 related factor 2 (Nrf2) signaling system has emerged as perhaps the most important cellular defense and survival pathway against oxidative stress *[Zhang et al., 2015].* Nrf2 is a basic leucine zipper transcription factor which regulates a coordinated transcriptional program that maintains cellular redox homeostasis and protects the cell from oxidative injury *[Cano et al., 2010].* Much of the protective antioxidant response of Nrf2 may be mediated by the upregulation and activation of a number of enzymes including “direct” response enzymes such as catalase or superoxide dismutase, “indirect” enzymes such as heme oxygenase-1, glutathione and thioredoxin generating enzymes including the regulatory and catalytic subunits of glutamate-cysteine ligase, the rate limiting step in glutathione biosynthesis, and xenobiotic metabolism enzymes that produce

reducing equivalents, such as NADPH quinine oxidoreductase. There is some evidence for antioxidant protection by Nrf2 in RPE cells *[Nakagami, 2016].*

Non-enzymatic antioxidants

The non-enzymatic antioxidants are free radical scavengers (Table 7 and Figure 8). They slow down oxidation reactions, trapping free radicals and transforming them into less aggressive compounds. They are hydrosoluble and cytosolic e.g., glutathione (GSH) and ascorbic acid (vitamin C) or liposoluble and membrane-bound e.g., α-tocopherol (vitamin E) and carotenoids (β-carotene, lutein, zeaxanthin, and lycopenes) *(Pinazo-Duran, et al., 2014).*

- **Glutathione** is a naturally occurring tripeptide that acts as a reductant of peroxides either by a nonenzymatic reaction or by a reaction catalyzed by glutathione peroxidase. The major activity of GSH peroxidase is catalyzed by selenoenzymes that are active on fatty acid hydroperoxides, phospholipid hydroperoxides, cholesterol hydroperoxides and hydrogen peroxide. GSH can also be used to detoxify reactive aldehydes generated from lipid peroxidation *[Winkler et al., 1999].*

- **Vitamin C** is a major water-soluble antioxidant. Like GSH, vitamin C is stable in nitrogen and undergoes metal-catalyzed oxidation. The oxidation products are, however, unstable in aqueous solution at physiological temperature and pH. The instability of dehydroascorbic acid is a potential problem since this is the only oxidation product that is reduced back to vitamin C in a GSH-dependent manner *[Winkler et al., 1999].* Vitamin C also helps regenerating other antioxidants within the body, including vitamin E *[McCusker et al., 2016].*

- **Vitamin E** and **carotenoids** are the principal liposoluble antioxidants. Vitamin E is the major chain-breaking lipid-soluble antioxidant in membranes, and thus is expected to play the most important role in minimizing effects of oxidation of PUFAs. Both vitamin E and the carotenoids scavenge free radicals, particularly hydroxyl radical and singlet oxygen. Both types of compounds are stable in nitrogen, but unstable in oxygen. Vitamin E is recycled by redox coupling with vitamin C. It was shown that deprivation in vitamin E leads to lipofuscin accumulation, retinal damages, and loss of photoreceptors *[Corusupudi et al., 2017].*

- The antioxidant function of **β-carotene** is due to its ability to quench singlet oxygen, scavenge free radicals and protect the cell membrane lipids from the harmful effects of oxidative degradation. The ability of β-carotene and other carotenoids to quench excited oxygen, however, is limited, because the carotenoid itself can be oxidized during the process (autoxidation).

The ability to defend against oxidative stress by up-regulating the antioxidant defense response is likely to be the pivotal event that mediates the initiation and progression of AMD *[Cano et al., 2010]*. The molecular damage from oxidative modification suggests that the antioxidant response in the macula becomes unable to neutralize oxidative stress.

An increase in oxidative stress due to a reduction in protective mechanisms or an increase in number and concentration of active photo-oxidative reaction species are believed to contribute to the pathogenesis of AMD *[Strauss, 2005]*.

It is postulated that the antioxidant response becomes insufficient with aging, favoring ROS generation and degenerative diseases. Increased production of free radicals with age, while some of the endogenous defense mechanisms decrease, creates an imbalance that leads to progressive damage of cellular structures *[Cano et al., 2010, Zhang et al., 2015]*.

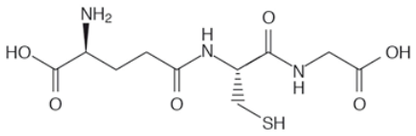
Table 7 : Basic properties of antioxidants

Glutathione	• Undergoes metal-catalyzed oxidation; • Its oxidation product, GSSG (oxidized glutathione), is stable at physiological temperature and pH; • Neither GSH nor GSSG readily passes through membranes • GSSG is reduced back to GSH by NADPH-dependent pathways involving glutathione reductase and cell metabolism of glucose (hexose monophosphate shunt pathway) and other substrates
Vitamin E	• A group of eight fat-soluble compounds • α-tocopherol is the biologically most active form • Protects lipids from peroxidative damage • A chain-breaking antioxidant that reacts with, $\cdot O_2$, $^1O_2$, peroxyl ($ROO^\bullet$), and alkoxyl ($RO^\bullet$) radicals • Major lipid-soluble antioxidant protecting membranes and lipoproteins from injury • Vitamin E$\cdot$ (radical form) is reduced back to vitamin E by vitamin C
Macular carotenoids	• Absorb blue light, protective against short wavelength visible light • Quench singlet oxygen • Quench triplet state of photosensitizers • Inhibit autoxidation of lipids • β-carotene, an effective antioxidant at low oxygen pressure; assume same for macular carotenoids • Undergo autoxidation

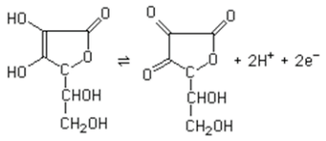
Ref: Winckler et al., 1999

Figure 8 : Main cellular antioxidants

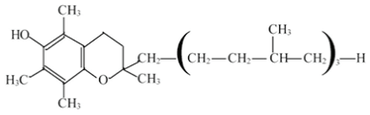
Glutathione



Ascorbic acid

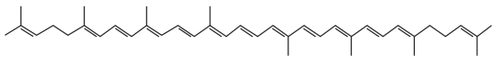


Alpha-tocopherol

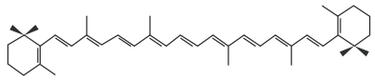


Carotenoids

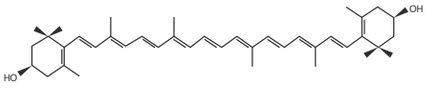
Lycopene



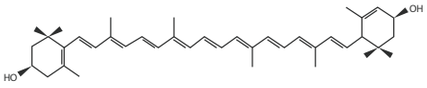
β-carotene



Zeaxanthin



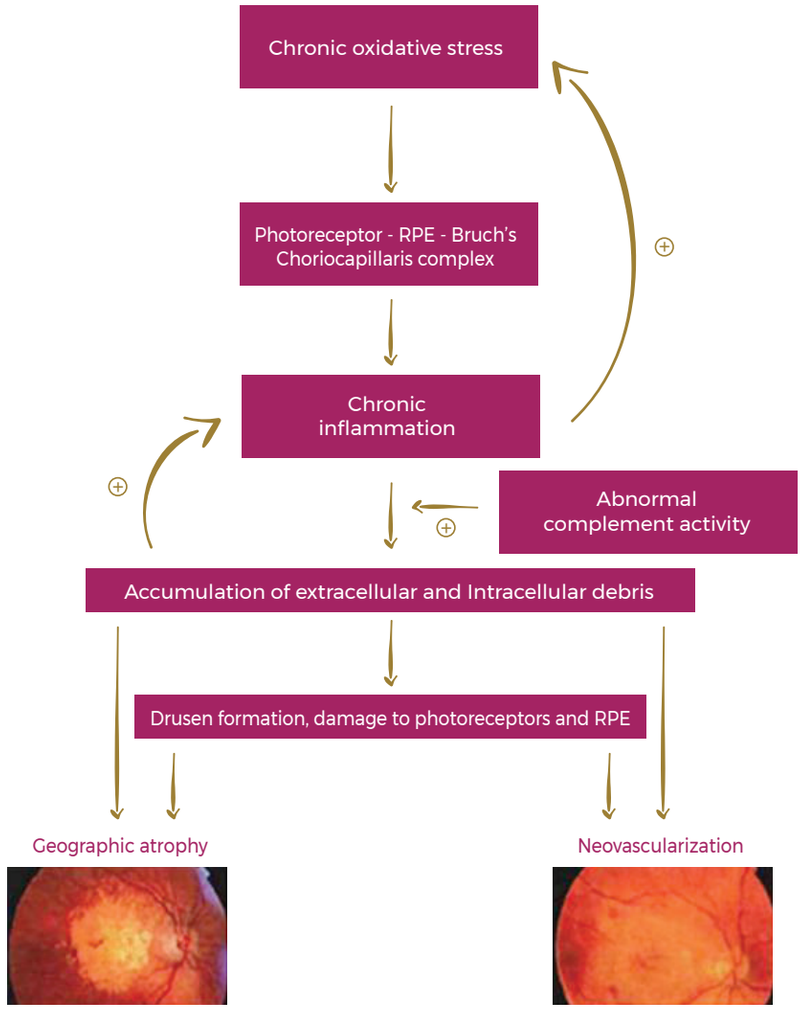
Lutein



3.4.4 Oxidative Stress and AMD

Several key processes involved in the pathogenesis of AMD are illustrated in Figure 9. These include: oxidative damage; accumulation of toxic visual cycle products (i.e. lipofuscin); impaired RPE function; abnormal immune system activation; chronic inflammation (including complement activation); senescent loss of homeostatic control; abnormalities in BM and choroidal vascular insufficiency *[Ding et al., 2009; Zhang et al., 2012].*

Figure 9 : Proposed pathophysiology of AMD



Adapted from Zhang et al., 2012

The lesions induced by oxidative injury (including blue light-induced photochemically released oxidants) may accumulate over time and trigger affected RPE cells to undergo apoptosis. The cellular debris under the RPE cells is thought to activate the complement system, chronic inflammation, and toxic reactive oxygen species (ROS) generation leading to RPE damages and further malfunction of this cell layer, propagating a vicious cycle of local deterioration. Inflammatory and immune-mediated processes are considered to play a central role in the development of drusen and subsequent events [Hageman et al., 2001; Anderson et al., 2002]. The complement alternative pathway contributes to the structural changes observed in RPE and BM, including drusen formation and to VEGF-mediated mechanisms in wet AMD. There is probably inadequate inhibition of the alternative pathway leading to spontaneous complement activation and generation of pro-inflammatory activation fragments. Decreased levels of tissue oxygenation and hypoxia may induce accumulation of detrimental RPE-associated deposits, inflammation and neovascularization processes in retina *[Arjamaa et al., 2009; McLeod et al., 2009].*

Oxidative stress has been proposed in AMD through several mechanisms, including blue light-induced photochemically released oxidants that damage cells, cigarette smoke-related oxidants such as hydroquinone that alter the BM, iron-induced oxidative damage to the outer retina, and possibly advanced glycation end-products (AGEs) within the BM. The lesions induced by oxidative injury may accumulate over time and trigger affected RPE cells to undergo apoptosis. Chronic oxidative stress was shown to upregulate drusen-related protein expression *[Rabin et al., 2013]*.

Oxidative stress in senescent RPE is a key event in initiating and maintaining macular damages, and developing AMD *[Parmegianni et al., 2012]*. Direct and indirect effects of oxidative damages can be seen in all layers of the fundus in AMD eyes *[Cano et al., 2010]*. As reported by Ding et al., experimental studies have shown that retina of human donor AMD contained higher levels of protein adducts resulting from the oxidative modification of carbohydrates and lipids and higher levels of antioxidant enzymes compared with non-AMD eyes, which indicate that oxidative stress plays an important role in AMD *[Ding et al., 2009]*.

Rabin et al. demonstrated that eyes of patients with AMD exhibit a greater amount of oxidative modifications to proteins and DNA in BM and RPE compared to age-controlled eyes *[Rabin et al., 2013, Raman et al., 2017]*. When exposed to a regimen of ROS, human RPE cells showed an increased concentration of drusen, a disrupted cell morphology, decreased cell viability and epithelial integrity *[Rabin et al., 2013]*.

Oxidative stress and mitochondria

A drastic decrease in normal mitochondria can be seen in photoreceptors and RPE cells of AMD eyes as compared to normal age-controlled eyes *[Ding et al., 2009]*. Mitochondrial defects in the RPE cells in patients with AMD include DNA mutations, impaired structural integrity, and defective mitochondrial function *[Ambati & Fowler, 2012]*. This leads to decreased energy production and imbalance between pro-and anti-apoptotic signals leading to cell death. The increase in the amount of ROS is believed to destabilize intracellular membrane compartments such as lysosomes and mitochondria *[Strauss, 2005]*. The resulting decrease in metabolic efficiency produces more lipofuscin and ROS. In a vicious cycle, these mechanisms further destabilize RPE cells leading to a loss of RPE, which denotes the beginning of the formation of drusen *[Strauss, 2005]*.

Indirect effect of oxidative stress

In addition to direct toxicity, oxidative stress has also been shown to potentiate complement-induced RPE secretion of VEGF *[Thurman et al., 2009]* to modulate the immune-inflammatory system, through enhanced expression of pro-inflammatory genes. Inflammation in turn, enhance oxidative stress *[Cano et al., 2010]*. ROS have been shown to reduce RPE barrier integrity by phosphorylation of cadherin-β-catenin complexes. With reduced RPE barrier integrity, VEGF can access the neural retina and potentially attract activated CECs to migrate and form CNV *[Wang & Hartnett, 2016]*.

Oxidative stress and lipofuscin

Oxidative stress in the retina is aggravated by lipofuscin which accumulates in the RPE with age *[Bowes Rickman et al., 2013]*. Lipofuscin normally accumulates inside RPE cells and this site is not readily accessible to complement. Oxidized lipofuscin when excreted by RPE cells into the subretinal space, can interact with complement proteins. It was suggested that products of the photooxidation of bis-retinoid lipofuscin pigments (e.g. A2E) in RPE cells could serve as a trigger for the complement system that would predispose the macula to disease and over time to chronic inflammation *[Zhou et al., 2009]*.

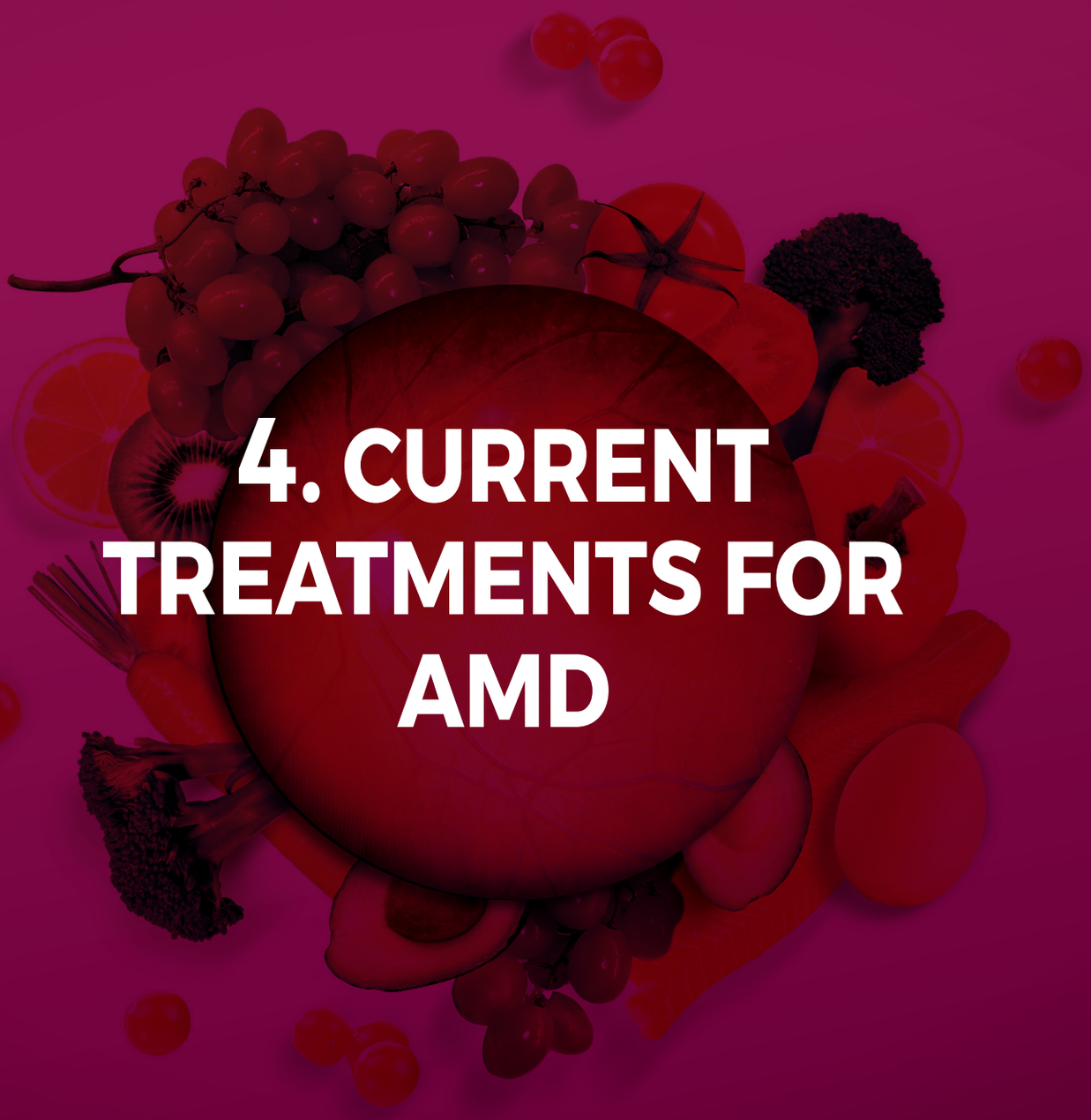
Oxidative stress and advanced glycation end products

There is evidence from experimental studies suggesting an interaction between oxidative stress and complement cascade activation in the development of AMD *[Khandhadia et al., 2012]*.

Extracellular drusens contained advanced glycation end-products (AGE), high levels of oxidized lipoproteins, and oxysterol *[Kinnunen et al., 2012]*. The oxidative stress may accelerate AGEs formation and the accumulation of AGEs in the BM is believed to promote choroidal neovascularization by activating VEGF expression from RPE cells *[Strauss, 2005]*.

Key messages

- The retina is a highly oxidative environment.
- The balance between radical oxygen species production and antioxidant defenses determines the degree of oxidative stress.
- This balance may be reduced with aging.
- The ability to defend against oxidative stress by up-regulating the antioxidant defense response probably plays a pivotal role in the initiation and progression of AMD.



4. CURRENT TREATMENTS FOR AMD

There is no treatment to prevent or to cure AMD. There are currently only palliative treatment options for the late-stage neovascular form of the disease with anti-angiogenic agents, photodynamic therapy and thermal laser. There are no current therapies for the more common dry AMD, except for the use of antioxidants that delay progression in 20-25% of eyes with intermediate AMD *[AREDS report no 8, 2001; AAO, 2015; Gehrs et al., 2006]* (See Section 5.1). New emerging therapeutic options are actually under investigation.

4.1 ANTI-VEGF THERAPY

Neovascular AMD may be partially reversible with intravitreal injection therapy using anti-VEGF agents (e.g. aflibercept, bevacizumab, ranibizumab). This is the most effective way to manage neovascular AMD and represents the first line-treatment *[AAO, 2015]*. Anti-VEGF therapy is generally well tolerated and rarely associated with serious adverse events such as infectious endophthalmitis or retinal detachment. However, its efficacy seems highly dependent on an early diagnosis of neovascular lesions before major retinal damage has occurred *[Delcourt et al., 2017]*, and depends from frequency of injections; moreover delivery of anti-VEGF is burdensome, costly and invasive *[Lee et al., 2018]*.

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 (Table 8) *[San Giovanni & Chew, 2005]* Furthermore, VEGF is known to increase levels of MMPs, which not only contribute to ECM breakdown, but also may increase VEGF expression and further secretion from RPE cells via a feedback mechanism *[Miller, 2013]*.

The primary goal of anti-VEGF agents, injected intravitreally, is to block the growth of abnormal blood vessels in the eye to prevent vision loss and, in some instances, improve vision *[Solomon et al., 2014]*. As reviewed by Miller et al. in 2013, treatments with anti-VEGF can halt vision loss in more than 90% of patients and improve vision in one third *[Miller, 2013]*. There are currently several anti-VEGF agents with different potencies. The clinician, therefore, can choose another agent if efficacy is not found following one treatment regimen *[Wang & Hartnett, 2016]*. Currently, anti-VEGF agents are administered most commonly via monthly/bimonthly intravitreal injections, as needed (pro re nata, PRN) or in a Treat and Extend protocol after three consecutive monthly injections. There is apparently no difference regarding efficacy between these different modalities *[AAO, 2015]*. Nevertheless, intravitreal injection of anti-VEGF agents is not only burdersome, costly and invasive, but is also associated with eventual visual reduction in some cases *[Rofagha et al., 2013, Lee et al., 2018]*.

It was recently confirmed that anti-VEGF treatment was often experienced with some anxiety related to treatment, regardless of the number of injections received and clinical levels of depression seem to be more frequent in patients at early stage of anti-VEGF treatment *[Senra, 2017]*. The source of anxiety may be related to fear of going blind because of injections, concerns about vision getting worse from treatment failure, and waiting in the waiting room *[Senra et al., 2017]*.

Given the burden and the cost of treatment, prevention of neovascular AMD like ocular nutrition and antioxidants seems an attractive strategy to avoid the chronic and costly anti-VEGF therapy *[Lee et al., 2018]*.

New therapeutic drug are actually under investigations and results from randomized clinical trials are awaited, as discuss later in future treatment options chapter.

Table 8 : Main VEGF functions

Angiogenesis	↑ Migration of endothelial cells ↑ Mitosis of endothelial cells ↑ Matrix metalloproteinase activity ↑ Integrin αvβ3 activity ↑ Creation of blood vessel lumen creates fenestrations
Chemotactic for macrophages and granulocytes	
Vasodilation (indirectly by NO release)	

Ranibizumab

Ranibizumab is an anti-VEGF medication developed for ocular administration. It is a humanized antibody fragment which can block the VEGF protein and prevent it from binding to its receptor, thus inhibiting angiogenic activity. Ranibizumab was the first treatment for neovascular AMD that offered a realistic hope for vision improvement; it was approved in the USA by the Food and Drug Administration (FDA) in 2007 and in the European Union by the European Medicines Agency (EMA) in January 2007 *[Schmidt-Erfurth et al., 2014]*.

Bevacizumab

Bevacizumab is used to treat choroid neovascularization secondary to neovascular AMD. Bevacizumab is a humanized monoclonal antibody against VEGF. Bevacizumab is currently approved for the treatment of conditions such as colorectal cancer, but is also widely used by ophthalmologists as an off-label drug for neovascular AMD. Bevacizumab showed almost same efficacy as ranibizumab, but at lower cost, although it remains still off label and the modality of vial preparation still unsafe. A multicentre, randomized clinical trial in 1107 patients with neovascular AMD showed that ranibizumab and bevacizumab had similar effects on visual acuity over a two-year period. In this clinical trial, treatment as needed may result in less gain in visual

acuity, whether instituted at enrollment or after one year of monthly treatment. There were no differences between drugs in rates of death or arteriothrombotic events *[Martin et al., 2012]*.

