



The Lutein – Prevention and Treatment for Age-related Diseases 2006

Published by Transworld Research Network

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Transworld Research Network

T.C. 37/661(2), Fort P.O.,

Trivandrum-695 023, Kerala, India

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ISBN: 81-7895-219-X

Preface

Many fruits and vegetables contain abundantly the xanthophyll lutein and zeaxanthin, which can reduce the risk for developing age-related cataract and other age-related diseases such as hypertension, heart failures of myocardial infarction, diabetes (mellitus), many cancers and aging also.

Especially, age-related macular degeneration (ARMD) is the leading cause of the vision loss and blindness worldwide. Many recent results of researches show that dietary phytochemicals such as lutein and zeaxanthin could greatly prevent, reduce or improve the ARMD and suggest that the prevention of ARMD is very essential to choose the intake of our daily dietary and food lifestyle. Antioxidant lutein and zeaxanthin as macular pigments have selectively the antioxidant effects for the risk of cataract, which might cause the vision loss. These dietary carotenoids lutein and zeaxanthin exist mainly within the retina and lens.

The purpose of this volume is to show the possibility that how we could smoothly prevent, improve or alter the ARMD from our daily various diets.

March, 2006

Professor, Noboru Motohashi
Editor

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The Lutein - Prevention and Treatment for Age-related Diseases, 2006: 49-77
ISBN: 81-7895-219-X Editor: Noboru Motohashi

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Age Related Macular Degeneration (AMD), oxidative stress and nutritional factors

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Abstract

The Age-related Eye Disease Study (AREDS) study has shown that the pathogenesis of Age-Related Macular Degeneration (AMD), either atrophic or neovascular, involves nutritional factors related to oxidative stress. Indeed, antioxidant micronutrients, free radical scavengers or carotenoids protecting from the blue light such as lutein, play a role, probably in slowing the development of the disease. These nutritional factors interact with environment factors such as smoking or chronic blue light exposure that are oxidative stress factors. In addition, the ongoing intervention studies involving a

supplementation with Omega-3 polyunsaturated long chain fatty acids (PUFAs) such as Docosahexaenoic acid (22: n-3) (DHA) are probably also of great interest for the primary prevention of the disease.

In daily practice, a supplementation with omega-3 PUFAs could probably be proposed to patients with a known risk of AMD as a primary prevention of the disease. On the basis of the results of the Lutein Antioxidants Supplementation Trial (LAST) study, a supplementation with Lutein could be proposed to patients with an atrophic AMD. A supplementation with an "AREDS type" antioxidant formulation of micronutriments could be proposed to patients presenting an AMD at stages 3 and 4 as a secondary prevention. Above all, the basis of these possible supplementations need to be explained to the patients since only the AREDS formulation has nowadays