Aflibercept

Aflibercept is a relatively new anti-VEGF for neovascular AMD that was approved by the FDA in November 2011 and by the EMA in November 2012 *[Schmidt-Erfurth et al., 2014; Sarwar et al., 2016)*. It showed comparable visual acuity outcomes when compared to ranibizumab in randomized controlled clinical trials *[Sarwar, 2016]*. Aflibercept acts as a VEGF decoy to inhibit the growth of new blood vessels. It binds very tightly both ends of activated dimerized VEGF and prevents it from interacting and activating native VEGF receptors and cross linking. This binding results in blockage of the biological activity of VEGF and inhibits abnormal growth of blood vessels. Aflibercept also binds to the placental growth factor (PIGF) and prevents it from activating VEGF receptors. Analyses of available data indicate that aflibercept is equally effective compared to ranibizumab or bevacizumab and safe and may be considered as a first-line treatment for patients with neovascular AMD because of the potential for fewer injections needed to achieve similar results *[Sarwar et al., 2016]*.

Pegaptanib

Pegabtanib is historically the first anti-VEGF agent and seems less effective than bevacizumab, ranibizumab, or aflibercept *[Wang & Hartnett, 2016]*. This is a chemically synthesized 28-base ribonucleic acid molecule. It is an aptamer (foldable single-strand nucleic acid) with a capability to change its three-dimensional structure to fit VEGF. By binding to VEGF, pegaptanib blocks and inactivates VEGF, thus, halting the process of neovascularization. Pegaptanib was approved for the treatment of neovascular AMD by the FDA in the United States in December 2004 and by the European Medicines Agency (EMA) in Europe in January 2006 *[Schmidt-Erfurth, 2014]*. No study has compared Pegaptanib with the other anti-VEGFs, but clinical data have shown that the most recent anti-VEGFs (ranibizumab and aflibercept) improved visual acuity whereas pegaptanib merely reduced the loss of visual acuity. Pegaptanib is no longer recommended in therapeutic use for the management of exudative AMD *[Schmidt-Erfurth et al., 2014]*.

4.2 LASER PHOTOCOAGULATION

Photocoagulation can be used to obtain immediate closure of subretinal neovascular membranes resulting in permanent cessation of exudation, haemorrhage and vessel growth *[Schmidt-Erfurth et al., 2014]*. Laser photocoagulation to treat exudative forms of AMD should be used only in extrafoveal forms of the disease. Nowadays, its use is almost non-existent due to the best results of anti-VEGF therapies, being limited to

exceptional cases and usually as a combination therapy with anti-angiogenesis therapy. In the presence of subfoveal or juxtafoveal neovessels, laser photocoagulation may cause major risks and complications such as enlargement of the scar, or permanent scotoma recurrences. A previous Cochrane systematic review concluded that laser photocoagulation could effectively slow the progression of neovascularization in non-subfoveal lesions compared with observation alone *[Virgili & Bini, 2007]*. A more recent Cochran systematic review in 2015 confirmed that laser photocoagulation of drusen, using a variety of different laser sources and techniques, leads to their disappearance. However, treatment does not result in a reduction in the risk of developing CNV, and was not shown to limit the occurrence of geographic atrophy or visual acuity loss *[Virgili et al., 2015]*. Clearly, drusen reduction using laser photocoagulation has no clinical benefits, including improvement or stabilization of visual acuity, delayed or reduced CNV, or harms such as the onset of atrophy.

Nevertheless, the prophylactic use of laser therapy to prevent or reduce progression of AMD remains under investigation. Although traditional thermal laser results in clearance of drusen, but does not alter the risk of progression to advanced AMD, there is a now interest in short pulse duration lasers, delivered in a subthreshold manner without causing incidental retinal damage associated with conventional laser photocoagulation.

These new modalities in laser technology may provide promising treatments of AMD through RPE-mediated mechanisms. A multi-center randomized clinical trial using a nanosecond laser to investigate progression of AMD in 240 participants, is underway *[Findlay et al., 2018; Eng et al., 2019]*.

4.3 PHOTODYNAMIC THERAPY

An alternative method of closing subretinal CNV is photodynamic therapy (PDT), which combines intravenous infusion of a photosensitive dye (verteporfin) that releases free oxygen radicals when exposed to targeted illumination of the area of the fundus where the new vessels are located.

In patients with predominantly visible subfoveal CNV, PDT with verteporfin offers an alternative if anti-VEGFs are contraindicated or if there is no response to them and in certain clinical forms in combination with anti-VEGFs (polypoidal vasculopathy, for example).
According to previous guidelines, PDT was recommended for the treatment of wet AMD for individuals who have a confirmed diagnosis of classic with no occult sub-foveal CNV (i.e., whose lesions are composed of classic CNV with no evidence of an occult component) and best-corrected visual acuity 6/60 or better *[NICE, 2014]*. A Cochrane review of verteporfin PDT concluded that PDT was effective in preventing

clinically significant vision loss *[Wormald et al., 2007].* However, as laser photocoagulation, PDT did not offer any significant chance for vision improvement.

Nowadays, the use of PDT is limited only to the polypoidal subtype in association with anti-VEGF.

4.4 ANTIANGIOGENIC STEROIDS

Steroids have gained attention in their role for the treatment of neovascular AMD for their antiangiogenic and anti-inflammatory properties. However, there is currently no evidence that intravitreal steroids (e.g. dexamethasone implant; triamcinolone) prevent visual loss in patients with neovascular AMD *[Geltzer et al., 2013]*. According to the American Academy of Ophthalmology (AAO) in 2015, there is currently no data to support the use of intravitreal steroids in combination therapy with anti-VEGF or PDT phototherapy. Moreover, there are some concerns about the development of glaucoma or cataract associated with the long-term use of corticosteroids *[AAO, 2015].*

4.5 FUTURE TREATMENT OPTIONS

Considering the limitations of current anti-VEGF approaches, including the need for frequent injections, inadequate response in some patients, and a relatively short duration of effect, several new therapeutic modalities are under evaluation *[Falavarjani et al., 2017; Schlottmann et al., 2017]*.

The trends in wet AMD therapy included the use of alternate anti-VEGFs such as brolucizumab (a single-chain antibody that inhibit all form of VEGF-A), abicipar (an ankyrin repeat protein that also inhibits all forms of VEGF-A), and the ranizumab port delivery system *[Wykov et al., 2018; Souied et al., 2014].* Novel molecular targets in combination with VEGF-A blockade are being investigated, including faricizumab (RO6867461; RG7716), inhibiting angiopoietin-2 and VEGF-C plus VEGF-D blockade (NCT03038880) and X-82 (an oral tyrosine kinase inhibitor which blocks the VEGF and PDGF receptors) (NCT02348359).

Non-invasive eye drop treatment is also subject to intensive research *[Zeitz & Joussen, 2017]*. This includes squalamine (OHR-102) a small molecule, with antiangiogenic effects and the ability to inhibit several growth factors such as VEGF, basic fibroblast growth factor, and platelet-derived growth factor (PDGF) *[Schlottmann et al., 2017]*.

Although, there is no treatment for dry AMD, a number of different approaches are under development for treating non exudative manifestations of AMD. This included antioxidants, visual cycle inhibitors (targeting lipofuscin; fenretinide), anti-inflammatory agents (e.g. monoclonal antibodies targeting complement factors),

drugs that increase choroidal blood flow (e.g. hydralazine), neuroprotective therapy (brimonidine), and stem cell therapy to replenish the lost or degenerating RPE cells *[for review see Querques et al. 2014a and Hanus et al., 2016].*

Despite the recent failure of lampalizumab (a complement factor D inhibitor), the complement cascade remains a clear target and multiple alternative pharmaceuticals are progressing through human trials *[Holz et al., 2018; Apellis Pharmaceuticals, 2018]*. A complement C3 inhibitors (APL-2) is to date the only treatment for geographic AMD currently investigated in a phase 3 clinical trial *[Rosenfeld, 2018]*.

Key messages

- There is no definitive treatment to prevent or to cure AMD, especially dry AMD and GA.
- Intravitreal administration of anti-VEGF antibodies is the most effective way to stabilize or even improve visual acuity in the majority of patients with neovascular AMD.
- The efficacy of anti-VEGF is highly dependent on early diagnosis of neovascular lesions.
- Nevertheless, anti-VEGF administration is burdensome, costly and invasive.

5. OCULAR NUTRITION AND AMD

The initial hypothesis that antioxidant dietary and nutritional factors could influence AMD was based on the known influence of daily insults of free radical formation and oxidation, and that the retina was a set up for these oxidative processes due to the abundance of PUFAs in the photoreceptor outer segment membranes. Thus, it was thought that dietary antioxidants could potentially block the damaging effect of oxidation and scavenge, decompose, or reduce the formation of these harmful compounds in the macula *[Sobrin & Seddon, 2014].*

Diet is an excellent source of antioxidants, vitamins, and minerals necessary for healthy living and a wide variety of nutrients, such as minerals, vitamins, omega-3 (n-3) fatty acids, and various carotenoids, have been associated with reducing the risk of AMD in population-based studies. Nutritional supplements containing antioxidants, minerals, and essential fatty acids are available over the counter and can be consumed as an alternative source.

The interest of ocular nutrition in the prevention of AMD was suggested in observational studies, based on semi quantitative food questionnaires, adjusting for confounding risk factors such as age, smoking status, or genetic predisposition. Information derived from frequency food questionnaires (FFQs) is considered as valid for measuring long-term dietary intake in epidemiological studies *[Chiu et al., 2009].* Current evidence indicates that all patients, regardless of their disease severity, should be given dietary advice to increase the consumption of green leafy vegetables, to consume low-glycemic index diets, and to consume fish at least twice a week *[Broadhead et al., 2015].*

Some cross-sectional and case-control observational studies have suggested that a reduced risk of early or advanced AMD was associated with dietary intake of essential micronutrients including minerals (zinc), vitamins (vitamins A, B,C, D, E, carotenoids, and omega-3 fatty acids *[for review see Raman et al., 2014; Connell et al., 2009; Gorusupudi et al., 2017].* The association between minerals, antioxidants, or omega-3-fatty acids and the risk of early or late AMD was confirmed in some, but not all, prospective population-based cohort studies with a follow-up of 5 to 10 years *[van Leuwen et al., 2005; Tan et al., 2008; Tan et al., 2009].* Other nutrients, such as vitamin D and polyphenols (e.g. resveratrol) may also have a beneficial effect in AMD *[Merle et al., 2017].*

Even if diet has a prominent role, it is extremely unlikely that a single nutrient is directly responsible for a chronic disease or, conversely, that the addition of a single nutrient will eliminate disease risk *[Christen et al., 2014].* On the other hand, observational data cannot provide causal effects, but can only determine associations. It is possible that the associations between nutrition and AMD are due to other confounding factors. Thus, nutrients must be studied in randomized controlled interventional trials to evaluate their potential therapeutic effects on AMD *[Chew, 2017]*..

The use of multivitamins (vitamin E, vitamin C, β-carotene, zinc, folic acid, vitamin B6, vitamin B12) at recommended dietary allowance (RDA) levels (See Table 9) has been investigated in a randomized double-blind placebo-controlled study (the Physicians Health Study II) *[Christen et al., 2014]*. In this study, 14,641 US male physicians took a daily multivitamin supplementation for an average of 11 years. The results did not show any evidence for a protective effect of this vitamin supplement on development of AMD in primary prevention (hazard ratio, HZ=1.19; 95% CI: 0.94-1.50) *[Evans & Lawrenson, 2014]*.

To date, only one randomised placebo-controlled interventional study (AREDS) demonstrated a beneficial effect of minerals (zinc, copper) and vitamins (vitamin C, E and β-carotene) supplementation at pharmacological doses to reduce the risk of AMD in patients with intermediate or late AMD at baseline *[AREDS report no.8, 2001]*.

The absence of benefit on AMD may reflect, at least in part, the lower dosage of the multivitamin used but also the inclusion of patients with lower risk profile for advanced AMD compared with the AREDS population. The absence of effect of some dietary supplementation may be due to an inadequate dose, inadequate duration of treatment, or both *[AREDS-2, 2013]*.

Table 9 : Recommended dietary allowance (RDA) levels of minerals and vitamins for adult men and women (age > 50 years)

Micronutrients	Recommended dietary allowance*	
	Men	Women
Zinc	11 mg	8 mg
Iron	8 mg	8 mg
Copper	0.9 mg	0.9 mg
Selenium	0.055 mg	0.055 mg
Vitamin A	0.9 mg (Retinal equivalent)	0.7 mg (Retinal equivalent)
Vitamin B6	1.7 mg	1.5 mg
Vitamin B12	2.4 mg	2.4 mg
Folate	0.4 mg	0.4 mg
Vitamin C	90 mg	75 mg
Vitamin E	15 mg (22.5 IU)	15 mg (22.5 IU)
Vitamin D	400 IU	400 IU

*IU: International unit, *daily intake level of a nutrient that is considered to be sufficient to meet the requirements of 97-98% of healthy individuals in every demographic in the United States (Merck Manual, 2006)*

Key messages

- The association between minerals, antioxidants, or omega-3-fatty acids and the risk of early or late AMD was confirmed in some prospective population-based cohort studies.

- Some cross-sectional and case-control observational studies have suggested that a reduced risk of early or advanced AMD was associated with higher dietary intake of essential micronutrients including minerals (zinc), vitamins (vitamins A, B, C, D, E, carotenoids, and omega-3 fatty acids.

- All patients, regardless of their disease severity, should be given dietary advice to increase the consumption of fruits, green leafy vegetables and to consume fish at least twice a week.

5.1 ANTIOXIDANT VITAMINS

Natural antioxidants may be defined as molecules that prevent cell damage against free radicals and are critical for maintaining optimum health in both animals and humans *[Puertollano et al., 2011]*. Vitamins, such as vitamins C, E, B-6, and B-12 and folic acid, are hypothesized to decrease the risk of AMD progression *[Gorusupudi et al., 2017]*.

Results from epidemiologic studies, support the possibility that an increased intake of dietary antioxidants, specifically carotenoids, vitamin E, and vitamin C may reduce the risk of advanced AMD *[Raman et al., 2017]*.

This suggested that dietary antioxidants may delay the development of early AMD and, possibly, of AMD in general.

- Vitamin C

  Vitamin C (ascorbic acid) is considered to be essential for protection against disease processes caused by oxidative stress. It is the most effective aqueous phase antioxidant in human blood. Foods rich in vitamin C include berries, citrus fruits, broccoli, brussel sprouts, bell peppers and potatoes *[van Leeuwen et al., 2005; Connell et al., 2009]*. The National Institute of Medicine (IOM) recommends a daily intake of 75-90 mg of vitamin C daily for an adult woman and man, respectively. This is approximately equivalent to a cup of orange or grapefruit juice. Larger doses are needed for smokers *[McCusker et al., 2016]*.

  Low plasma levels of vitamin C have been associated with an increased risk of AMD, but that high concentrations were not protective *[see Gorusupudi et al., for review]*. There is no evidence that dietary consumption of vitamin C may be beneficial in primary prevention *[Evans & Lawrenson, 2014]*.

- Vitamin E

  Vitamin E exists naturally as eight distinct fat-soluble compounds of tocopherols. Nuts and seeds such as sunflower seeds, almonds and hazelnuts are rich sources of vitamin E in addition to dark leafy vegetables like spinach and collard greens. The recommended intake of vitamin E is 22.4 IU/day (about 20 mg/day). High intake of vitamin E can be achieved by consumption of whole grains, vegetable oil, eggs, and nuts *[van Leeuwen et al., 2005]*. In the Rotterdam Study, a population-based cohort of all inhabitants aged ≥55 years in the Netherland, dietary intake of vitamin E was inversely associated with incident AMD (regrouping early and wet AMD) with a hazard ratio (HR) per standard deviation increase of intake for vitamin E of 0.92 (95% CI: 0.84-1.00) after a mean follow-up of 8.0 years *[van Leeuwen et al., 2005]*.

- β-carotene

  β-carotene is the predominant source of vitamin A (retinol and its esters). Carrots, kale, and spinach are the main suppliers of β-carotene *[van Leeuwen et al., 2005].* In a recent case-control study in Japanese population, using a brief-type diet history questionnaire, it was suggested that a high dietary intake of n-3 fatty acid, α-tocopherol, zinc, vitamin D, vitamin C, and β-carotene was associated with a reduced risk of neovascular AMD *[Aoki et al., 2016].* In general, intake of a single vitamin has not been associated with a significant effect in preventing AMD, although the combined intake of vitamins and other antioxidants does appear to reduce the risk of AMD *[Broadhead et al., 2015].* In the Rotterdam Study, a median intake of all 4 nutrients, β-carotene (3.6 mg/d), vitamin C (114 mg/d), vitamin E (13 mg/d), and zinc (9.6 mg/d), was associated with a 35% reduced risk (HR=0.65; 95% CI: 0.46-0.92) of AMD *[van Leuwen et al., 2005].*

Antioxidant therapy and genetics

It has been proposed that the response to antioxidant therapy may be influenced by the genotype. For example, results from the Rotterdam Study indicate a greater reduction in risk for incidence of early AMD for patients with either Y402H CFH or A69S ARMS2 variants who consumed diets high in antioxidant substances *[Ho et al., 2011].* Further research is required to better investigate any potential gene-treatment interactions *[Broadhead et al., 2015]* and the use of genetic testing in routine practice is not supported by existing literature and thus is not recommended at this time *[AAO, 2015].*

Key messages

- An increased intake of dietary antioxidants, specifically carotenoids, vitamin E, and vitamin C may reduce the risk of early and possibly advanced AMD.
- A combined intake of vitamins instead of one single vitamin may be beneficial in early and late AMD.
- The response of antioxidants may be dependent on some genetic polymorphisms.

5.2 MINERALS

Minerals such as selenium, copper, zinc and iron also act in the antioxidant network, primarily as cofactors for enzymes with antioxidant activity. As reviewed by Gorusupudi et al., iron, zinc, copper, and selenium are essential trace elements that play key roles in retinal physiology. The cellular homeostasis of iron, zinc, and copper is tightly interlinked. If one of these metals becomes deficient, another metal may accumulate *[Gorusupudi et al., 2017].*

- Iron

  There is a higher concentration of iron in AMD retinas than there is in age-matched control retinas. Although these results do not demonstrate that iron overload is a reason for AMD, iron may contribute to oxidative stress, which might lead to AMD. An increase in iron deposition was also reported to lead to photoreceptor loss, but deficiency was not associated with adverse effects.

- Zinc

  Zinc is the second most abundant metal after iron in the human retina, suggesting an important physiologic role. Zinc depletion results in poor dark adaptation and reduced photopic and scotopic responses. Concentrations of zinc affect the progression of AMD, and its concentrations in human retina and RPE also decrease with age *[Erie et al., 2009; Gorusupudi et al., 2017]*. Zinc is known to be a cofactor of many metabolically active enzymes within the eye, including superoxide dismutase and catalase, which are important in protecting the retina from oxidative damage. Zinc also binds complement factor H, inducing large multimeric forms that lose complement component 3b inhibitory activity *[Nan et al., 2013; Aoki et al., 2016]*, theoretically reducing the risk of neovascular AMD by suppressing chronic inflammation at the RPE/choroidal interface *[Aoki et al., 2016]*. Oysters (and other seafood) contain the most amount of zinc per serving than any other food, with 3 oz. containing 74 mg *[NIH factsheets].* Meat (beef, poultry and pork) provides the necessary intake for most people. Beans, cereals and nuts are other sources, but plant-based phytates can inhibit their absorption. In regions of the world where meat and seafood are scarce, zinc consumption may not be adequate *[McCusker et al., 2016].* Increased zinc intake has been associated with a decreased risk of both early and late AMD in the Rotterdam study *[van Leeuwen et al., 2005]* and the BMES study *[Tan et al., 2008]* and with a decreased risk of early AMD (OR=0.6, 95%CI: 0.4-1.0) in the Beaver Dam Eye Study *[Mares-Perlman et al., 1996].*

- **Copper**

  Copper is an essential trace element with the specific ability to easily accept and donate electrons; thus, it plays an important role in oxido-reduction and the scavenging of free radicals *[Zampati et al., 2014]*. Copper is also necessary for the synthesis of melanin, a storage protein for iron, zinc, and copper in RPE and melanocytes.

- **Manganese and selenium**

  Evidence for implication of manganese and selenium in antioxidant functions of the retina is not as strong as zinc or copper. Selenium is an essential component of selenocysteine-containing protein. It plays a crucial role in balancing the redox state of the cell and removing ROS. The antioxidant activity of this element is determined by glutathione peroxidase, the enzyme that recycles glutathione, which depends on the presence of selenium for exerting its antioxidant activity. However, no significant benefit was associated with supplementation with selenium for AMD. One report suggested an inverse association of manganese with AMD, whereas in other studies, no significant association was observed *[See Sobrin et al., for review]*.

5.3 LUTEIN AND ZEAXANTHIN

Lutein (L), zeaxanthin (Z), and meso-zeaxanthin (MZ) are xanthophyll carotenoids found within the retina and throughout the visual system. Subsequently, the effect of xanthophylls in the prevention and treatment of various eye diseases (retinopathy, cataract) has been examined through epidemiological studies, animal studies, and clinical trials. Macular pigments are xanthophyll carotenoids that provide the macula lutea with its yellow appearance. There is evidence that dietary intake of L and Z is associated with enhanced visual function (including visual acuity and contrast sensitivity), increased macular pigment optical density (MPOD), and a lower risk of late AMD *[Scripsema, 2015]*. However, the basic clinical science supporting such recommendations is underappreciated by clinicians and vision scientists *[Bernstein, 2016]*.

5.3.1 Sources of xanthophyll carotenoids

L and Z cannot be synthesized by the body de novo and must be acquired from the diet. MZ is a metabolite of L but also can be absorbed from the diet. The richest sources of L and Z are from leafy green vegetables, such as spinach and kale, and orange or yellow fruits and vegetables (Table 10) *[Lawrenson & Evans, 2013; Humphries & Khachik, 2003].*

Table 10 : Qualitative and quantitative distributions of lutein and zeaxanthin in fruits, vegetables, wheat, and pasta products

	Concentration of lutein and zeaxanthin (µg/100g)		
Foods	Lutein (L)	Zeaxanthin (Z)	L/Z ratio
Greens			
Beans, green	418	35	12
Beans, lima (canned)	356	16	22
Broccoli	1511	43	35
Collards	5120	140	37
Kale	15000	240	63
Lettuce, romaine	170	8	21
Parsley	10820	502	22
Peas (canned)	719	51	14
Spinach	9157	525	17
Yellow-orange			
Corn (canned)	198	333	0.6
Mango	10	10	1.0
Nectarine	20	170	0.1
Oranges	350	250	1.4
Oranges, mandarin	70.5	60	1.2
Papaya	22.1	22.1	1.0
Peaches	20	20	1.0
Plum, red	40	-	-
Squash, acorn	50	-	-
Squash, butternut	2400	280	8.6

Adapted from Humphries et al, 2003

L and Z are found in lower amount in egg yolk, however its absorption is very efficient due to their fat content *[Goodrow et al., 2006].* There has been no recommended daily intake (RDI) established for lutein and zeaxanthin. Only 1-3 mg per day are obtained from the typical American diet *[McCusker et al., 2016]*. A randomized, crossover study of 33 men and women (mean age 79 years) found that consuming 1 egg a day for 5 weeks raised L and Z levels by 26% and 38%, respectively without a change in LDL, HDL or triglycerides. LDL is the main transporter for both L and Z *[Goodrow et al., 2006]*. As fat soluble molecules, L and Z bioavailability is greatly increased by dietary fat *[Goodrow et al., 2006].*

Macular pigments account for 20-30% of total carotenoids in the human serum, but 80-90% of carotenoids in the human retina. The concentration of L, Z, and MZ in the macula is much higher than concentrations in the serum and liver. This suggests a specific uptake and storage mechanism for L, Z, and MZ in the retina and emphasizes their essential role in retinal function *[Scripsema et al., 2015]*. In addition, the ratio of Z to L is much higher in the central retina (1:1 in the macula and 2:1 in the fovea) than it is in the plasma (1:5), suggesting that the eye preferentially accumulates zeaxanthin *[Delcourt et al., 2006].*

Ref: Humpries et al., 2003

5.3.2 Biological rationale for lutein/zeaxanthin supplementation in AMD

The macular pigment plays an important role in visual function and also possesses essential antioxidant and protective blue-light filtering properties *[Bernstein et al., 2010].* Xanthophyll carotenoids have two main functions in the retina:

- blue light absorption (thus reducing light-induced oxidative stress),
- direct antioxidant properties by quenching reactive oxygen species

L and Z have a peak absorbance near 460 nm. In the inner retina they serve as a filter for high energy, short wavelength blue light. This protects the outer retina from photochemical injury easily induced by these high energy wavelengths. They also enhance visual performance by decreasing chromatic aberration and enhancing contrast sensitivity *[Scripsema et al., 2015].*

Lutein has been shown to block H_2O_2-induced apoptosis of cultured retina photoreceptors *[Chucair et al., 2007].* Membrane-bound lutein is considered to scavenge the oxygen intermediates, whereby the oxygen scavenging property of lutein is caused by quenching of reactive oxygen intermediates via the numerous unconjugated double bonds in the lutein molecule. A transfer of protein-bound lutein into the lipid membrane of retinal cells is likely and the close proximity of transmembrane lutein with polyunsaturated phospholipids allows optimal protection against ROS *[Kijlstra et al., 2012].* Lutein has been shown to decrease lipofuscin accumulation in cultured RPE cells possibly by inhibiting peroxidation of membrane phospholipids of the photoreceptor outer segments. Peroxidation of phospholipids and polymerization is thought to affect degradation into simple molecules leading to a build-up of lipofuscin in RPE cells.

In a recent experimental study, a dietary supplement containing lutein, antioxidants, minerals, and omega-3 fatty acids (Nutrof®total, Laboratoires Théa, France) *[See Patient information leaflet in Annexes]*, was able to produce a recovery effect on oxidative stress damage and to reduce the pro-inflammatory markers in human RPE cells (ARPE-19), human retinal endothelial cells and monkey choroidal endothelial cells in culture *[Recalde et al., 2018].*

5.3.3 Lutein/Zeaxanthin supplementation and improved visual function in AMD

Previous studies have shown that the macular pigment was significantly lower in eyes with AMD than in control eyes, and a statistically significant relationship was found between the MPOD and the risk of progression *[Ma et al. 2012a].* Thus, elevated MPOD has been suggested as a potential therapeutic intervention to slow the progression

of AMD. L and Z supplementation from foods can increase macular pigment density, but this ability was shown to vary considerably among individuals.

- In a randomised controlled study, Hammond et al. reported that daily supplementation with L (10 mg/d) and Z (2 mg/d) for 3 months resulted in significant increase in serum levels of L and Z and MPOD and improvements in chromatic contrast and recovery from photostress in 57 young and healthy subjects as compared with 58 controls *[Hammond et al., 2014].*
- Ma et al. reported that the intake of L (10 or 20 mg/d for 48 weeks) and Z (10 mg/day) supplements in patients with early AMD could improve the MPOD and visual function (contrast sensitivity and best far visual acuity) as compared with placebo *[Ma et al., 2012a].*
- In a randomized double-blind, placebo-controlled trial, L supplementation (10 mg/d for one year) significantly increased the MPOD levels in patients with early stage AMD *[Murray et al., 2013].*
- Consistent results were reported by Huang et al. showing that supplementation with L (10 or 20 mg/d for two years) or L (10mg/d) + Z (10mg/d) increases MPOD, and supplemental lutein enhances retinal sensitivity in patients with early AMD *[Huang et al., 2015].*
- In the LIMPIA study, among first-generation of offsprings of parents with neovascular AMD, a statistically significant increase in plasma lutein and zeaxanthin was shown after 3 and 6 months after dietary supplementation with capsules containing lutein (5 mg), zeaxanthin (1 mg), vitamin C (90 mg), vitamin E (15 mg), zinc (7.5 g), copper (<0.5 mg), and resveratrol (0.5 mg), as well as fish oils (that included 50% of omega-3 essential fatty acids (Nutrof®total capsules *[See Patient information leaflet in Annexes]*) taken twice daily. However, there was no increase in MPOD when measured using the modified MPD-visucam 200 (Carl Zeiss Meditec) or the modified Heidelberg Retina Angiograph (Heildelberg Engineering) compared with patients receiving placebo capsules *[Korobelnik et al., 2017].*
- A meta-analysis of eight randomized controlled clinical trials in 2014 confirmed that L and Z is a safe strategy to improve visual performance in patients with AMD *[Liu et al., 2014].* In 1176 AMD patients included in the meta-analysis, xanthophyll carotenoids supplementation was associated with significant decrease in logMAR levels compared with the placebo group (weighted mean difference of -0.04; 95%CI: -0.06 to -0.03), and during intervention, each 1-mg/day increase in these carotenoids supplementation was related to a 0.003 reduction in logMAR level of visual acuity. Remarkable benefit was also observed at all four spatial frequencies of contrast sensitivity compared with placebo. Furthermore, association was observed between the post-intervention increase in MPOD and

improvements in visual acuity (linear regression coefficient, r = -0.58; P=0.02), and in contrast sensitivity at 12 cycles/degree as well (r= 0.94; P < 0.001).

- In the Veterans LAST study *[Richer et al., 2004]*, visual function was improved with lutein supplementation (10 mg per day for 12 months) (L group) and with a lutein plus a broad spectrum of antioxidant/ vitamins and minerals (L/A group). The MPOD was increased by 0.09 log units from baseline, and Snellen visual acuity improved 5.4 letters in L groups and 3.5 letters in L/A group. According to Richer et al., those individuals with the lowest MPOD were most likely to benefit from either lutein or lutein plus antioxidant supplementation *[Richer et al., 2007]*.

5.3.4 Lutein/Zeaxanthin supplementation and risk of AMD

Epidemiologic evidence evaluating the relation between dietary L and Z intake and the risk of AMD is still inconsistent.

Cross-sectional studies

- The Eye Disease Case-Control Study showed that subjects with the highest quintile of carotenoid intake had a 43% reduced risk of AMD compared with subjects in the lowest quintile.
- Seddon et al. showed an inverse association between neovascular AMD and dietary intake of carotenoids from foods. The median intake of this highest quintile was 6 mg lutein. Higher frequency of intake of spinach and collard greens (≥5 times per week as compared with <once per month) was associated with a substantially lower risk of AMD (OR=0.12, 95% CI: 0.01-0.09, P for trend = 0.001) *[Seddon et al., 1994]*.
- In a cohort of 899 subjects aged ≥ 60 years (POLA study), Delcourt et al. showed a strong inverse association between plasma carotenoids concentration, particularly zeaxanthin, with age-related maculopathy (early or late) suggesting a protective role of L and Z *[Delcourt et al., 2006]*.
- Gale et al. reported that the risk of AMD (early or late) was significantly higher in people with lower plasma concentrations of zeaxanthin. Compared with those whose plasma concentrations of zeaxanthin were in the highest third of the distribution, people whose plasma concentration was in the lowest third had an OR for risk of AMD of 2.0 (95% CI: 1.0-4.1), after adjustment for age and other risk factors. However, the relationship between the risk of AMD and lutein was not significant *[Gale et al., 2003]*.

- In AREDS, baseline data showed that dietary L/Z intake was inversely associated with neovascular AMD (OR=0.65; 95% CI: 0.45-0.93), geographic atrophy (OR=0.45; 95% CI: 0.24-0.86), and large or extensive intermediate drusen (OR= 0.73; 95% CI: 0.56-0.96), comparing the highest versus lowest quintiles of intake, after adjustment for total energy intake and non nutrient- based covariates. Other nutrients (e.g. dietary vitamin C, vitamin E, β-carotene, lycopene) were not independently related to AMD *[AREDS report 22, 2007]*.

Prospective studies

- In a meta-analysis of six longitudinal cohort studies performed in 2012, it was found that early and late AMD have different relationship with the intake of L and Z. In the late AMD, the pooled relative risk (RR) was 0.74 (95% CI: 0.57-0.97), which indicated that increase in the intake of L/Z was significantly associated with a 26% risk reduction for late AMD. Furthermore, a significant inverse association was observed between L/Z intake and neovascular AMD risk (RR: 0.68; 95% CI: 0.51-0.92), but not with geographic atrophy. The meta-analysis found that dietary intake of L/Z was not significantly associated with a reduced risk of early AMD *[Ma et al. 2012b]*.

- The Blue Mountain Eye Study reported a 65% reduced risk of incident neovascular AMD (RR=0.35; 95%CI, 0.13-0.92) between subjects with the highest and lowest intake of L/Z at baseline. Subjects above the median carotenoid intake also had a reduced risk of indistinct soft or reticular drusen (RR= 0.66; 95% CI, 0.48-0.92), suggesting that high dietary L/Z intake can reduce the risk of long-term incident AMD *[Tan et al., 2008]*.

- A nested case-control study including participants of the Rotterdam Study was performed to investigate whether dietary nutrients could reduce the genetic risk of early AMD. After a median follow-up of 8.6 years, a significant interaction was found between the CFH Y402H genotype and lutein/ zeaxanthin dietary intake assessed at baseline. Homozygotes in the lowest tertile of dietary L/Z had an HR of 2.63 (95% CI: 1.60-4.32); a higher intake reduced this risk to a HR of 1.72 (95% CI: 0.97-3.03) in the highest tertile. Heterozygotes and noncarriers showed nonsignificant trends with higher intake. This study suggests that higher dietary intake of L/Z and other nutrients (e.g. zinc, β-carotene, omega-3 fatty acids) can attenuate the incidence of early AMD in those carrying important genetic risk variants *[Ho et al., 2011]*.

- Similarly, pooled data from participants of the Blue Mountains Eye Study and Rotterdam Study showed significant interaction between AMD genetic risk status *[classified according to the number of risk alleles of CFH (rs1061170) or ARMS2 (rs10490924)]*

and L/Z intake (P=0.0009) for risk of any AMD. Among participants with high genetic risk, the highest intake tertile of L/Z was associated with a >20% reduced risk of early AMD. No similar association was evident among participants with low genetic risk *[Wang et al., 2014].* This raises the possibility of personalized preventive interventions according to genetic risk.

Interventional study

- In AREDS2 (See Section 5.4.3), a dose of 10 mg lutein plus 2 mg zeaxanthin (ratio 5:1) was chosen for the carotenoid arm. This dose was based on a small pilot-scale, 6-month dose-ranging study, which observed a 4-time (400%) increase in serum lutein with the 10 mg lutein/d treatment *[Rosenthal et al., 2006].* This dosage of L and Z is much higher than in a typical American diet (1-2 mg/d range for lutein and 0.2 mg/d for zeaxanthin); however, many vegetarian populations consume these carotenoid amounts regularly and safely *[Gorusupudi et al., 2017].* The study failed to demonstrate a significant effect of L/Z supplementation on the primary efficacy endpoint (i.e. a 25% incremental improvement beyond the previous AREDS regimen) progression of AMD *[AREDS2, 2013].* However, a secondary analysis comparing L/Z versus no L/Z supplementation clearly revealed definitively positive results *[AREDS2, 2014].* In participants who received the L/Z supplementation, the HR for the development of late AMD was 0.90 (95% CI: 0.82-0.99). In the exploratory subgroup analysis, comparing L/Z + AREDS formulation without β-carotene and the original AREDS formulation with β-carotene, the hazard ratios were 0.82 (95% CI: 0.69-0.96) for development of late AMD, 0.78 (95% CI: 0.64-0.94) for development of neovascular AMD, and 0.94 (95% CI: 0.70-1.26) for development of central geographic atrophy.

  Thus, removal of β-carotene improved efficacy suggesting that competitive absorption may exist among different carotenoids *[AREDS2, 2013].* Based on these results, the AREDS2 investigators concluded that substitution with 10 mg of lutein and 2 mg of zeaxanthin for 15 mg of β-carotene was an appropriate modification of the original AREDS formulation for smokers, former smokers, and non-smokers, and these recommendations have been rapidly incorporated into clinical practice *[Bernstein et al., 2016].*

Key messages

- Lutein/Zeaxanthin supplementation was shown to increase the macular pigment and thus is believed to protect the outer retina from photochemical injury.

- Lutein/Zeaxanthin supplementation also enhances visual performance by decreasing chromatic aberration and enhancing contrast sensitivity.

- Patients with AMD may have the lowest level of plasma Lutein/Zeaxanthin concentration.

- Patients with low plasma Lutein/Zeaxanthin concentration may have an increased risk of progression to late AMD.

- The AREDS regimen with Lutein/Zeaxanthin (AREDS2) but without β-carotene is recommended in clinical practice.

5.4 AREDS REGIMENS

The AREDS has demonstrated that a combination of antioxidants (vitamin C, E and β-carotene) and zinc supplements, at levels beyond what can be achieved through diet alone (Table 11), reduced the risk of severe AMD and vision loss in humans *[AREDS, Report no 8, 2001]*.

Table 11 : Supplementation therapy for AMD (AREDS formulation)

Supplement	Daily dose	RDA
β-carotene	15 mg	5.4 mg in males and 4.2 mg in females*
Vitamin C	500 mg	75 mg
Vitamin E	400 IU	22.5 IU
Zinc oxide	80 mg	11 mg (males) and 8 mg (females)
Cupric oxide	2 mg	0.9 mg

** Equivalent to vitamin A RDA (900 mg in males, and 700 mg in females)*

AREDS was a multi-center double-blind placebo controlled trial sponsored by the National Institute of Eye (NEI) including 3,640 individuals from 11 retinal subspecialty clinics, which was completed in 2001. Participants were aged between 55 and 80 years, and all were required to have a baseline visual acuity of 20/32 or better in one eye. Participants were randomized in 4 treatment groups: 1) a high-dose antioxidant vitamin combination (vitamins C, E and β-carotene), 2) the antioxidant vitamin combination with zinc supplementation, 3) zinc supplementation alone, or 4) placebo. Patients receiving zinc were also given copper supplementation to prevent zinc-induced copper deficiency anemia (See Section 8.2 Safety of Long-Term Supplementation).

5.4.1 AREDS supplement and early AMD

The use of antioxidants, vitamins and minerals did not reduce the progression of early AMD to the intermediate stage of AMD, and there was insufficient power to determine the effects of the combination treatment on the progression of more advanced AMD. Therefore, there is no evidence to support the use of these supplements for patients who have less than intermediate AMD. In early AMD (AREDS category 2), only 1.3% of participants progressed to advanced AMD in 5 years *[AREDS report no 8, 2001]*.

5.4.2 AREDS supplement and intermediate AMD

In AREDS, participants with extensive intermediate (i.e; medium-sized) drusen in one or both eyes, one or more large drusen in at least one eye, nonsubfoveal geographic atrophy in one eye, the rate of development of advanced AMD in 5 years was reduced by 25% in the participants using the combination treatment of antioxidant vitamins with zinc and copper. The risk of losing vision of 3 or more lines (doubling of the visual angle) was reduced by 19% with this combination treatment. Although zinc alone or antioxidants alone reduced progression, the therapy that resulted in a statistically significant reduction in both the development of advanced AMD and vision loss was the combination treatment of antioxidant vitamins and minerals (Table 12) *[AREDS report no 8, 2001].*

Table 12 : Effect of treatment on risk of progression to advanced AMD at 5 years

Treatment	Participants with Grade 3 and 4 at baseline OR [95%CI]	P-value
Antioxidants vs. no antioxidants	0.83 [0.66 – 1.06]	0.05
Zinc versus no zinc	0.79 [0.62 – 0.99]	**0.009**
Antioxidants vs. placebo*	0.73 [0.54 – 1.05]	0.03
Antioxidants + zinc vs. placebo*	0.66 [0.47 – 0.93]	**0.001**

*Advanced AMD indicates photocoagulation or other treatment for choroidal neovascularization, central geographic atrophy, non drusenoid retinal pigment epithelial detachment, serous or hemorrhagic retinal detachment, hemorrhage under the retina or pigment epithelium, or subretinal fibrosis; OR: odds ratio; CI: confidence interval. Analysis by repeated-measures logistic regression, P≤0.01 is considered statistically significant. *Adjusted for age, sex, race, AMD category, and baseline smoking status.*

The 10-year follow-up of AREDS, published in 2013, indicates that these benefits were sustained over a prolonged period. Patients with categories 2, 3 or 4 at baseline receiving AREDS supplements for 10 years demonstrated reduced progression to advanced AMD (OR=0.69, 99%CI: 0.56-0.86) or neovascular AMD (OR=0.64, 99%CI: 0.50-0.82). No effect was seen on the rate of progression to geographic atrophy (OR=0.99, 99%CI: 0.69-1.43) *[Chew et al., 2013; Broadhead et al., 2015].*

Following the publication of these results, the NEI continues to recommend the use of the AREDS formulation in persons with intermediate AMD or advanced AMD in one eye, and in persons at moderate to high risk of developing advanced AMD. Although much of the benefit of the AREDS formulation is driven by efficacy in decreasing the development of NV AMD and not CGA, it is believed that all participants with AREDS AMD category 3 and 4 should consider taking the AREDS formulation. The development of neovascularization in patients with central GA may occur as

frequently as 40% in 10 years *[Chew et al., 2013]*. Thus, the simultaneous occurrence of both forms of advanced AMD is common.

As discussed by Chew et al., several factors need to be considered when interpreting results from the follow-up study. First, the AREDS population differs from the general population in several respects. It is better nourished, more highly educated and healthier. The effect of this on generalizability of the results to the general population is unknown. Secondly, the treatment effect is relatively modest and AMD and vision loss events continue to occur in participants taking the AREDS formulation. Thirdly, we still do not know how long someone at risk of advanced AMD should take the supplements.

In addition to the benefit in AMD, both initial and long-term follow-up of the original AREDS cohort showed decreased mortality in those participants who received zinc supplementation. This was largely due to fewer circulatory disorder-related deaths (10-y HR for all-cause mortality in those who consumed vs those who did not consume zinc (OR=0.83, 95%CI: 0.73-0.95) *[Chew et al., 2013]*.

Table 13 : Main conclusions of the AREDS supplementation

- Reduced risk of developing advanced AMD by about 25% at 5 years.
- Reduced overall risk of moderate vision loss by 19% at 5 years.
- No statistically significant benefit on the progression of dry AMD or the development of geographic atrophy at 5 and 10 years.
- The AREDS formulation is the best-validated supplementation therapy for atrophic AMD in patients with moderate to advanced AMD.

5.4.3 AREDS2 regimen

Following the results of the AREDS trial, the AREDS formulation quickly became the standard of care for AMD. This formula contains about 5, 6 and 18 times the RDA levels of zinc, vitamin C and vitamin E, respectively *[Chiu et al., 2009]* leading to some safety concerns for long-term use including increased risk of cancer in smokers due to β-carotene supplementation and genitourinary complications and anemia due to high zinc supplementation (See Section 8.2 Safety of Long-Term Supplementation).

The AREDS2 study was designed to test whether the original AREDS supplement formulation could be made safer and more effective. This study showed that β-carotene

in the AREDS formulation could be substituted by lutein (L) and zeaxanthin (Z) in a new formulation with vitamin C, vitamin E, zinc and copper. This study included 4203 participants with either bilateral large drusen or large drusen in one eye and advanced AMD in the other eye followed for 5 years. The study design included a double randomization: Participants were first randomized assigned to one of the following 4 treatment groups: 1) L+Z, 2) L+Z+EPA+DHA, 3) EPA+DHA, and 4) placebo; then, all nonsmoking enrolled subjects were offered secondary random assignment and were assigned to the original AREDS formula or to a modified AREDS formula with no β-carotene and/or lower concentrations of zinc. Enrolled subjects who were smokers were randomly assigned to the AREDS supplement with no β-carotene with or without low concentrations of zinc.

Substituting L (10 mg/day) and Z (2 mg/day) for β-carotene (15 mg/d) was shown to be a safe and appropriate modification of the original AREDS formulation for all subjects, including smokers and former smokers.

The main effect analyses of low zinc (25 mg as zinc oxide) versus high zinc (80 mg as zinc oxide) showed no difference in the primary outcome of progression to late AMD or adverse effects. Non inferiority analyses were not considered because of insufficient statistical power. Therefore, it was not possible to conclude that the 2 doses were similar in effect. Because of data from the original AREDS, which was a placebo-controlled trial demonstrating the beneficial effects of 80 mg of zinc, it was decided that AREDS2 supplement would continue to maintain 80 mg of zinc *[Chew, 2017].*

Thus, clinicians and consumers have rapidly adopted these AREDS nutritional recommendations *[Gorusupudi et al., 2017]* which remain the most effective treatment currently available to slow the progression to advance AMD (Table 14). It is possible that, when used in tandem with other dietary antioxidants, notably B group vitamins, future supplementation therapies may be even more effective, though further research on this topic is needed *[Broadhead et al., 2015].*

Table 14 : Treatment recommendations for patients with macular degeneration

AMD category	Recommendations
Normal/early AMD (AREDS grades 1 and 2)	Consume a diet high in green leafy vegetables. Attempt to restrict consumption of unsaturated fats and high-glycemic index foods. Increase fish intake.
Intermediate AMD (AREDS grade 3)	Use AREDS-based supplements without β-carotene. Supplements containing zeaxanthin and lutein as β-carotene substitutes should be recommended. Dietary advice per early AMD
Advanced AMD in one eye (AREDS grade 4)	Use AREDS-based supplements without β-carotene. Supplements containing zeaxanthin and lutein as β-carotene substitutes should be recommended. Dietary advice per early AMD

Key messages

- AMD patients who have at least one eye with intermediate disease who take a daily high-dose of vitamin plus zinc supplements (AREDS regimen) are at reduced risk of developing neovascular AMD.
- There was a trend for a beneficial effect of the AREDS supplement on the progression to late atrophic AMD.
- Lutein and zeaxanthin may be more appropriate than β-carotene because of the potential risk of cancer in ex- or current smokers.

5.5 OMEGA-3 FATTY ACIDS

Fatty acids are divided into three broad categories, saturated, monounsaturated and polyunsaturated. Although humans can synthesize saturated and monounsaturated fats, they do not have the enzyme systems required to synthesize PUFAs and therefore dietary sources are essential.

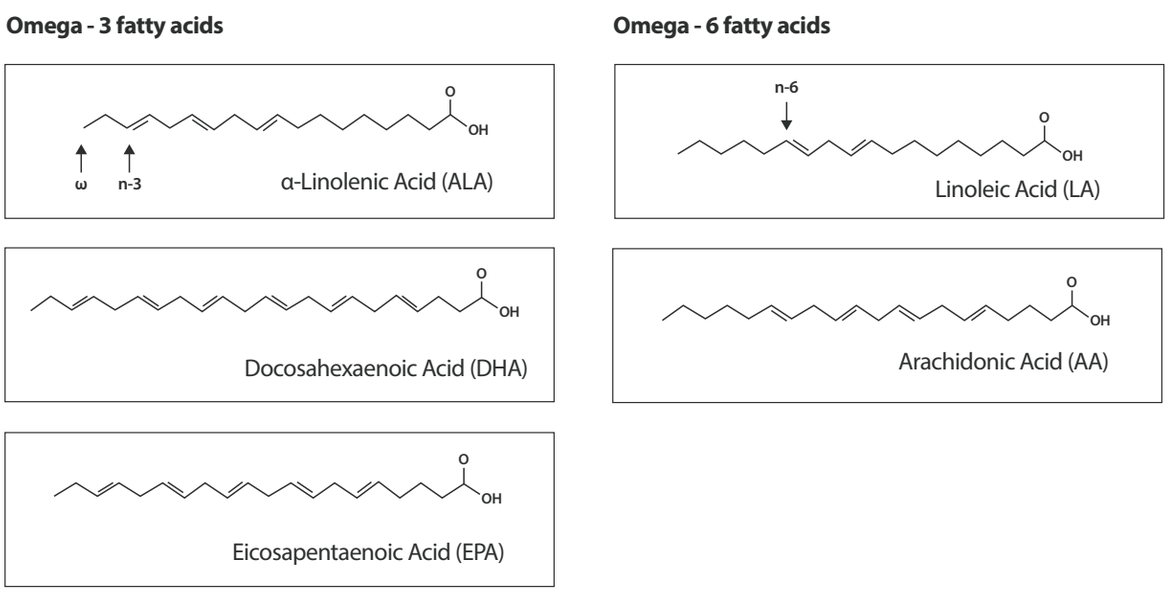
PUFAs are classified according to their chemical structure (Figure 10):

- The omega-3 PUFAs include the short-chain alpha linolenic acid (ALA) and long-chain EPA and DHA.
- The ω-6 PUFA include the short-chain linoleic acid (LA) and long-chain arachidonic acid (AA).

ALA and LA compete for the same biosynthetic enzymes (delta-6-desaturase, elongase and delta-5-desaturase) to generate EPA/DHA and AA, respectively. Thus dietary lipid balance and composition affect production and tissue accretion of these nutrients *[Kishan et al., 2011].*

Increased intake of omega-3 fatty acids, especially long-chain omega-3 fatty acids, such as DHA and EPA, found in fish, has been associated with amelioration of a number of chronic diseases, including AMD *[Schleicher et al., 2013].*

Figure 10 : Chemical structure of the main omega-3 and omega-6 fatty acids



The omega carbon is the methyl end of the carbon backbone of the fatty acid, as depicted. n-3 fatty acids have their final double bond at the n-3 position, whereas n-6 fatty acids have their final double bond at the n-6 position (Adapted from Kishan et al., 2011).

5.5.1 Dietary source of omega-3 fatty acids

Omega-3 fatty acids are obtained principally from dietary sources (Table 15). They can also be synthesized from ALA, but this synthesis is very limited in humans. Deep-sea fish, such as salmon (1.5 g) and sardines (1.3 g) contain a high concentration of DHA and EPA. Other excellent sources of omega-3 fatty acids include flaxseeds (3.2 g) and walnuts (2.25 g). The ideal intake of ω-6 to omega-3 fats is modeled by the Mediterranean diet at 4:1. Intakes of the omega-3 fats in the typical American diet reach 10-20:1 *[McCusker et al., 2016]*.

Although a diet rich in oily fish, eggs, nuts and particular vegetable oils provides a plentiful supply of omega-3 fatty acids, dietary intakes of omega-3 PUFA remain below the recommendations (1.0% of the total energy intake for ALA and 250 mg/d each for DHA and EPA) in the French elderly population, as in other industrialized countries *[Merle et al., 2013]*. Thus, there has been a great deal of interest in the health benefits of omega-3 supplementation, and commercially available supplements in the form of oils and capsules are widely available. Capsules typically contain a mixture of DHA and its precursor EPA, often in combination with antioxidant vitamins and minerals *[Lawrenson & Evans, 2015]*.

Table 15 : Dietary source of omega-3 fatty acids

Fish	DHA (g/100g)	EPA (g/100g)	DHA+EPA (g/100g)	DHA:EPA
Tuna (bluefin)	1.141	0.363	1.504	3.1 : 1.0
Tuna (light, canned in water)	0.223	0.047	0.270	4.8 : 1.0
Tuna (albacore, canned in water)	0.629	0.233	0.862	2.7 : 1.0
Salmon (Atlantic, farmed)	1.457	0.690	2.147	2.1 : 1.0
Salmon (Atlantic, wild)	1.429	0.411	1.840	3.5 : 1.0
Salmon (Chinnook)	0.727	1.010	1.737	1.0 : 1.4
Salmon (Rockeye)	0.700	0.530	1.230	1.3 : 1.0
Mackerel (Atlantic)	0.699	0.504	1.203	1.4 : 1.0
Herring (Atlantic)	1.105	0.909	2.014	1.2 : 1.0
Trout (rainbow, farmed)	0.820	0.334	1.154	2.5 : 1.0
Trout (rainbow, wild)	0.520	0.468	0.988	1.1 : 1.0
Halibut	0.374	0.091	0.465	4.1 : 1.0
Cod	0.154	0.004	0.158	38.5 : 1.0
Haddock	0.162	0.076	0.238	2.1 : 1.0
Shrimp	0.144	0.171	0.315	1.0 : 1.2

5.5.2 Biological rationale for omega-3 supplementation in AMD

There is strong biologic plausibility for an association of DHA and EPA intake with AMD and multiple mechanisms have been described. They demonstrate anti-angiogenic, anti-vasoproliferative, and neuroprotective actions on factors and processes implicated in the pathogenesis of proliferative and degenerative retinal diseases *[San Giovanni & Chew, 2005]*.

- DHA reaches its highest concentration in the membranes of photoreceptors and is important in photoreceptor differentiation and survival as well as in retinal function *[San Giovanni & Chew, 2005]*. DHA accounts for 50% to 60% of the total fatty acid content of the outer segments of photoreceptors. The constant turnover of outer segment membranes requires a continuous dietary supply of DHA, or its precursors, and a deficiency may predispose a person to the development of AMD *[Lawrenson & Evans, 2015]*. As a result of its biophysical and biochemical properties, DHA may affect the permeability, fluidity, thickness, and lipid phase properties of the photoreceptor membrane, and may also be involved in signaling cascades, acting to enhance activation of membrane-bound retinal proteins, and may be involved in rhodopsin regeneration. There is evidence that tissue DHA insufficiency is associated with changes in retinal function *[Querques & Souied, 2014b]*.

- DHA and EPA could affect AMD occurrence by modulating inflammatory and immune processes thought to play a role in AMD pathogenesis. One protective effect of omega-3 PUFA may be mediated by inhibition of phospholipase A2 (PLA2) activity as shown in nerve growth cones of nerve growth factor-differentiated PC12 cells *[San Giovanni & Chew, 2005]*. PLA2 is an enzyme activated in response to ischemia, light exposure, oxidative stress, apoptosis, inflammation, cell signaling molecules, and developmental processes associated with aging. This enzyme preferentially cleaves AA, leading to pro-inflammatory and pro-angiogenic mediators *[San Giovanni & Chew, 2005]*.

- Dietary omega-3 fatty acids have been shown to reduce the inflammatory response by competing with AA metabolism and altering the eicosanoid profile *[Querques & Souied, 2014b]*. Higher intake of omega-3 fatty acids is believed to reduce the production of AA-derived eicosanoids which are generally pro-inflammatory (Leukotriene B4 *[LTB4]*, prostaglandin E2 *[PGE2]*), and increases levels of EPA-derived eicosanoids which are 10 to 100 fold less active *[Christen et al., 2011, Kishan et al., 2011]*. The potential for DHA or EPA to modulate production of AA-derived eicosanoids is also important because LTB4 is associated with TNFα production, known to mediate production of a number of potent proinflammatory and immunoregulatory cytokines.

- In a mouse model of AMD-like retinal lesions, progression of the disease was slowed, and in some cases reversed, in a group of mice fed on a diet rich in DHA and EPA. The protective effect of omega-3 long-chain PUFA was associated with a reduction in pro-inflammatory mediators and an increase in the levels of anti-inflammatory metabolites. An EPA-rich diet has also been shown to suppress experimental choroidal neovascularization (CNV) and CNV-related inflammatory molecules both *in vitro* and *in vivo* *[Lawrenson & Evans, 2015].*

- *In vitro* and ex vivo studies from humans and rodents have established that EPA and/or DHA suppress the activation of CD4+ T cells in response to an activating stimulus and cause the inflammatory environment from a pro-inflammatory Th1 to an anti-inflammatory Th2 phenotype *[Shaikh et al., 2012].*

- Experimental research demonstrates that EPA and DHA have the potential to affect AA-derived eicosanoids implicated in abnormal retinal neovascularization, vascular permeability, and inflammation. EPA also depresses VEGF-specific tyrosine kinase receptor activation and expression *[San Giovanni & Chew, 2005].*

5.5.3 Consumption of fish and foods rich in omega-3 and risk of AMD

There is evidence from animal models and observational studies in humans that there is an inverse relationship between dietary intake of omega-3 long-chain PUFA and risk of developing AMD or progression to advanced AMD *[Lawrenson & Evans, 2015].* Both protection against early AMD *[Tan et al., 2009; Chong et al., 2009]* and advanced AMD (including geographic AMD and exudative AMD) *[Reynolds et al., 2013]* have been shown in participants with high omega-3 long-chain PUFAs (EPA/DHA). In addition, there is increasing evidence of a benefit from regular dietary fish and omega-3 PUFA intake on the risk of AMD particularly in people with a lower ratio of ω-6 to omega-3 PUFAs *[Tan et al., 2009].* Some studies also suggested possible gene-nutrient interactions *[Ho et al., 2011, Reynolds et al., 2013].*

The benefit of omega-3 PUFA on the risk of AMD was demonstrated in cross-sectional studies including case-control studies, and in prospective studies where the progression to early or advanced AMD was determined after a follow-up of 5-15 years. One major limitation of all these studies is also the determination of dietary levels of omega-3 PUFA using food questionnaires filled and there are concerns about dietary changes during the follow-up *[Chiu, et al. 2009].*

Case-control studies

- The Eye Disease Case Control Study (EDCC), which consisted of 349 cases and 504 controls, found that, in subjects with a low linoleic acid (ω-6 fatty acid) intake, there was a trend of retinal protection in those with higher intake of omega-3 fatty acids (P = 0.05). Without adjusting for ω-6 intake, this trend became non-significant (p = 0.29). This trend suggests that ω-6 and omega-3 fatty acids may be in a state of metabolic competition. When comparing those with the highest and lowest EPA and DHA consumption, EPA and DHA did not confer significant protection from neovascular AMD before adjusting for linoleic acid intake (OR = 0.75; 95% CI: 0.44, 1.25) or in those with low (OR = 0.61; 95% CI: 0.26-1.42) or high linoleic acid intake (OR = 0.78; 95% CI: 0.39-1.56) *[Seddon et al., 2001; Schleicher et al., 2013]*.

- In another case-control study, in 1024 patients with exudative AMD and 275 controls, use of cooking oils rich in omega-3 fatty acids was significantly associated with a reduced risk of exudative AMD (OR=0.55, 95% CI: 0.36-0.84, P=0.006), as well as a high consumption of fruits (OR=0.60, 95% CI: 0.37-0.98, P=0.04), but not the consumption of fish, vegetables or oils rich in ω-6 *[Zerbib et al., 2014]*.

Cross-sectional studies

- In EUREYE (European Eye Study) involving 2277 participants aged ≥ 65 years, it was found that eating oily fish more than once weekly decreased the odds of neovascular AMD by two (OR=0.50; 95%CI: 0.28-0.88; P=0.02). A further reduction was seen with greater weekly consumption, but results were not statistically significant. Those with consumption levels of DHA (OR = 0.32; 95% CI: 0.12-0.87) and EPA (OR = 0.29, 95% CI: 0.11-0.73) in the highest quartile had a reduced risk of neovascular AMD *[Augood et al., 2008]*.

- The US Twin study found that compared to those consuming the least amount of omega-3 fatty acids, those consuming the highest amount had a reduced risk for any stage of AMD (OR=0.55; 95%CI: 0.32-0.95). This association was driven mostly by those with a low linoleic and ω-6 fatty acid intake (p < 0.001), as the association disappeared in those with an intake of linoleic acid above the median. Reduction in risk of AMD with higher intake of omega-3 fatty acids was seen primarily among subjects with low levels (below median) of linoleic acid intake, an ω-6 fatty acid (P for trend=0.001). The preventive fraction was 22% for higher omega-3 intake *[Seddon et al., 2006b]*.

Prospective studies

- Analysis of 6339 participants from the Melbourne Collaborative Cohort indicated that those consuming the highest amounts of omega-3 fatty acids were at a slightly reduced risk for early AMD (OR=0.85; 95% CI: 0.71-1.02; P=0.03, for trend) after a mean follow-up of 13 years, but there was no association between particular fatty acids, such as EPA, DHA and ALA, and early or late AMD *[Chong et al., 2009]*.

- In the Blue Mountains Eye Study, weekly consumption of fish was associated with a significant risk reduction in the development of early AMD (RR=0.69, 95% CI: 0.49-0.98). Compared to those with the lowest intakes of omega-3 fatty acids, those with the highest intakes were at reduced risk for incidence of early AMD (RR = 0.63; 95% CI: 0.42-0.95) Smoking, hypertension, diabetes or intakes of lutein and zeaxanthin did not significantly affect this observation *[Tan et al., 2009]*. It was concluded that protection against early AMD may be obtained from regularly eating fish, greater consumption of omega-3 PUFA, and low intakes of foods rich in linoleic acid.

- In the Rotterdam study, participants with the highest EPA+DHA intake had reduced risk of progression to early AMD if they had the ARMS2/HTRA1 homozygotous risk phenotype. Carriers of LOC387715 A69S with the highest intake of EPA/DHA reduced their risk from 1.59 to 0.95 (P trends <0.05). In addition, homozygotes of CFH Y402H with dietary intake of EPA/DHA in the highest tertile reduced their hazard ratio of early AMD 1.97 to 1.30, thus attenuating the genetic risk *[Ho et al., 2011]*.

- In the ALIENOR study, a population-based epidemiologic study in France, high intake of long-chain omega-3 PUFA was associated with a reduced risk of early AMD (OR=0.83, 95% CI 0.71-0.98, p = 0.03), neovascular AMD (OR=0.26, 95%CI: 0.08-0.83, p < 0.02), atrophic AMD (OR=0.74, 95%CI: 0.52-1.06, p = 0.10) and late AMD (OR=0.59, 95%CI 0.39-0.88, p = 0.01) *[Merle et al., 2011]*. Measurement of plasma concentrations of omega-3 PUFA (considered as a biomarker of dietary intake) showed that high plasma total omega-3 PUFA was associated with a 38% reduction of the odds of late AMD (OR=0.62 for 1-SD increase, 95%CI: 0.44-0.88, p = 0.008) *[Merle et al., 2013]*.

- In a large prospective cohort of 38,022 female health professionals (Women's Health Study), regular consumption of DHA and EPA and fish was associated with a 35%-45% lower risk of visually-significant AMD during 10 years of follow-up. The study population was comprised of women without a prior diagnosis of AMD, and the large majority of cases documented during follow-up were characterized by some combination of drusen and RPE changes signifying an early stage of disease development. Thus, these findings suggest that dietary intake of DHA and EPA and fish may be beneficial in the primary prevention of AMD. In this study, the ratio of ω-6/ omega-3 fatty acids was directly associated with risk for AMD.

The inverse relation between DHA and EPA intake and AMD appeared stronger among participants with intake of linoleic acid above, as opposed to below, the median level although the tests of interaction were not significant *[Christen et al., 2011]*. This supports the conclusion that both the level of omega-3 fatty acids and its ratio to ω-6 fatty acids are important in determining risks of AMD.

- In 4,519 AREDS participants, compared to those in the lowest quintile of intake, those in the highest quintile of intake for EPA (OR = 0.72; 95% CI: 0.51-1.01; $p < 0.05$), DHA (OR = 0.60; 95% CI: 0.42-0.85) and total long-chain omega-3 fatty acids (OR = 0.63; 95%CI: 0.45-0.89) were at a reduced risk for neovascular AMD. However, subgroup analysis of the population found that the reduction of risk associated with high consumption of EPA and DHA became non-significant when the cohort was separated by intake of arachidonic acid, another omega-6 fatty acid *[San Giovanni et al., 2007]*.
- Analysis of 1,837 AREDS participants with moderate risk to develop sight-threatening AMD found that increasing intake of DHA + EPA was associated with a decreased rate of progression to central GA over 12 years (P=0.026), and progression to central GA was lowest in subjects with intakes in the highest quintiles of intake of DHA (OR=0.68; 95% CI: 0.47-0.99), EPA (OR=0.70; 95% CI: 0.49-1.00) and DHA + EPA (OR=0.66; 95% CI: 0.46-0.94). Increasing intakes of DHA alone (P=0.001) or DHA + EPA (P=0.032) were also associated with a decrease in risk of progression to neovascular AMD (P=0.001) *[SanGiovanni et al., 2009a]*. This analysis suggests that patients with a moderate to high risk of progressing to late AMD who reported the highest consumption of omega-3 PUFA were 30% less likely to develop GA and neovascular AMD when compared to those reporting the lowest consumption *[San Giovanni et al., 2009a]*.
- Analysis of 2,924 AREDS participants revealed that those consuming more than 64 mg/day of DHA, compared to less than 26 mg/day, had a reduced risk for progression to advanced AMD (HR= 0.73; 95% CI: 0.57-0.94). Those consuming at least 42.3 mg/day EPA, compared to less than 12.7 mg/day, were at a reduced risk for progression to advanced AMD (HR = 0.74; 95% CI: 0.57-0.94). Participants who were healthy at baseline also benefitted from a high DHA diet, as indicated by reduced progression of early AMD (HR = 0.58; 95% CI: 0.37-0.92) *[Chiu et al., 2009]*.
- Another analysis in 2,531 AREDS participants without advanced AMD at baseline was performed to investigate the associations between dietary omega-3 fatty acids and genes related to AMD. It was shown that a higher intake of DHA was associated with reduced risk of progression to GA, controlling for known genetic variants associated with AMD. Increased DHA intake also significantly reduced risk of progression among individuals with the ARMS2/HTRA1 homozygous risk genotype, but not the non-risk ARMS2 genotype, with a suggestive interaction between DHA intake and ARMS2/HTRA1 *[Reynolds et al., 2013]*.

5.5.4 Effect of omega-3 supplementation in clinical trials

Since the concentration of retinal long-chain omega-3 PUFAs is modifiable by and dependent on dietary composition, it was suggested that these nutrients could be an easily implemented approach to modifying risk of AMD progression *[San Giovanni et al., 2009b].*

Nutritional AMD Treatment 2 [NAT-2] study

In NAT-2, patients were given either 840 mg/day DHA and 270 mg/day EPA (as fish oil capsule) or placebo (olive oil capsule) for 3 years. For patients that showed the highest levels of EPA + DHA in red blood cell (RBC) membranes, there was a 68% lower risk for choroidal neovascularization (CNV), but not for other indicators of AMD status. For the full cohort of patients, supplementation with DHA and EPA was without effect relative to placebo *[Souied, 2013].* However, in the DHA group, patients steadily achieving the highest tertile of EPA/DHA levels in RBC membrane had significantly lower risk (-68%; P=0.047; HR = 0.32, 95% CI: 0.10-0.99) of developing CNV over 3 years.

The AREDS 2 Study

AREDS2 was a five-year study (starting in 2006) designed to test whether the original AREDS formulation could be improved by adding omega-3 fatty acids; adding lutein and zeaxanthin; removing ω-carotene; or reducing zinc. In AREDS2, participants took one of four AREDS formulations: 1) the original AREDS formulation, 2) the AREDS formulation with no ω-carotene, 3) AREDS with low zinc, 4) AREDS with no ω-carotene and low zinc. In addition, they took one of four additional supplement or combinations including L and Z, DHA/EPA (350/650 mg), L/Z and DHA/EPA, or placebo. The study reported that there was no overall additional benefit from adding omega-3 fatty acids or lutein and zeaxanthin to the formulation.

This result was unexpected and could be explained by the study design and methodological consideration. In AREDS2, control subjects received a nutritional formula already found to be effective in AREDS1, but no placebo for DHA/EPA supplementation, contrary to NAT-2 study which included a true placebo group *[Souied et al., 2015]*. Thus caution should be taken in closing the chapter on omega-3 and instead more detailed exploration are required.

Meta-analysis of interventional studies

Based on these two randomized placebo-controlled trials (AREDS2 and NAT-2), a meta-analysis in 2015 showed that there is still no evidence that omega-3 long-chain PUFA supplementation for periods up to 5 years reduces the risk of progression to advanced AMD or the development of moderate to severe visual loss *[Lawrenson & Evans, 2015]*. Thus,

despite the epidemiologic data support, the beneficial role of consuming omega-3 fatty acids in fish, oral supplementation with DHA+EPA in persons with at least intermediate AMD did not have a beneficial or harmful effect *[Chew, 2017]*. Since, there is no published clinical trial investigating the dietary omega-3 fatty acids supplementation (either by eating more foods rich in omega-3 or by taking nutritional supplements) for prevention of AMD, this remains to be determined.

Key messages

- There is compelling evidence that omega-3 fatty acids (DHA/ EPA) play an important role in modulating inflammatory and immune processes.

- Omega-3 fatty acids demonstrate anti-angiogenic, anti-vasoproliferative, and neuroprotective actions on factors and processes implicated in the pathogenesis of proliferative and degenerative retinal diseases.

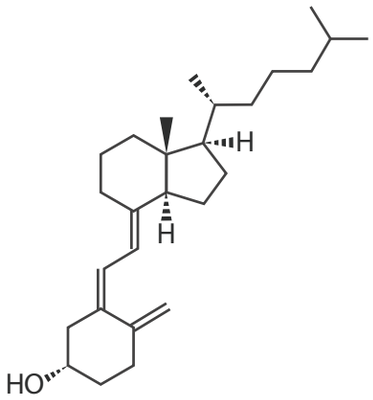
- There is evidence from animal models and observational studies in humans that there is an inverse relationship between dietary intake of omega-3 long-chain PUFA and risk of developing AMD or progression to advanced AMD.

- Omega-3 fatty acids supplementation (either by eating more foods rich in omega-3 or by taking nutritional supplements) is an interesting approach for prevention of AMD, but this remains to be confirmed in clinical studies.

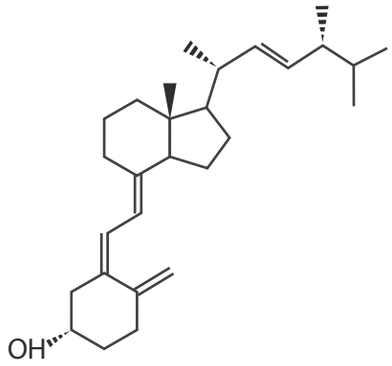
5.6 VITAMIN D

There are two main forms of vitamin D which differ by their side chains on the sterol skeleton (Figure 11). Vitamin D3 (cholecalciferol) is produced in the skin after sun exposure by the conversion of 7-dehydrocholesterol (7-DHC), while vitamin D2 (ergocalciferol) is provided only by foods. Vitamin D without a subscript relates to either or both vitamin D2 or vitamin D3 and its metabolites.

Figure 11 : Molecular structure of vitamin D2 and D3



Vitamin D3 (Cholecalciferol)



Vitamin D2 (Ergocalciferol)

Vitamin D is well known for its major role in bone mineral homeostasis by promoting the transport of calcium and phosphate, ensuring adequate bone mineralization. However, for about two decades, compelling evidence has been collected showing that vitamin D could play a role in acute and chronic diseases including: respiratory infections, cardiovascular diseases, diabetes, skin diseases and cancers *[Hossein-nezhad & Holick, 2013a, Bikle, 2014]*, and more recently eye diseases including AMD *[Morrison et al., 2011].*

5.6.1 Main dietary source of vitamin D

Very few foods naturally contain vitamin D. The major sources are wild caught salmon, other oily fish, cod liver oil and mushrooms which have enhanced vitamin D when exposed to UVB radiation (Table 16). In some countries, some foods are fortified with vitamin D including milk and some other dairy products including yogurt and some cheeses, cereals and some juices *[Holick et al., 2011].* Foods that are fortified with vitamin D are often inadequate to satisfy vitamin D requirement. Diets rich in oily fish are known

to prevent vitamin D deficiency *[Holick, 2007]*. The IOM in USA considered RDAs of 600 IU/day (15 µg/day) for ages 1-70 years and 800 IU/day (20 µg/day) for age above 70 years *[NIH, Vitamin D Fact Sheet 2019]*.

Table 16 : Dietary source of vitamin D

Source	Amount	Vitamin D content*	
Salmon (Fresh, wild)	100 g	600 – 1000 IU	i.e. 15 - 25 µg
Salmon (Fresh, farmed)	100 g	100 – 250 IU	i.e. 2.5 - 6.25 µg
Salmon (canned)	100 g	300 – 600 IU	i.e. 7.5 - 15 µg
Sardines (canned)	100 g	300 IU	i.e. 7.5 µg
Mackerel (canned)	100 g	250 IU	i.e. 6.25 µg
Tuna (canned)	100 g	230 IU	i.e. 5.75 µg
Cod liver oil	1 teaspoon	400 – 1000 IU	i.e. 10 - 25 µg
Shiitake mushrooms (Fresh)	100 g	100 IU	i.e. 2.5 µg
Shiitake mushrooms (Sun-dried)	100 g	1600 IU	i.e. 40 µg
Egg yolk	-	20 IU	i.e. 0.5 µg

** 1 international Unit (IU) = 25 ng; Adapted from Holick MF, 2007*

According to a meta-analysis of 55,844 individuals of 18 nutritional and health surveys from various European Union countries, 40% of adults in the European Union have insufficient status in vitamin D and 13% are deficient in vitamin D *[Cashman et al., 2016]*. In the general population, increasing age, female sex, non-white race, diabetes, current smoking and a high BMI, are independently associated with an increased odds of being deficient in vitamin D *[Melamed et al., 2008]*. Elderly individuals are at risk of vitamin D deficiency because of poor dietary vitamin D intake, reduced mobility, decreased exposure to sunlight (because of reduced outdoor activity) and decline in renal function *[Noguchi et al, 2013]*. Capacity of skin to produce vitamin D is also decreased in the elderly and by 70 years of age vitamin D synthesis is reduced approximately by 75% *[Holick, 2007]*. Vitamin D deficiency is in part due to the inadequate fortification of foods with vitamin D and the misconception that a healthy diet contains an adequate amount of vitamin D *[Holick, 2014]*.

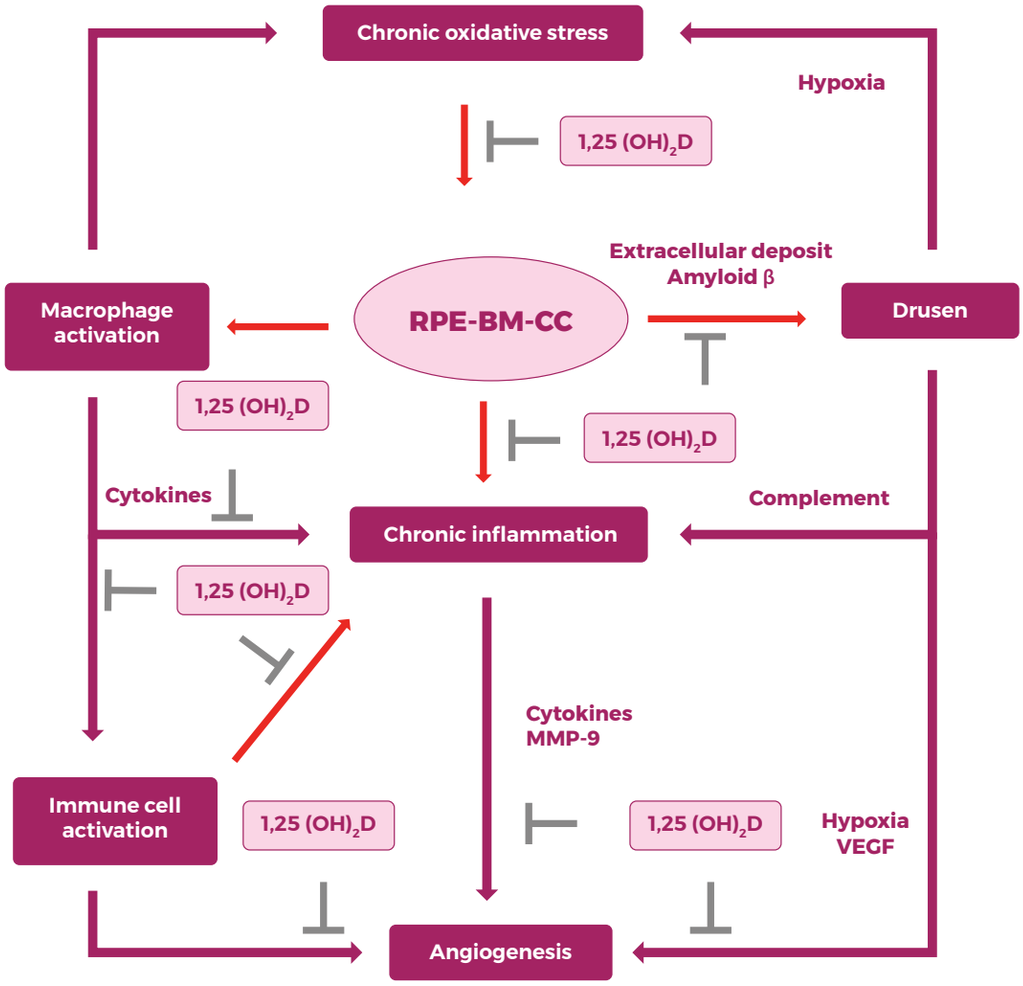
5.6.2 Biological rationale for vitamin D supplementation in AMD

Vitamin D, produced by the skin or supplied by food, is biologically inert and requires two subsequent hydroxylation processes in the liver and kidneys to produce active metabolites: 25(OH)D (25-hydroxy vitamin D) is the major circulating form of vitamin D and is used by clinicians to determine Vitamin D status *[Layana et al., 2017].* 25(OH)D is converted to the active metabolite 1,25(OH)2D through the action of the enzyme 1-alpha-hydroxylase (CYP27B1) located in the proximal tubule of the kidney, although other tissues have 1-alpha-hydroxylase enzymatic activity. The degradation of 25(OH) D and 1,25(OH)2D to inert metabolites is initiated by a 24-hydroxylase (CYP24A1). As for steroid hormones, the biological effects of vitamin D are mediated by binding to a specific vitamin D receptor (VDR). When activated, the VDR acts as a transcriptional factor *[Bikle, 2014; Haussler et al., 2011]* and may directly or indirectly control 200 to 2000 genes in various tissues and cells *[Hossein-nezhad & Holick, 2013a]*. It was recently demonstrated that vitamin D3 supplementation (400 to 2000 IU/day for 8 weeks) was associated with related alterations of 291 genes, including 17 genes known to play important roles in transcriptional regulation, immune function, apoptosis, and responses to stress *[Hossein-nezhad et al., 2013b].*

A possible physiological role of vitamin D in the retina is supported by evidence that the VDR and the enzymes involved in the metabolism of vitamin D (CYP27B1 and CYP24A1) are expressed in the retina, RPE and choroidal endothelial cells *[Layana et al., 2017].*

The protective effects of vitamin D to prevent the development of early and/or late AMD, are based on experimental *in vitro* and *in vivo* studies, and include actions on pathogenic steps centered on oxidation, inflammation and angiogenesis *[Layana et al., 2017],* as illustrated in Figure 12. Vitamin D may prevent the risk of developing early and intermediate AMD, by inhibiting oxidative stress, inhibiting extracellular amyloid deposits and inhibiting macrophage activation. Vitamin D may also reduce the risk or slow the development of neovascular AMD by inhibiting angiogenesis or immune cell activation.

Figure 12 : Main biological mechanisms in AMD and putative vitamin D effects



Inhibitory activities of vitamin D are indicated in green truncated arrows and mechanisms of AMD pathophysiology in red arrows. RPE: Retinal pigmentary epitelium; BM: Bruch's membrane; CC: Chorio capillaries

Inhibition of chronic oxidative stress

Vitamin D has been shown to be protective against oxidative stress including serum starvation, hypoxia, oxidative stress, and apoptosis induction in various cell lines and animal models *[Peng et al., 2010; Diker-Cohen et al., 2006]*. In a mouse cone cell line (661W), vitamin D (1,25(OH)2D) was shown to decrease the generation of ROS in H2O2-stimulated cells, by modulating the expression of antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase) *[Tohari et al., 2016]*.

Inhibition of amyloid beta protein deposits

Amyloid beta protein (Aβ) is considered a primary activator of the complement cascade and inflammation *[Akiyama et al., 2000]*. Vitamin D has been recently associated with the clearance of Aβ deposits and the improvement of retinal function in aged mice treated with subcutaneous injections of vitamin D. The proposed mechanism involves the activation of macrophage phagocytosis of Aβ deposits by 1,25(OH)2D, and their removal from the Bruch's membrane *[Lee et al., 2012]*.

Inhibition of chronic inflammation

Cells of the myeloid lineage are known to produce CYP27B1 hydroxylase, leading to local production of 1,25(OH)2D. Activated macrophages express the VDR and are thus vitamin D responsive cells. One major effect of vitamin D on activated macrophages is potent suppression of pro-inflammatory events mediated by interferon-gamma (INF-γ) *[Helming et al., 2005]*. Vitamin D has been shown to suppress proinflammatory cytokines, in part by altering T-cell function toward a Th2 (anti-inflammatory) rather than a Th1 (pro-inflammatory) response *[Hewison, 2010]*. As reviewed by Prietl et al. *[Prietl el al., 2013]*, treatment of T cells with 1,25(OH)2D or its analogs, inhibits the secretion of proinflammatory Th1 (Interleukin [IL]-2, INF-γ, TNF-α), Th9 (IL-9) and Th22 (IL-22) cytokines, but promotes the production of more anti-inflammatory Th2 cytokines (IL-3, IL-4, IL-5, IL-10). The production of IL-17 produced by Th17 cells is also inhibited by vitamin D *[Joshi et al., 2011]*.

The addition of vitamin D to Nutrof®total *[See Patient information leaflet in Annexes]* containing antioxidants, macular pigments (L and Z), and omega-3 was shown to maintain a significant reduction in oxidative stress related adverse events. It was concluded that vitamin D contributed to a novel synergistic action especially on the reduction of pro-inflammatory markers (especially IL-12 and INF-γ) expression under inflammatory conditions *[Recalde et al., Poster, 2018]*.

Vitamin D and Angiogenesis in AMD

As demonstrated in tumor cells, vitamin D is a potent inhibitor of angiogenesis due to its effects on endothelial cells and interruption of the angiogenesis signaling pathway *[Majewski et al., 1996]*. As demonstrated by Mantel et al., Vitamin D inhibits VEGF-induced endothelial cell sprouting and elongation and also has a small, but significant, inhibitory effect on VEGF-induced endothelial cell proliferation *[Mantel et al., 2000]*. In experiments carried out by Albert et al. using a mouse model of oxygen-induced ischemic retinopathy, animals treated with 1,25(OH)2D had significant decreases in retinal neovascularization relative to control animals, although the levels of ocular VEGF were similar in treated and control animals *[Albert, 2007]*. A possible mechanism may include induction of endothelial cell apoptosis *[Mantel et al., 2000; Albert et al., 2007]*.

5.6.3 Relationship between vitamin D status and AMD

The association between vitamin D and AMD is a relatively new investigational field. Cross-sectional studies conducted over the past decade, to investigate the relationship between vitamin D status and AMD risk, have led to controversial results *[Layana et al., 2017]*.

Association between vitamin D and early AMD

- Parekh et al. were the first to suggest a role of vitamin D in AMD. In a cross-sectional study involving subjects of the National Health and Nutrition Examination Survey (NHANES) III, they showed a significant correlation between reduced serum vitamin D levels and prevalent early AMD *[Parekh et al., 2007].* In analyses including more than 7,700 participants (mean age: 57 years; 56% female), the OR for early AMD, after adjustment for age and smoking (plasma cotinine levels), was 0.64 (95% CI: 0.5-0.8; P <0.001) among participants with the highest level of serum 25(OH)D (above 34 ng/mL) compared to the participants with the lowest level (below 17 ng/mL). The association with soft drusen was also statistically significant (adjusted OR: 0.76; 95% CI: 0.60-0.96; P=0.006). However, there were no associations between vitamin D serum levels and pigmentary abnormalities or advanced AMD, possibly due to the small sample size (185 and 54 patients, respectively).

- In another cross-sectional study *[Millen et al., 2011]*, Millen et al. reported a significant association between vitamin D status and prevalent early AMD in 1,313 post-menopausal women (50-79 years old; (823 early AMD and 54 late AMD). However, this association was modified by age with a strong interaction (P=0.0025). In the multivariate models adjusting for AMD risk factors, women <75 years (N=968) with the highest vitamin D concentrations (above 75 nmol/L) had 48% decreased odds of early AMD. Similarly, the odds of pigmentary abnormalities were 57% lower (OR: 0.43; 95% CI: 0.18–0.96; P=0.02; for quintile 5 vs. 1). Results from this study suggest a protective effect of vitamin D in early AMD for post-menopausal women aged < 75 years.

Association between vitamin D and advanced AMD

- An inverse relationship between blood vitamin D (25(OH)D) levels and the risk of prevalent AMD was suggested by several observational studies.

- In a single center, retrospective, case-control study, Itty et al. compared the vitamin D status among patients (mean age: 79.5 years; 78% female) with neovascular AMD (NVAMD), non neovascular AMD (NNVAMD), and control subjects *[Itty et al., 2014]*. They also found that the highest quintile (> 40 ng/mL) of blood vitamin D levels was associated with a 65% decreased likelihood (OR=0.35; 95% CI: 0.18-0.68) of NVAMD compared to the lowest quintile (<18 ng/mL).

- In a national cross-sectional Korean study conducted on 17,045 participants aged 40 years and older (mean age: 61.6 years; 48% male), Kim et al. showed that high levels of vitamin D (25(OH)D) in the blood were inversely associated with late AMD in men, but not in women *[Kim, 2014]*. In men, the risk of late AMD was significantly decreased in the highest quintile of vitamin D (> 24.3 ng/mL) with an OR of 0.32 (95% CI: 0.12-0.81; P for trend=0.018) compared with the lowest quintile (<13.1 ng/mL) after adjusting for potential confounders, including age, smoking status, hypertension, heart problems, stroke and sunlight-exposure time. In this study, early AMD was associated with blood 25(OH)D levels only in the unadjusted model.

- A meta-analysis of 11 observational studies performed in 2016 *[Annweiler et al., 2016]* indicated that low circulating levels of 25(OH)D (<50 nmol/L; 20 ng/mL) were significantly associated with late AMD with an OR of 2.18 (95% CI: 1.34–3.56). However, the association was not statistically significant in early AMD. These results were not confirmed by another meta-analysis performed in 2016 *[Wu et al., 2016]*. Thus, further studies are needed to confirm the link between vitamin D status and AMD risk.

- Recently, an interaction between vitamin D status and SNPs in the CFH (Y402H) and complement factor I (CFI) genes has been suggested *[Millen et al., 2015]*. Overall, compared to subjects with adequate blood vitamin D levels (i.e. ≥ 30 ng/mL), there was a 2.6-fold (95% CI: 1.3-5.2) increased likelihood of AMD in vitamin D deficient subjects (i.e. < 12 ng/mL), a 3.4-fold (95% CI: 1.1-10.9) increased likelihood in subjects carrying one risk allele for CFH Y402H versus 6.7-fold (95% CI: 1.6-28.2) increased likelihood in subjects carrying two risk alleles. Similar trends were found with a CFI SNP.

5.6.4 Vitamin D supplementation and AMD

To date, interventional studies assessing the effect of vitamin D supplementation in preventing the onset or progression of AMD are lacking. Thus, there are no specific dietary recommendations regarding vitamin D for primary or secondary prevention

of AMD, despite the high prevalence of vitamin D deficiency or insufficiency in the general population *[Layana et al., 2017]*.

However, the relationship between dietary vitamin D and AMD has been investigated in several studies.

- Parekh et al. found that milk intake (fortified in vitamin D in the USA) was inversely associated with early AMD (OR=0.75; 95% CI: 0.6-0.9) and fish intake was inversely associated with advanced AMD (OR=0.41; 95% CI: 0.2-0.9) *[Parekh et al., 2007]*.

- In Caucasian male monozygotic twin pairs with discordant AMD phenotypes, Seddon et al. reported that higher dietary intakes of vitamin D (assessed using a food frequency questionnaire) were present in twins with less severe AMD (P=0.01) and smaller drusen sizes (P=0.05) relative to co-twins – adjusted for smoking and age *[Seddon et al., 2011]* – providing evidence that vitamin D could be involved in the etiology of AMD.

- In a recent Japanese case-control study, patients with neovascular AMD and control subjects, randomly selected from the population, aged ≥ 65 years, were assessed, using a brief-type self-administered questionnaire on diet history. Logistic regression analyses, adjusted for smoking, age, sex, chronic diseases, supplement use, and alcohol consumption, demonstrated that a low intake of vitamin D, together with other nutrients, including n-3 fatty acid, alpha-tocopherol, zinc, vitamin C, and β-carotene, was significantly ($p < 0.001$) associated with neovascular AMD *[Aoki et al., 2016]*.

A recent prospective study looked for the association of vitamin D intake (from food or supplementation) and progression to incident advanced AMD in 2,146 participants with early or intermediate AMD *[Merle et al., 2017]*. After a mean follow-up of 9.4 years (range 1.0 to 24.9 years), there was a 40% lower risk of progression to advanced AMD in the highest versus lowest quintile of vitamin D intake from food (but not from supplement source) after adjustment for demographic, behavioral, ocular and nutritional factors (HR=0.60, 95%CI: 0.43-0.83, P for trend = 0.0007). Similar results were shown for progression to neovascular AMD (HR=0.59, 95%CI: 0.39-0.89, P for trend = 0.005), but not for geographic atrophy. This suggests that a diet rich in vitamin D may prevent or delay the progression to advanced AMD, especially neovascular AMD. In this study, total vitamin D intake (dietary plus supplemental) was not significantly associated with a reduced risk of progression to advanced AMD, although the estimates of effect were in the protective direction. This result could be explained by the fact that vitamin D from food may differ from supplemental vitamin D with regard to its bioavailability, or other correlates of vitamin D which remains to be determined *[Merle et al., 2017]*.

Key messages

- The protective effect of vitamin D on AMD pathogenic steps are centered on oxidation, inflammation and angiogenesis.

- Vitamin D may prevent the risk of developing early and intermediate AMD, by inhibiting oxidative stress, inhibiting extracellular amyloid deposits and inhibiting macrophage activation.

- Vitamin D may also reduce the risk or slow the development of neovascular AMD by inhibiting angiogenesis or immune cell activation.

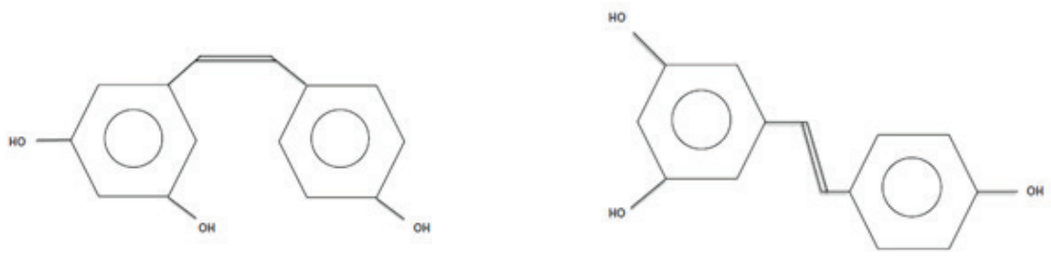
- High serum vitamin D levels have been associated with a lower risk of early and/or late AMD.

- In some studies, vitamin D supplementation was shown to be inversely associated with early or advanced AMD.

5.7 RESVERATROL

Resveratrol (3,5,4'-trihydroxystilbene) is a natural non-flavonoid polyphenol with a stilbene structure produced naturally by several plants in response to injury or when the plant is under attack by pathogens, such as bacteria or fungi *[Bola et al., 2014; Abu-Amero et al., 2016]*. It can be found in the cis or trans configurations (Figure 13). The trans-isomer appears to be the more predominant and stable natural form. Cis-isomerisation can occur when the trans-isoform is exposed to solar, artificial light or ultraviolet radiation *[Gambini et la., 2015]*.

Figure 13 : Resveratrol structure



Cis-resveratrol **Trans-resveratrol**

As reviewed recently by Abu-Amero et al., resveratrol due to its potent antioxidant and anti-inflammatory properties was shown to be cardio-protective, chemotherapeutic, neuroprotective, and display anti-aging effects in clinical studies. Oxidative stress and inflammation play a critical role in the initiation and progression of age-related ocular diseases (glaucoma, cataract, diabetic retinopathy and macular degeneration) that lead to progressive loss of vision and blindness.

Although, there are still no observational studies (cross-sectional or prospective) or interventional studies investigating the protective effect of this nutrient in prevention or progression of AMD, there is cumulative data from *in vitro* and *in vivo* (animal model) experimental studies providing evidence for the biological effects of resveratrol on numerous pathways including oxidative stress, inflammation, mitochondrial dysfunction, apoptosis, pro-survival or angiogenesis that are implicated in the pathogenesis of age-related ocular disorders *[Abu-Amero et al., 2016]*.

5.7.1 Dietary source of resveratrol

The richest source of resveratrol is the Polygonum cuspidatum herb, whose root extract has played a very important role in Japanese and Chinese traditional medicine. Resveratrol is mainly found in red wine but it is also found in some fruits (e.g. blueberries, and blackberries) and peanuts (Table 17). Resveratrol content in red wine comes from grapes, mainly the skin. Red wine is richer in resveratrol than white wine, because during the production of red wine, parts of the grape where resveratrol is concentrated are macerated. This does not happen in white wine. Alcohol formation during grape fermentation facilitates its solubility and thus its extraction *[Gambini et al., 2015].*

Table 17 : Trans-resveratrol concentration estimates in selected sources

Source	Trans-resveratrol
Red wines	0.1-14.3 µg/mL
White wines	<0.1–2.1 µg/mL
Cranberry raw juice	~0.2 µg/mL
Grapes	0.16–3.54 µg/g
Blueberries	up to ~0.032 µg/g
Bilberries	up to ~0.016 µg/g
Peanuts	0.02–1.92 µg/g
Boiled peanuts	5.1 µg/g
100% Natural peanut butters	0.65 µg/g (average)
Polygonum cuspidatum herb	524 µg/g

Ref: Abu-Amero et al., 2016

Absorption, metabolism, and bioavailability of resveratrol have been previously reviewed by Gambini et al. Resveratrol is poorly soluble in water but exhibits lipophilic characteristics, which lead to its absorption. Intestinal resveratrol absorption can vary depending on the kind of food ingested. The bioavailability of resveratrol is determined by its rapid elimination and the fact that its absorption is highly effective, but the first hepatic step leaves little free resveratrol (Low bioavailability of resveratrol is a factor that may reduce the efficacy of resveratrol). Although *in vitro* studies show high efficacy in biologically beneficial effects of resveratrol in cells, it is known that its distribution in tissues is very low. Consequently, *in vitro* studies must be interpreted with caution when trying to extrapolate its effect in *in vivo* studies *[Gambini et al., 2015].*

The concentrations used *in vitro* are too high to be reached in the organism after red wine consumption. However, it is possible to achieve such high concentration of resveratrol in plasma by administering resveratrol supplements and that is how many of the *in vitro* results have been verified in animal tests. Although there is still no recommendation for use of resveratrol in human disease, many nutritional supplements containing resveratrol as a single component or in combination with other nutrients are promoted as being of benefit in long-term eye health *[Bola et al., 2014].*

5.7.2 Biological rationale for resveratrol supplementation in AMD

In vitro and animal studies suggest that resveratrol has the potential to be used in a range of ocular disease, including AMD *[Lançon et al., 2016]*. The major actions of resveratrol on the eye include antioxidant, anti-apoptotic, anti-tumorigenic, anti-inflammatory, anti-angiogenic, and vasorelaxant effects *[Bola et al., 2014].*

Antioxidant effects

It is well established that resveratrol is able to act on various cell types of the eye by increasing the level of natural antioxidant enzymatic and molecular defenses *[Lançon et al., 2016].*

The antioxidant effects of resveratrol contribute substantially to the health benefits of this compound. Resveratrol has been shown to be a scavenger of a number of free radicals. However, the direct scavenging activities of resveratrol are relatively poor. The antioxidant properties of resveratrol *in vivo* are more likely to be attributable to its effect as a gene regulator. Resveratrol inhibits NADPH oxidase-mediated production of ROS by down-regulating the expression and activity of the oxidase. It reduces mitochondrial superoxide generation by stimulating mitochondria biogenesis. In addition, resveratrol increases the expression of various antioxidant enzymes *[Xia et al., 2017]* as shown in RPE cells.

- In human retinal D407 RPE cells, resveratrol was shown to exhibit a dose-dependent protective effect against H_2O_2-induced cytoxicity by increasing superoxide dismutase, glutathione peroxidase, and catalase activities that inhibit the levels of intracellular ROS. Moreover, resveratrol significantly enhanced the level of reduced glutathione under both basal and oxidative stress conditions. The significant inhibition of the intracellular ROS generation supports the hypothesis that resveratrol can also contribute to the antioxidant defense by directly scavenging the ROS in RPE cells *[Pintea et al., 2011]*.

- In an experimental study, King et al. reported that treatment with 50 and 100µmol/L resveratrol significantly reduced proliferation of RPE cells by 10% and 25%, respectively ($P < 0.05$). This reduction in proliferation was not associated with resveratrol-induced cytotoxicity. Resveratrol (100 µmol/L) inhibited basal and H_2O_2-induced intracellular oxidation and protected RPE cells from H2O2-induced cell death. The observed reduction in cell proliferation was associated with inhibition of mitogen activated protein kinase/ERK (MEK) and extracellular signal-regulated kinase (ERK 1/2) activities at concentrations of resveratrol as low as 5 µmol/L. These results suggest that resveratrol can reduce oxidative stress and hyperproliferation of the RPE *[King et al., 2005]*.

- In another study, resveratrol was shown to positively affect mitochondrial biogenesis leading RPE cells protection against acrolein (a major toxicant in cigarette smoke) - induced oxidative cytotoxicity by increasing mitochondrial bioenergetics. In cultured ARPE-19 cells, resveratrol rescued acrolein-induced cell death, and reversed acrolein-induced SOD expression. Resveratrol increased the mitochondrial bioenergetics, including basal respiratory rate, adenosine triphosphate synthesis via oxidative phosphorylation, and maximal mitochondrial capacity. In animal experiments, choroidal neovascularization lesions were created in rats by laser-induced photocoagulation and the effects of cigarette smoke alone or with additional resveratrol treatment on chorio-neovascular lesions were quantified by fundus fluorescein angiography. Cigarette smoke induced a significant increase in CNV following laser injury, and this was prevented following peripheral infusion of resveratrol *[Sheu et al., 2013]*.

- In mice experiments, Kubota et al. showed that resveratrol had a protective effect against light-induced retinal degeneration. It was found that light exposure caused an activation of retinal activating protein-1 (AP-1) and inhibition of surtuin 1 (SIRT 1) (a known regulator of aging which was shown to be activated by resveratrol in other studies *[Howitz et al., 2003]*) activation, both of which were reversed by resveratrol.

- In addition, after exposure to light, a significant reduction in apoptotic cells was detected in the outer nuclear layer (ONL) of the retina in the presence of resveratrol, supporting the potential use of resveratrol as a therapeutic agent to prevent retinal degeneration related to light damage *[Kubota et al., 2010]*.

Anti-inflammatory and anti-angiogenic effects

Resveratrol anti-inflammatory effects are due to its capacity to limit the expression of pro- inflammatory factors, such as interleukins and prostaglandins, and also to decrease the chemo-attraction and recruitment of immune cells to the inflammatory site *[Lançon et al., 2016]*.

Resveratrol has been shown to modulate inflammation, or at least to influence the levels of several inflammatory response markers. As reviewed by Toré-Carneiro et al., significant changes on the level of these markers were reported in various studies performed on rodents when resveratrol was administered in doses ranging from 1 mg/kg up to more than 1 g/kg of body weight per day (human equivalent dose from 11 mg to >5 g for a 70 kg) and exposure times varied from a few days up to 30 weeks). Resveratrol reversed the rise in the levels of important pro-inflammatory cytokines and other inflammation-related markers in several disease-induced animal models, such as obesity, hypertension, diabetes and colon colitis, and exposure to carcinogens. Resveratrol treatment was generally found to reduce TNF-α, IL-1β, IL-6, MCP-1, COX-2, and iNOS. In addition, resveratrol exposure diminished the activity of T- and B-cells, and macrophages, due to a significant inhibition of their proliferation, antibody production, and lymphokines secretion. Moreover, resveratrol inhibited NF-κB, and NF-κB-related inflammatory and autoimmune markers *[Tomé-Carneiro et al., 2013]*. Nevertheless, studies investigating the anti-inflammatory effect of resveratrol in humans led to inconsistent results *[Poulsen et al., 2015]*. This was suggested to be due to differences in the study populations including gender, age, dosage of resveratrol, length of the study, and health status of the participants. Especially, a large difference has been found in the concentration and dosage of resveratrol among the clinical studies. No consensus exists on which concentration of resveratrol may be ideal in relation to anti-inflammatory effects *[Poulsen et al., 2015]*.

- RRPE cells adjacent to drusen deposits in the AMD eye are known to contain CXCL11, a chemokine involved in inflammatory cell recruitment. Kutty et al. investigated CXCL11 production by the human RPE (ARPE-19) cells under inflammatory conditions and tested its response to resveratrol. They found that a pro-inflammatory cytokine mixture consisting of IFN-γ, IL-1β and TNF-α highly increased CXCL11 mRNA expression and CXCL11 protein secretion by ARPE-19 cells. Resveratrol substantially inhibited the pro-inflammatory cytokines-induced CXCL11 production while partially blocking NF-κB activation. This inhibitory

action of resveratrol was also observed for the cytokines-induced expression of chemokines CXCL9, CCL2 and CCL5. This indicates that resveratrol could potentially attenuate RPE inflammatory response implicated in the pathogenesis of AMD *[Kutty et al., 2015]*.

- In another study, Losso et al. demonstrated the ability of resveratrol to inhibit RPE cell inflammation, caused by hyperglycemia *in vitro* *[Losso et al., 2010].* They found that hyperglycemia generated the expression and downstream upregulation of several inflammatory molecules. Treatment of the ARPE-19 cells with resveratrol significantly inhibited the accumulation of these molecules, including VEGF, TGF-β1, COX-2, IL-6 and IL-8 in a dose-dependent manner. Activity of protein kinase Cβ, an enzyme that up-regulates VEGF in hypoxic conditions further contributing to blood-retina barrier degradation, was reduced in the presence of resveratrol. Resveratrol was also found to prevent hyperglycaemic down-regulation of Connexin 43 (Cx43) protein in RPE cells *[Bola et al., 2014]*. Cx43 is abundantly present in the retinal vascular cells, where it maintains intercellular communication and most likely helps maintain the integrity of the blood-retinal barrier *[Losso et al., 2010].* This suggests that resveratrol may enhance gap junction intercellular communication, crucial to the integrity of the blood-retinal barrier.

- Using primary culture of RPE cells prepared from aged human donor eyes, Nagineni et al. showed that resveratrol, in a dose dependent (10-50 μM) manner, significantly suppressed VEGF secretion induced by inflammatory cytokines. In this experimental study, TGF-β and cobalt chloride (hypoxia mimic) upregulated RPE cell production of VEGF, and this was inhibited by resveratrol. In contrast, resveratrol had no effect on anti-angiogenic molecules, endostatin and pigment epithelial derived factor secretion. Thus, it was concluded that resveratrol may be a useful nutraceutical in controlling choroidal neovascularization processes in AMD *(Nagineni et al., 2014).*

- In animal studies, a modified AREDS 2 nutritional supplement containing resveratrol (15 mg), omega-3 fatty acids (190 mg EPA, 95 mg DHA), lutein (5 mg), zeaxanthin (1 mg), copper (0.5 mg), zinc (7.5 mg), selenium, vitamin C (300 mg) and E (15 mg) appeared to decrease early CNV progression in mice with laser-induced lesions. This effect was accompanied by a decrease in VEGF and MMP-9 gene expression and protein activity levels. The oral dose was adapted to each animal body weight and treatment started 10 days before laser application and continued until 4 weeks after laser application. Treatments were administered alone or in combination with intravitreal anti-VEGF administered 48h after laser *[Ivanescu et al., 2015].*

In summary, resveratrol was shown to possess anti-VEGF effects and to inhibit proliferation and migration on vascular endothelial cells *[Lançon et al., 2016]*.

Effect on retinal ischemia/reperfusion (I/R)

Recently, using a rat model of retinal ischemia/reperfusion (I/R) induced by endothelin 1 injection or by increased IOP by pumping air into the anterior chamber, Kiseleva et al. reported that animals supplemented with resveratrol (20 mg/kg per os one month before and after I/R), had zones of normal retina event 30 days after ischaemia and significantly ($p<0.05$) lower level of apoptosis and inflammation markers compared to animals without supplementation. This suggests that resveratrol may have neuroprotective and anti-inflammatory properties after retinal I/R injury regardless of the mechanism of ischaemia *[Kiseleva et al., 2018]*.

5.7.3 Resveratrol supplementation and AMD

A report on resveratrol-based nutritional supplementation on three octogenarians with AMD observed a short-term effect similar to that found with anti-VEGF treatment including anatomical restoration of retinal structure, improved RPE function, and a suggested improved choroidal blood flow *[Richer et al., 2014]*. In another recent study, Richer et al. reported broad bilateral improvements in ocular structure and function in three patients with AMD over a long-term follow-up of two to three years suggesting its efficacy in AMD *[Richer et al., 2013]*.

Key messages

- Resveratrol possesses potent antioxidant and anti-inflammatory properties and was shown to display anti-aging effects.

- Resveratrol was shown to possess anti-VEGF effects and to inhibit proliferation and migration on vascular endothelial cells.

- In animals, oral supplementation containing resveratrol associated with other antioxidants, minerals, and macular pigment, protected against early choroidal neovascularization in mice with laser-induced lesions.

5.8 SAFFRON

Saffron is a spice made from the dried stigmas of the plant Crocus sativus L. Saffron is mainly used for cooking. From time immemorial, it has also been considered a medicinal plant with therapeutic properties *[Bagur et al., 2017].* In recent years there has been growing demonstration of saffron's bioactivity, attributed to its main components that is crocetin and to its glycosidic esters that are crocins, and safranal. These constituents belong to the carotenoid family and bear structural similarities to natural antioxidant substances, such as zeaxanthin. Saffron also contains other carotenoids, anthocyanins, flavonoids, vitamins (riboflavin and thiamine), minerals and many other elements with beneficial nutritional properties *[Bagur et al., 2017, Heitmar et al., 2019].*

Oral supplements containing saffron are currently available in some countries. Nevertheless, its effects remain to be determined when used in association with other carotenoids especially lutein and zeaxanthin because of possible interactions..

Pharmacological properties

The beneficial effects of saffron have been demonstrated in experimental models. Saffron improves endothelial function, increases oxygen diffusion in tissues, and presents anti-inflammatory properties by decreasing the serum level of pro-inflammatory factors (TNF-α) and increasing anti-inflammatory factors (as IL-10). Saffron also has protective anti-apoptotic and antioxidant effects. It enhances endogenous antioxidant activity (superoxide dismutase) and decrease of lipid peroxidation activity *[Bagur et al., 2017].*

Animal models

In an animal model of light induced retinal degeneration *[Maccarone et al., 2008]* saffron pre-treatment was able to reduced photoreceptors death maintaining both morphology and function suggesting multiple ways of action. This idea was confirmed in subsequent microarray experiments *[Natoli et al., 2010; Marco et al., 2013]*. Saffron given in albino rats before retinal exposure to damaging light up or down regulated significant numbers of genes and non-coding RNAs. Following these results many ways of action have been tested confirming the complex nature of neuroprotective activities of saffron and its chemical components *[Di Marco et al., 2019]*. Also the ratio among crocins components resulted critical in providing neuroprotective efficacy. The best composition has been patented.

Subsequent experiments, in a variety of experimental models, confirmed the neuroprotective action of saffron and its components.

In experimental model of ischemia/reperfusion by artery ligation in anesthetized rats, pre-treatment with crocetin prevented retinal cell death and apoptosis, reduced the expression of 8OHdG (a marker of oxidative stress) in retinal cells, and prevented the reduction in ERG a- and b waves amplitude *[Ishizuka et al., 2013].*

In another experimental model of ishemia/reperfusion obtained by raising the IOP in anesthetized rats, pre-treatment with crocin enhanced retinal ganglion cells (RGC) survival by 36% and decreased RGC apoptosis by 44% *[Qi et al., 2013].*

In an experimental model of autosomal dominant retinitis pigmentosa (P23H rat), pre-treatment with safranal preserved photoreceptors morphology and density, the ERG a- and b waves amplitude and the capillary network *[Fernandez-Sanchez et al., 2012].*

In an experimental model of retinal oxidative stress in mice, treatment with crocin improved spatial visual acuity and visual contrast sensitivity. Crocin also protected retinal integrity and Muller cells and modulated the cone cellular function *[Liou et al., 2018].*

In an experimental model of susceptibility to oxidative stress (apoE -/- mice fed a high-fat diet), treatment with saffron (20 mg/kg/day for 20 weeks) enhanced the glycemic control and preserved the retinal thickness *[Doumouchtis et al., 2018].*

Clinical studies

In a randomized, crossover study in 25 AMD patients, oral saffron supplementation (20 mg/day for 3 months) improved focal electroretinograms (fERGs) amplitude (mean change: 0.25 log µV vs -0.003 log µV; $p < 0.01$) and decreased fERG threshold (-0.26 log units vs 0.0003 log units) *[Falsini et al., 2010].*

In a longitudinal, interventional open-label study in 29 patients with early AMD, saffron oral supplementation (20 mg/day) improved the mean fERG in focal region (18°) sensitivity by 0.3 log units compared to baseline values ($p < 0.01$), and the mean visual acuity by two Snellen lines compared to baseline values (0.75 to 0.9, $p < 0.01$). These changes remained stable over the follow-up period. These results indicate that in early AMD, saffron supplementation induces macular function improvements from baseline that are extended over a long-term follow-up *[Piccardi et al., 2012].*

In a longitudinal study involving 33 consecutive patients with a diagnosis of bilateral early AMD screened for CFH (rs1061170) and ARMS2 (rs10490924) polymorphisms, the fERG-derived (18°) macular flicker amplitude and sensitivity improved significantly after 3 months of saffron oral supplementation (20 mg/day). These changes remained stable throughout the follow-up period (6-12 months). This suggests that genotype had no impact on the therapeutic effect of saffron *[Marangoni et al., 2013].*

In a randomized, double-blind, placebo-controlled study, 60 patients with wet or dry AMD were assigned to saffron oral supplementation (30 mg/day) or placebo. After 6 months of treatment, no statistically significant decrease in OCT results was observed between the groups with dry AMD ($p = 0.282$). However, there was a statistically significant increase in ERG results between the groups at 3 months after treatment ($p = 0.027$). In addition, there was a significant decrease in OCT results between groups

with wet AMD at the follow-up (p = 0.05). Finally, there was a significant increase in ERG findings between the groups with wet AMD at 3 months after treatment (p = 0.01), but these changes decreased at 6 months after treatment (p = 0.213). It was concluded that daily supplementation with 30 mg of saffron for 6 months may result in a mid-term, significant improvement in retinal function in patients with AMD *[Lashay et al., 2016]*.

In a randomised, double-blinded, placebo-controlled crossover trial with mild/moderate AMD and vision > 20/70 Snellen equivalent in at least one eye, 100 participants were given oral saffron supplementation (20 mg/day) for 3 months or placebo for 3 months, followed by crossover for 3 months. Mean BCVA improved 0.69 letters (p = 0.001) and mean-pooled mfERG latency reduced 0.17 ms (p = 0.04) on saffron compared to placebo. Amongst participants on AREDS supplements, mean BCVA improved 0.73 letters (p = 0.006) and mean-pooled mfERG response density improved 2.8% (p = 0.038). There was no significant difference in adverse event occurrence. In this study, saffron supplementation modestly improved visual function in participants with AMD, including those using AREDS supplements. It was concluded that given the chronic nature of AMD, longer term supplementation may produce greater benefits *[Broadhead et al., 2019]*. All trials showed a positive trend, the discrepancy in efficacy reported in different trials might be due to the chemical characteristics of saffron used: a wrong ratio among crocins reduces the protective efficacy and in extreme cases abolishes it.

Safety

Daily 30 to 400 mg doses used in clinical trials have shown no difference in tolerance compared with a placebo. Adverse effects including nausea, vomiting, bleedings and headaches have been reported with doses ≥ 5g.

Saffron showed some effects on blood coagulation and platelet aggregation *in vitro* and *in vivo* studies. In a double-blind, placebo-controlled study, 60 healthy subjects received 200 mg, 400 mg of saffron (1 tablet per day) for 1 weeks or placebo. Saffron supplementation had no effect on plasma level of fibrinogen, factor VII (as coagulant agent), C and S protein (as anti-coagulant agent), PT and PTT *[Ayatollahi et al., 2014]*.

All these studies demonstrated the importance of saffron supplementation both in animal model and in AMD patients as well, and pointed out the potential and safety for using dietary saffron to treat retinal degeneration either AMD *[Bisti et al., 2014]* or hereditary macular dystrophy *[Piccardi et al., 2019]*.

Key messages

- There is growing evidence of saffron short and middle term beneficial functional effects in AMD.
- These effects are mainly recorded through fERG and also BVCA.
- The effects on “hard” signs progression in AMD (neovascularization, drusen, atrophy...) have not been evaluated yet.

5.9 OTHER NUTRIENTS OF INTEREST AND AMD

5.9.1 Vitamin B

Vitamin B12 and foalte (vitamin B9) act as essential coenzymes during homocysteine metabolism. It has been suggested that elevated serum homocysteine, folate and vitamin B12 deficiencies could predict increased risk of incident AMD *[Gopinath et al., 2013]*. Treatment with folic acid, vitamin B6 (pyridoxine hydrochloride) and vitamin B12 (cyanocobalamin) was shown to reduce homocysteine levels *[Homocysteine Lowering Trialists' Collaboration, 2005]*. In the Women's Antioxidant and Folic Acid Cardiovascular Study (WAFACS), participants were randomly assigned to receive a combination of folic acid (2.5 mg/d), vitamin B6 (50 mg/d), and vitamin B12 (1 mg/d), or placebo *[Christen et al., 2009]*. After a mean follow-up of 7.3 years of treatment, the study indicates that those assigned to active treatment had a statistically significant 35% to 40% decreased risk of developing AMD. However, the authors suggest that the results should be interpreted cautiously as AMD diagnosis was self-reported, and that findings in this group of females at increased risk of cardiovascular disease may not be applicable to the general population. Further investigation is warranted to determine the role of B vitamins in AMD risk *[Krishnadev et al., 2010]*.

5.9.2 Hydroxytyrosol

In a prospective population-based study involving 6,734 participants aged between 58 and 69 years (Melbourne Collaborative Cohort Study), olive oil intake (≥100 mL/week versus < 1 mL/week) at baseline was associated with decreased prevalence of late AMD over 13 years (odds ratio, 0.48; 95%CI: 0.22-1.04; P=0.03) after adjustments for age, smoking, energy, vitamins C and E, β-carotene, zinc, lutein, zeaxanthin and supplements. There was no association with early or intermediate prevalence *[Chong et al., 2009]*. In another prospective study involving 654 participants from the ALIENOR study, regular use of olive oil was significantly associated with a decreased risk of late AMD (OR=0.44; 95%CI: 0.21-0.91), but not with early AMD *[Cougnard-Grégoire et al., 2016]*.

Although olive oil contains approximately 85% oleic acid, neither monounsaturated fatty acids nor oleic acid intakes were associated with late AMD in the first study. Thus, other non fat components of olive oil have been suggested to contribute to this apparent protective effect.

Hydroxytyrosol (3,4-dihydroxyphenyl ethanol) (HT) is a major polyphenol of olive oil and has known cytoprotective beneficial effects *[Echeverria et al., 2017]*. The antioxidant properties of HT are associated with its high degree of absorption and bioavailability.

The effect of HT on RPE cells submitted to oxidative stress induced by acrolein indicated that hydroxytyrosol was able to increase the translocation of Nrf2 to the nucleus, which resulted in increased protein expression and antioxidant enzyme (including heme oxygenase-1, GSH reductase, GSH peroxidase and catalase). These results demonstrate that HT confers additional indirect antioxidant protection in addition to its already known direct antioxidant properties.

HT has been described as one of the polyphenols of extra virgin olive oil with the most potent anti-inflammatory effects, including inhibition of nitric oxide (NO) and PGE2 production, decreased secretion of pro-inflammatory cytokines and chemokines and decreased gene expression of inducible NO synthase, IL-1, CXCL10/IP-10, macrophage inflammatory protein-1 (MIP-1), MMP-9, and PGE2 synthase.

Olive leaves are the richest source of olive phenolic compounds, and olive leaf extract is now a popular nutraceutical taken either as liquid or capsules *[De Bock et al., 2013].* One study showed that regular intake of 15 mg/day of HT changed body composition parameters and modulated the antioxidant profile and the expression of inflammation and oxidative stress-related gene *[Colica et al., 2017].* Although one study showed that HT was able to prevent the degeneration of retinal pigment epithelial cells (ARPE-19) induced by oxidative stress *[Zhu et al., 2010]*, there is currently no published information on the effect of HT supplementation and the risk of AMD.

Key messages

- Other nutrients including Vitamin B and hydroxytyrosol have potential interest in supplementations to prevent early AMD or progression to late AMD.

5.10 COST-EFFECTIVENESS OF OCULAR NUTRITION IN AMD

Cost is one of the most important components for the patient when considering a nutritional supplement, since they are not reimbursed. This was confirmed in an European survey performed to evaluate ophthalmologists' opinion of the use of micronutritional dietary supplements, 10 years after the publication of the first AREDS study. Micronutrition (especially AREDS and AREDS2 regimens) was considered as a part of the routine management of AMD for many ophthalmologists. Patients rarely refused nutritional supplements (average, 14% for early/intermediate stage, 13% for geographic atrophy stage, 11% for neovascular stage), but cost was identified as an issue limiting compliance *[Aslam et al., 2014].*

A recent cost-effectiveness analysis in the UK showed that the intervention with AREDS supplements is likely to be a dominant cost-effective strategy in category 4a patients with neovascularization in one eye. For AREDS category 4 with neovascularization compared with no intervention, AREDS supplements are more effective (10.59 vs 10.43 quality-adjusted life years *[QALYs]*) and less costly (£52 074 vs 54 900) over the lifetime of the patient. Over the lifetime of the patient, this translates to a cost-negative yet beneficial intervention driven by a mean of 7.67 (lower cost) fewer anti-VEGF injections in the treated group compared with no use of supplements. In patients with bilateral intermediate AMD (AREDS category 3), the incremental cost-effectiveness ratio (ICER) was £30,197 versus current treatment practice i.e. just above the threshold (£20,000–30,000) per QALY, which is often considered the UK National Health Service's willingness to pay *[Lee et al., 2018].*

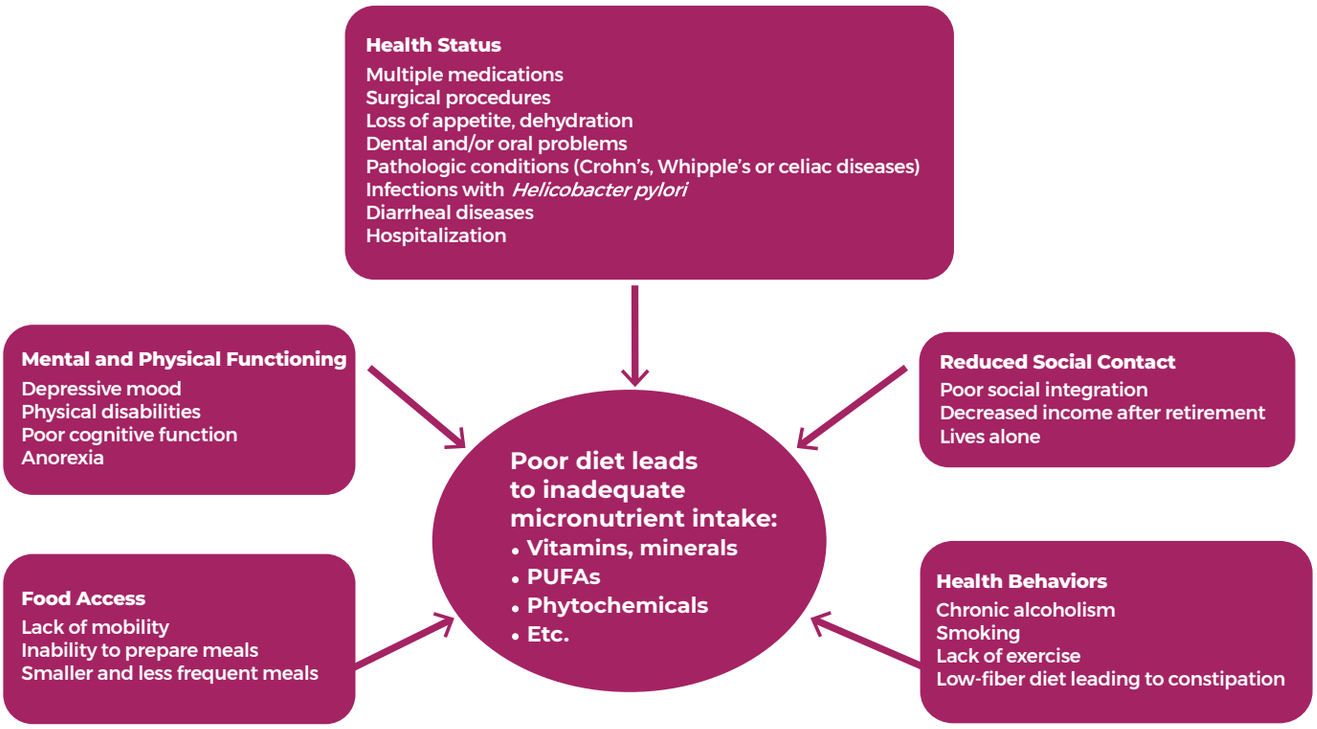
Thus, the recommendation to publicly fund AREDS supplements to category 3 patients depends on the healthcare system's willingness to pay. In contrast, initiating AREDS supplements in AREDS category 4 patients is both cost-saving and more effective than no supplement use and should therefore be considered in public health policy *[Lee et al., 2018].*

6. AMD PREVENTION: WHY IS IT NOT ENOUGH WITH DIET ?

Diet of various healthy foods may be optimal for reducing AMD risk *[Chiu et al., 2017]*. Patients should be advised that a healthy diet rich in green, leafy vegetables and fish may decrease the risk of AMD. If a patient cannot include enough of these foods in his diet, supplements that include the key ingredients in these foods (antioxidants, carotenoids, and omega-3 fatty acids) could be helpful. If a patient has been diagnosed with intermediate AMD in one or both eyes, AREDS-type supplements with lutein and zeaxanthin could be prescribed along with a healthy lifestyle *[Sobrin & Seddon, 2014]*.

A study which aimed to compare the micronutrient usage over 10 years among participants with and without AMD, showed that participants with AMD did not appreciably increase fish, fruit and vegetable consumption and overall diet quality over a 10-year follow-up. Adherence to dietary recommendations was poor among older adults with AMD. However, uptake of antioxidant supplements increased significantly among those with late AMD *[Gopinath et al., 2015]*. As summarized in Figure 14, there are various reasons why diet is not enough to reduce the risk of AMD.

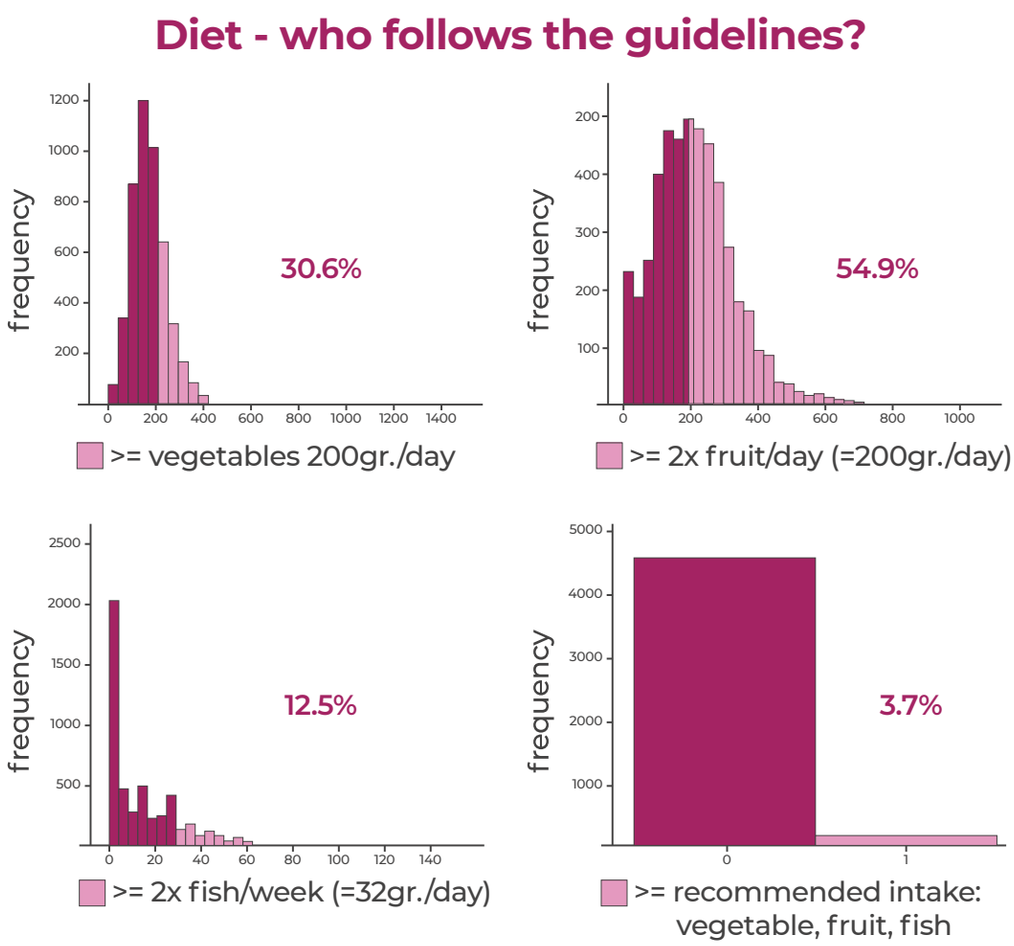
Figure 14 : Various factors causing poor coverage with essential nutrients and possible malnutrition in older adults



Adapted from Mohajeri et al., 2015

Among the 4,202 participants from the Rotterdam Study aged ≥ 55 years and without AMD at baseline, 754 patients developed AMD after a mean follow-up of 9.1 years. Intake of the recommended amounts of vegetables (≥200 g/day), fruit (2×/day), and fish (2×/week) were 30.6%, 54.9%, and 12.5%, respectively (Figure 15). However, intake of the recommended amounts of all 3 food groups was only 3.7%, and adherence to this pattern showed a reduction of the risk of incident AMD with an hazard ratio of 0.58 (95% CI: 0.36-0.93) *[de Koning-Backus et al., 2019]*.

Figure 15 : Poor adherence to recommended diet in the Rotterdam study



A large portion of the general population is inadequately supplied with various vitamins, even in industrialized countries (Table 18). Notably, elderly populations, especially those living in institutionalized settings, are at a higher risk of receiving insufficient levels of essential micronutrients *[Mohajeri et al., 2015]*. As reviewed by Mohajeri et al., NHANES data from 2003 to 2008 shows that intakes of vitamin A, C, D, E, K and folate are low in a significant proportion of the elderly US population. In Germany, vitamin D and folate appear to be the most critical vitamins in people aged 65 to 80 years, followed by vitamins E and C in both institutionalized and community-dwelling

elderly. The European Nutrition and Health Survey also reported that average intakes of vitamin D and folate for most countries are below the recommended levels, while around half of the countries have average intakes of vitamin E and C that are below recommendations *[Elmadfa et al., 2009]*. The situation in the USA and Europe is quite different, since in the US the nutrient-poor diet is partially compensated by fortified-food and the use of dietary supplements. This is not the case in most European countries. The 1999-2000 NHANES reported that more than 35% of people aged ≥ 60 years in USA take micronutrient supplements on a regular basis. By comparison, only around 3% of the elderly in Germany were taking supplements. The risk of nutrient intakes below the Estimated Average Requirements (EAR) is reduced four times in elderly persons who regularly used supplements of one or more micronutrients *[Sebastian et al., 2007]*. In elderly men (age ≥ 70 yrs), the prevalence of vitamin A, E and folate intakes below the recommendations decreased from 53% to 4%, from 93% to 14% and from 75% to 7%, respectively, with the use of supplements *[Sebastian et al., 2007]*. Thus, a significant proportion of seniors can be expected to be at risk for multiple micronutrient deficiencies.

Table 18 : Nutrient intake from food in non-institutionalized US individuals (supplement non users)

	EAR	% below EAR	
Men		Age 51-70 years	Age ≥70 years
Vitamin A	601 (RAE)	53%	53%
Vitamin B6	1.4 mg	17%	34%
Vitamin B12	2.0 µg	4%	10%
Vitamin C	75 mg	43%	48%
Vitamin E	12 mg (α-tocopherol)	90%	93%
Zinc	9.4 mg	26%	43%
Women			
Vitamin A	500 (RAE)	51%	47%
Vitamin B6	1.3 mg	48%	49%
Vitamin B12	2.0 µg	10%	25%
Vitamin C	60 mg	39%	37%
Vitamin E	12 mg (α-tocopherol)	>97%	>97%
Zinc	6.8 mg	25%	45%

Data from the US Department of Agriculture (1994-1996) (Sebastian et al., 2007)
EAR: Estimated Average Requirement; RAE: retinol activity equivalent

There are other reasons why diet alone is not enough for the prevention AMD. The elderly population above the age of 60-65 years shows a higher risk of developing nutritional disorders caused by the ageing process itself, coupled with a series of physiological, biochemical, biological and psychological changes, which in turn alter the individual physical activity as well as general behaviour, dietary habits and social interactions *[Mocchegiani et al., 2013]*. The elderly tend to be less capable of preparing meals for themselves, resulting in decreased food intake. Thus, older adults might be at increased risk of nutritional insufficiencies and malnutrition owing to lowered energy intake, lack of variety in the foods consumed, difficulties with chewing and swallowing, sensory losses, and pertinent comorbidities. For example vitamin B12 deficiency is highly prevalent among older adults (age ≥65 years), which is not only a result of poor diet but also diminished absorption associated with age *[Rautiainen et al., 2016]*.

Chronic poor food choices can lead to micronutrient insufficiencies and deficiencies regardless of age and demographic characteristics *[Rautiainen et al., 2016]*. When considering a certain food as a source for a given nutrient, bioavailability has an important role but may be difficult to estimate if information about factors reducing or enhancing it and the nutrient's chemical and binding form is not available. The oral bioavailability of a health-promoting dietary component (nutraceutical) may be limited by various physicochemical and physiological phenomena: liberation from food matrices, solubility in gastrointestinal fluids, interaction with gastrointestinal components, chemical degradation or metabolism, and epithelium cell permeability *[McClements et al., 2015]*. Intake of one food, with its many ingredients, might affect the bioavailability and nutritional value of another food or nutrient and that the benefits gained through nutrient intake could also vary by health status *[Chiu et al., 2017]*.

Thus, for certain individuals, it may be difficult to obtain adequate levels of antioxidants and other essential nutrients through diet alone. In such cases, there could be some advantage in augmenting particular nutrients through supplementation. Evidence supporting the use of supplementation in AMD comes primarily from the AREDS trial, which demonstrated that a supplement containing high doses of vitamin C, vitamin E, β-carotene and zinc could reduce progression to advanced AMD by 25% in populations with intermediate AMD or advanced AMD in one eye *[Lawrenson & Evans, 2013]*. The AREDS formulation contains much higher concentrations of vitamins and minerals than the recommended daily intake. The dose of vitamin C (500 mg) used in the formulation is about 5 times what the general population receives from diet alone. The 400-IU dose of vitamin E is about 13 times the RDA and the dose of zinc as zinc oxide is about 5 times the RDA. These levels of zinc and vitamins C and E generally can be obtained only by supplementation. The quantity of food to eat to reach the AREDS amount of nutrients is shown in Figure 16.

Figure 16 : Quantity of food to eat to reach the AREDS amounts of nutrients

AREDS2 Formula Supplement Vs Food

Nutriments	UK Labelling Nutrient Reference Value (NRV)	AREDS2 formulation (mg/day)	Difference between recommendation	Dietary equivalent of AREDS2 formulation
Vitamin C	80 mg	500 mg	420 mg	8 oranges 
Vitamin E	12 mg	400 IU (equivalent to 268 mg)	256 mg	45 tabletspoons of sunflower seeds 
Zinc	10 mg	80 mg	70 mg	14 rump steaks 
Copper	1 mg	2 mg	1 mg	200 mussels 
Lutein	No NRV	10 mg	10 mg	1 portion of spinach 
Zeaxanthin	No NRV	2 mg	2 mg	1 orange pepper 

Key messages

- A diet of various healthy foods may be optimal for reducing AMD risk.
- Adherence to dietary recommendations is poor among older adults with AMD, around 4%.
- Adequate levels of antioxidants and other essential nutrients through diet alone may be difficult to obtain in some subjects.
- There could be some advantages in augmenting particular nutrients through supplementation in some subjects.
- The recommended formulation (AREDS) contains much higher concentrations of vitamins and minerals than the recommended daily intake.

7. WHEN SHOULD OCULAR NUTRITION BE STARTED?

Nowadays, dietary supplements are widely marketed as a strategy for AMD prevention and treatment and very little reliable information is available to guide the public in making the decision as to whether or not to take these supplements *[Lawrenson & Evans, 2013].*

Bressler et al. estimated that as many as 300 000 cases of advanced AMD could be avoided in the USA over 5 years if all eligible patients took vitamin supplements containing antioxidants plus zinc *[Bressler et al., 2003, Lee et al., 2018].*

There are two key questions: should the general population take antioxidant supplements to reduce the risk of developing AMD later on in life (primary prevention) or should people with AMD take antioxidant supplements to slow down the progression of the disease (secondary prevention)? *[Evans & Lawrenson, 2014].*

For the majority, the type of supplement recommended did not comply with current best research evidence, based on the findings of the Age-related Eye Disease Study (AREDS) *[Lawrenson & Evans, 2013].* Evidence supporting the use of supplementation in AMD comes primarily from the AREDS trial *[AREDS, 2001],* which demonstrated that a supplement containing high doses of vitamin C, vitamin E, β-carotene and zinc could reduce progression to advanced AMD by 25% in populations with intermediate AMD or advanced AMD in one eye.

As reviewed by Evans et al., people with AMD may benefit from supplementation with antioxidant vitamins. However, there is currently no evidence from randomized intervention studies to support the use of nutritional supplements in primary prevention or to slow progression in patients with early AMD *[Evans & Lawrenson, 2014; Lawrenson & Evans, 2013].* But in populations at a higher risk of progression to advanced disease, the use of high dose antioxidant vitamin and zinc supplementation has been shown to be protective *[Evans & Lawrenson, 2014].*

In a cross-sectional survey of the current practice of UK eye care professionals (mainly optometrists) in 2012, practitioners were asked about the advice given to their patients according to the risk of AMD *[Lawrenson & Evans, 2013].* The results suggest that the likelihood of a patient being advised to take a supplement is dependent on their risk of progression to advanced AMD:

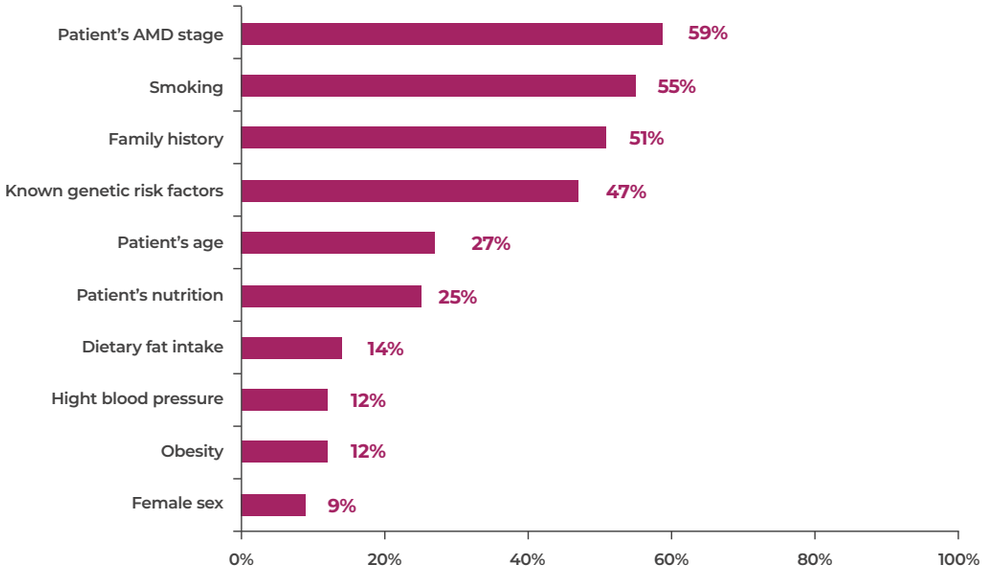
- 93% recommended supplements in 65-year old patients with advanced AMD in one eye and early AMD in the other,
- 45% recommended supplements in 75-year old patients with advanced AMD in both eyes
- 34% of practitioners recommended to take supplements in 55-year old patients with no evidence of AMD but with one or more parents and/or siblings affected by AMD,

Supplements containing macular carotenoids or macular carotenoids + vitamins were the most frequently recommended formula (about 60% whichever the risk). Omega-3 fatty acids were nevertheless recommended in 20-25% of patients according to the risk and the AREDS formula in 15-25%. However, compared to optometrists, it seems that ophthalmologists were less likely to recommend supplementation for primary prevention (only 9.6% of respondents recommending a supplement for a patient at risk of developing AMD according to family history versus 34.6% of optometrists, P=0.0061). The AREDS formula was the most frequently recommended supplement by ophthalmologists.

In another study, it was suggested that the timing of different supplementations may be important. A diet rich in omega-3 fatty acids or omega-3 fatty acid supplementation without the AREDS supplement might be more protective against progression of early AMD if started at the earliest stages of AMD, while when combined with the AREDS supplement, it might be protective with regard to preventing progression to advanced AMD *[Chiu et al., 2009]*.

A European survey (France, Germany, Italy, Spain, UK, Belgium, and Portugal) in 2014 was performed in general ophthalmologists and retinal specialists to determine the use of micronutritional supplement in AMD. These practitioners were selected because they managed at least 40 patients with AMD per month and prescribed nutrition supplements at least 4-times per month, thus they are not representative of ophthalmologists in general. Nutritional supplementation was most frequently initiated when diagnosis of early or intermediate AMD was confirmed (46% of patients), followed by neovascular AMD (18% of patients) and geographic AMD (13%). Only 3% of patients were initiated nutritional supplementation when anti-VEGF treatment was started. The most important risk factors for initiating treatment with nutritional supplements were AMD stage, smoking, family history, and known genetic risk factors (Figure 17). The patient's age and nutrition were also essential factors *[Aslam et al., 2014]*.

Figure 17 : Very important risk factors for the initiation of nutritional supplementation



Adapted from Aslam et al., 2014

Medical interest in nutritional supplements was important at all stages of the disease but was greatest in early/intermediate disease, at 78% (relatively high and very high medical interest) versus 58% for neovascular AMD, and 59% for geographic atrophy. Relatively similar opinions were observed in the different countries *[Aslam et al., 2014].*

Ophthalmologists were generally of the opinion that nutritional supplements were effective in slowing disease progression in early/intermediate-stage AMD. Expectations in terms of stopping disease progression and reducing lesions or damage were lower, particularly in geographic and neovascular stages.

Nutritional supplements were considered to have the most beneficial symptomatic effects on visual acuity (62% of patients), contrast vision (58%), and glare (31%). Nutritional supplements were expected to have a positive effect on both eyes, particularly in early/intermediate-stage disease.

In this study, there was a wide spectrum of opinions regarding the most important components for nutritional supplements. The most important components in nutritional supplements were lutein (77%), omega-3 (72%), and zeaxanthin (68%). Among the list of all the components, lutein, omega-3, zeaxanthin, zinc, and vitamin E were considered as very important by more than 50% of ophthalmologists. Resveratrol was considered to be a very important component by 26% of ophthalmologists. In Italy, ophthalmologists considered very important supplementation with anthocyanins (blueberries) (54% versus 13-25% in other countries), resveratrol (56.7%, versus 15-28%), vitamin E (83.3% versus 33-53%), vitamin C (73% versus 27-59%), β-carotene

(70% versus 31-47%). In France, 34.4% of ophthalmologists considered vitamin D as very important (versus 3 to 15% in other countries) *[Aslam et al., 2014]*.

Although most ocular nutritions are not yet validated for use in AMD, this survey showed that micronutrition has already become part of the day-to-day management of AMD for a considerable proportion of ophthalmologists. Ophthalmologists seem pragmatic in their expectations regarding the effect of nutritional supplements, with slowing of progression in the early stages of the disease being the strongest expectation, although more than half expected to see slowing of progression in the geographic atrophy stage.

Key messages

- A significant number of cases of advanced AMD could be avoided if all eligible patients took vitamin supplements containing antioxidants plus zinc.
- Micronutrition is part of the day-to-day management of AMD for most ophthalmologists.
- Supplements containing macular carotenoids or macular carotenoids + vitamins are probably the most frequently recommended formula in ophthalmologists' advice, followed by omega-3 fatty acids.
- Advice to take a nutritional supplement is dependent on the risk of progression to advanced AMD and patients food habits..
- Medical interest in nutritional supplements among ophthalmologists seems greater in early/ intermediate disease than late AMD.

8. EUROPEAN RECOMMENDATIONS AND REGULATIONS FOR FOOD SUPPLEMENTS: SAFETY PERSPECTIVE

8.1 EUROPEAN REGULATION OF FOOD SUPPLEMENTATION

Food supplements are concentrated sources of vitamins, minerals and/or other substances (such as amino acids, essential fatty acids, fibers and various plant and herbal extracts) sold as pills, tablets and other dose forms *[EFSA, 2017]*.

The European Commission has established harmonized rules to help ensure that food supplements are safe and properly labelled. In the EU, food supplements are regulated as foods and the legislation focuses on vitamins and minerals used as ingredients of food supplements. The main EU legislation is Directive 2002/46/EC related to food supplements containing vitamins and minerals. The Directive sets out labelling requirements and requires that EU-wide maximum and minimum levels are set for each vitamin and mineral added to supplements. As excessive intake of vitamins and minerals may result in adverse effects, the Directive provides for the setting of maximum amounts of vitamins and minerals added to food supplements..

Reference intakes of vitamins and minerals

Dietary recommendation intakes (DRIs) are intended to help individuals optimize their health, prevent disease, and avoid consuming too much of a nutrient. The DRIs included four nutrient-based reference values: estimated average requirement (EAR), the recommended dietary allowance (RDA), the adequate intake (AI) and the tolerable upper intake level (UL). The EAR is defined as the average daily nutrient intake level that is estimated to meet the requirements of half of the healthy individuals in a particular life stage and gender group. The RDA represents the average daily dietary nutrient intake level that is sufficient to meet the nutrient requirements of nearly all (97-98%) healthy individuals in a particular life stage and gender group. When an RDA cannot be determined, an AI is estimated which is the recommended average daily intake level based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate *[Aranceta & Perez-Rodrigo, 2012]*.

Essential fatty acids

According to the European Food Safety Authority (EFSA) in 2010, an intake of 250 mg/day of EPA + DHA appears to be sufficient for primary prevention in healthy subjects. Therefore, and taking into account that available data are insufficient to derive an EAR, an adequate intake of 250 mg for EPA + DHA was proposed for adults based on cardiovascular considerations *[EFSA, 2010]*.

According to the Food and Agriculture Organization (FAO) in 2008, the total n-3 fatty acid intake (ALA, EPA and DHA) can range between 0.5-2% of total energy (%E),

whereas the minimum dietary requirement for ALA (>0.5%E) prevents deficiency symptoms in adults. The higher value of 2%E includes the recommendation for ALA and long chain n-3 PUFA (AMDR for EPA and DHA 250 mg-2000 mg) can be part of a healthy diet. While ALA may have specific properties, there is evidence that the n-3 long-chain PUFA can contribute to the prevention of CHD and possibly other degenerative diseases associated with aging. For adult males and non-pregnant/non-lactating adult females, 250 mg/day of EPA+DHA is recommended. With insufficient evidence to set a specific minimum intake of either EPA or DHA alone, both should be consumed.

The upper value of acceptable macronutrient distribution range (U-AMDR) for EPA+DHA consumption is set at 2 g/day due to experimental evidence indicating that high supplement intakes of n-3 long-chain PUFA may increase lipid peroxidation and reduce cytokine production *[FAO, 2008]*.

Based on the evidence and conceptual limitation, there is no rational for a specific recommendation for n-6 to n-3 ratio, or LA to ALA ratio, if intakes of n-6 and n-3 fatty acids lie within the established recommendation.

8.2 SAFETY ISSUES

Vitamin supplements, which are widely marketed and consumed by the general population, may have harmful effects *[Evans & Lawrenson, 2014]*. The benefits or safety of using high dose antioxidants for long periods of time, as might be needed to prevent AMD or slow progression in the early stages, has not been established *[Meyers et al., 2015]*. But, there is some evidence that high intakes of supplemental form of antioxidants are damaging and studies are necessary to determine the appropriate dose, form and timing of antioxidant consumption *[Raman et al., 2017]*. Potential side effects include the following: vitamin C (kidney stones), vitamin E (fatigue, muscle weakness, decreased thyroid gland function and increased hemorrhagic stroke risk), β-carotene (yellow skin) and zinc (anemia, decreased high-density lipoprotein (HDL) cholesterol and upset stomach) *[Sin et al., 2013]*.

Vitamin E

A previous meta-analysis of 19 clinical trials including AREDS showed that high-dosage (> 400 IU/day) vitamin E supplementation may increase all-cause mortality *[Miller et al., 2005]*. In this meta-analysis, all-cause mortality progressively increased for dosages approximately greater than 150 IU/day. This dosage is substantially lower than the tolerable upper intake level for vitamin E i.e. 1000 mg of any form of supplementary alpha-tocopherol per day (corresponding to 1100 IU of synthetic vitamin E per day or

1500 IU of natural vitamin E per day) *[Miller et al., 2005].* The Heart Outcomes Prevention Evaluation (HOPE) Study found that, among people with vascular disease or diabetes, vitamin E supplementation was associated with a higher risk of heart failure *[Lonn et al., 2005, Evans 2012b].* Although vitamin E is considered relatively safe compared to other fat-soluble vitamins, plausible mechanisms of high-dose adverse effects included pro-oxidant effect, disruption of the natural balance of antioxidant systems and increasing vulnerability to oxidative damage and anti-coagulant activity *[Miller et al., 2005].*

β-carotene

Studies suggested potential harmful effects of β-carotene among smokers with increasing risks (four to fivefold) of lung cancer and cardiovascular disease (The ATBC Cancer Prevention Study Group, 1994; Omenn et al., 1996) compared with those in nonsmokers. Thus, the AREDS formula is contraindicated in smokers.

Zinc

A few studies have observed that high levels of zinc supplementation lead to genitourinary complications (urinary tract infections, stress incontinence, and prostatic hyperplasia) and self-reported anemia, which increased hospital admissions *[Gorusupudi et al., 2017; Broadhead et al., 2015].*

High zinc intake (>50 mg/day) can induce copper deficiency. In fact, metallothioneins, which bind to zinc, have a strong affinity for copper; where high levels of metallothioneins, induced by zinc excess, may cause a decrease in intestinal copper absorption. Similarly, copper intake can affect the zinc nutritional status, and zinc supplementation might even affect cysteine levels *[Zampatti et al., 2014].* Copper deficiency was found in an adult patient who had received excessive daily oral zinc for 10 months *[Hoffman et al., 1988].* The deficiency is characterized by hypochromic-microcytic anemia, leukopenia, and neutropenia. Although initially thought to be caused by iron deficiency, the anemia did not respond to oral or intravenous iron. Cessation of zinc tablets and ingestion of an oral copper preparation daily for 2 months failed to correct the anemia or leukopenia. It was not until shortly after intravenous administration of a cupric chloride solution during a 5-day period, at a total dose of 10 mg, that serum copper and ceruloplasmin levels increased and the anemia, leukopenia, and neutropenia resolved. This suggests that the elimination of excess zinc is slow and that, until such elimination occurs, the intestinal absorption of copper is blocked *[Hoffman et al., 1988].*

In conclusion, harmful effects associated with long-term vitamin supplementation, particularly in smokers and people with vascular diseases, cannot be ruled out.

Key messages

- A high dose of vitamins, minerals or other nutrients seems necessary to reduce the risk of AMD.

- Food supplementation in Europe is regulated by legislation.

- An excessive intake of vitamins and minerals may result in adverse effects. That's why food supplements should be recommended by health care professionnals.

9. PATIENT MANAGEMENT: INFORMATION ON AMD

Micronutrition is now part of the routine management of AMD for many ophthalmologists and people with AMD need to have reliable information in order to decide whether or not to take vitamin supplements *[Evans & Lawrenson, 2012a]*. Ophthalmologists choosing to use nutritional supplements are generally well-informed regarding current scientific studies *[Aslam et al., 2014]*. They are in a position where they have to provide information and advice to patients with diagnosed AMD or at risk of developing the disease, on the benefit of specific nutritional interventions or other lifestyle changes *[Lawrenson & Evans, 2013]*.

Current evidence indicates that all patients, regardless of their disease severity, should be given dietary advice to increase the consumption of green leafy vegetables, to consume low-glycemic index diets, and to consume fish at least twice a week *[Broadhead et al., 2015]*. The general public is often receptive to recommendations made by physicians regarding diet and supplements as a means of empowering themselves to avoid common and worrisome ailments such as AMD *[Gorusupudi et al., 2017]*.

Supplements are not designed to replace a healthy, balanced diet. So, patients should speak to their ophthalmologists, general practitioner or pharmacist first, if they are unsure about taking supplements alongside their prescription medicines.

Adherence to recommendations

- A cohort study investigating the micronutrient usage and other lifestyle behaviors over 10 years in 2007 showed that adherence to smoking and dietary recommendations was poor among older adults with AMD. Only one in four current smokers with any AMD at baseline quit smoking at 5 years and were still not smoking at 10 years, with the remaining 50% still smoking after 10 years *[Gopinath et al., 2015]*. The study also showed that participants with, compared to without, AMD did not appreciably increase fish, fruit and vegetable consumption and overall diet quality over the 10-year follow-up. The study also reported a low adherence to use of AREDS-type supplementation in patients with AMD *[Gopinath et al., 2015]*. The uptake of antioxidant supplements was lower in those who developed early AMD. This may be a cause for concern given that it is this subgroup which is at greatest risk of progression to advanced AMD and thus, irreversible vision loss. This study highlights the presence of a substantial gap between recommendations made by eye healthcare specialists and the recommendations actually practiced by AMD patients *[Gopinath et al., 2015]*. This suggested the need for educational strategies that target those with early AMD lesions in order to improve compliance to recommendations for antioxidant supplement use.

- Another prospective controlled survey was performed to assess the concordance with the AREDS recommendations in two retinal clinics with different education policy. In clinic 1, there was a formal policy of giving the

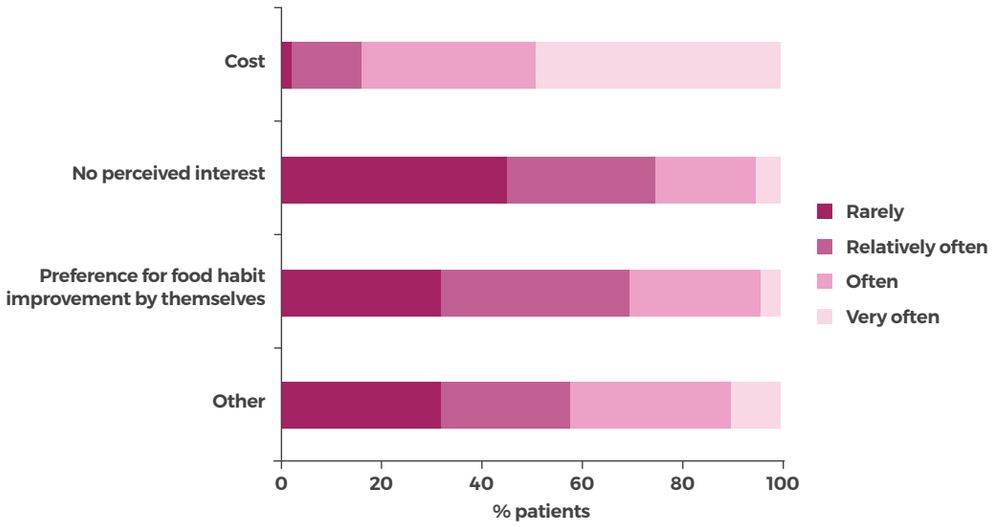
patient both verbal and written instructions and verbal repetition of these instructions from each staff member on each patient visit and in clinic 2, there was no specific education policy. Concordance was defined as taking the full AREDS dose of 2 tablets per day, and non-concordance was defined as taking 0 or 1 tablets per day. Clinic 1 had a concordance rate of 81.6% and clinic 2 of 44.1% with the AREDS recommendations. Thus, a high concordance rate can be achieved in clinical practice with rigorous patient education that includes a policy of having continual repetition of instructions. It seems very important to repeat and continue repeating instructions to older patients *[Weaver & Beaumont, 2015]*. In this study, the main reason (in 43% of cases) for not taking the AREDS supplementation was that patients were unaware of the supplement.

Thus, the lack of adherence could be due to patient failure to understand the importance of AMD-related recommendations, inadequate explanation and reinforcement from ophthalmologists, and lack of nutritionists' support and referral *[Gopinath et al., 2015]*.

- A European survey in 2014 examined the attitudes of ophthalmologists who prescribe nutritional supplements to AMD patients. The ophthalmologists reported that 58% of patients who were taking nutritional supplements were not aware of them before receiving advice or their first prescription. Information concerning the beneficial effects of nutritional supplements was regularly given by 67% of ophthalmologists. Patients rarely refused nutritional supplements (14% for early/intermediate stage, 13% for geographic atrophy stage, 11% for neovascular stage). Cost was the main reason of refusal (Figure 18). *[Aslam et al., 2014]*.

  Poor compliance was estimated as common concerning 40% of patients, and in most cases, this was judged to be a result of cost and this was worse in patients with early-stage disease (40-46%), than in patients with advanced disease (29%). Refusal of nutritional supplementation was relatively rare (10%-15% of participants), and was lower in patients with more advanced disease *[Aslam et al., 2014]*.

Figure 18 : Reasons for patients refusing nutritional supplementation



Adapted from Aslam et al., 2014

Key messages

- All patients, regardless of their disease severity, should be given dietary advice to increase their consumption of green leafy vegetables, to consume low-glycemic index diets, and to consume fish at least twice a week.
- Adherence to smoking and dietary recommendations is generally poor among older adults with AMD.
- Repeated patient education with instruction on the benefit of nutritional interventions or other lifestyle changes is necessary in older patients.
- The cost of antioxidant nutrients is a main cause of poor compliance especially in patients with early-stage disease.

10. CONCLUSIONS

AMD is a frequent disease with increasing prevalence and no treatment, except anti-VEGF for wet AMD. There is evidence that the role of oxidative stress and inflammation in the development of AMD and poor dietary habits, including lack of antioxidants and mineral intake, are important risk factors for the development of early AMD and/or progression to the late stages.

Vitamins, minerals, macular pigment (Lutein/Zeaxanthin), omega-3 fatty acids (EPA/DHA), and resveratrol have been shown to affect the disease processes, and a supplementation with these nutrients is of interest to prevent or to reduce the risk of progression of AMD. As supplementation with a single nutrient is not used or recommended, formulations with multiple nutrients are necessary. Nevertheless, the pharmacological doses used in antioxidant supplements are far above the Recommended Dietary Allowance. Excessive intake not only of fat-soluble vitamins and micronutrients, but also minerals and water-soluble vitamins, may be associated with toxicity, including undesirable interactions with other drugs and vitamins. In any case, a nutritional supplement cannot replace a balanced and varied diet. However, adherence to dietary recommendations is poor especially among older adults with AMD *[de Koning-Backus et al., 2019]* that's why food supplements may help elderly patients to reach the recommended daily intake.

The AREDS regimen containing vitamin C, vitamin E, lutein, zeaxanthin, zinc and copper is recommended in clinical guidelines in patients with intermediate to advanced AMD. This is currently the most commonly prescribed supplement in patients with AMD *[de Koning-Backus et al., 2019]*. Initiating an AREDS supplement in patients with intermediate or late AMD is effective and cost saving.

Treatment compliance is an important issue in patient with antioxidant supplements. It is thus important for practitioners to educate patients about the benefits of supplements, about why a certain supplement is being recommended for individual use, and that there can be risks when supplements are consumed in excess.

EN

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Zinc contributes to the maintenance
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DIETARY INFORMATION

	Daily dose (1 capsule)	% NRV*
Antioxidant vitamins and trace elements**		
Vitamin C	60 mg	75%
Vitamin E	10 mg	83%
Zinc	10 mg	100%
Copper	500 µg	50%
Selenium	25 µg	45%
Essential fatty acids		
Fish oil 4020 TG QUALITY SILVER class	330 mg	
Containing Omega 3: - EPA - DHA - DPA	231 mg 132 mg 66 mg ≤ 16.5 mg	
Lutein and zeaxanthin		
Lutein	10 mg	
Zeaxanthin	2 mg	
***Vitis Vinifera* extract containing resveratrol 5%**	**1 mg (resveratrol)**	
Vitamin D3		
Vitamin D3	5 µg	100%

* *Nutrient Reference Values (Regulation (EU) 1169/2011)*

***Zinc, copper, selenium, vitamin E and vitamin C contribute to the protection of cell constituents from oxidative damage.*

Net weight (30 capsules): 24 g

INGREDIENTS

Fish oil (Omega 3 essential fatty acids); bovine gelatine; purified water; humectant: glycerol; lutein, safflower oil; vitamin C (calcium ascorbate); emulsifier: glycerol monostearate; vitamin C (L-ascorbic acid); zinc (sulfate); *Vitis Vinifera* extract containing 5% resveratrol; vitamin E (alpha-tocopherol); selenium (as yeasts fortified with selenium); humectant: sorbitol; zeaxanthin, safflower oil; colourants: iron oxide black; iron oxide red; copper (sulfate); glutathione; vitamin D3 (Cholecalciferol).

ANTIOXIDANTS

Contribute to the protection of cell constituents from oxidative damage.

Vitamin E:
Vitamin E is found in vegetable oils.

Vitamin C:
Vitamin C is found in fruit and vegetables.

Zinc:
Zinc contributes to maintenance of normal vision.
It is found in meat and fish.

Copper:
Copper is found in offal, molluscs and dried fruits.

Selenium:
It is found in fish, shellfish, eggs, garlic, mushrooms, meat and cereals.

LUTEIN AND ZEAXANTHIN

Lutein and zeaxanthin are two yellow pigments that are found in large quantities in some green vegetables, such as spinach, broccoli and lettuce.

OMEGA 3 ESSENTIAL FATTY ACIDS (DHA AND EPA)

Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are two polyunsaturated fatty acids belonging to the family of Omega 3 fatty acids. Omega 3 acids are essential fatty acids: they must be provided by food or by dietary supplements in case of an unbalanced diet. Omega 3 acids are present in large quantities in fatty fish, such as tuna, salmon and herring.

VITIS VINIFERA EXTRACT CONTAINING 5% RESVERATROL

Grapes are rich in phenolic compounds including resveratrol.
Resveratrol is present in certain fruit, notably in grapes and in wine.

VITAMIN D3

Vitamin D3 is present in fatty fish, such as tuna, salmon and herring.

MODE OF ADMINISTRATION

One capsule once daily, preferably taken during meals with a small amount of water. Do not exceed the above recommended daily dosage without consulting your ophthalmologist or dietician.

NUTROF® TOTAL is a dietary supplement. It cannot replace a balanced and varied diet and a healthy lifestyle.

NUTROF® TOTAL can cause mild digestive disorders such as nausea, belching, diarrhoea. In this case, do not hesitate to consult your doctor.

PRECAUTIONS FOR USE

Do not take this food supplement if you are allergic to one of its components.

STORAGE INSTRUCTION

Store at temperatures below 25°C, protect from light and moisture.
Keep out of the sight and reach of children.
Do not use after expiration date marked on the container after best before end.

INFORMATION

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This product is not intended for the French market.

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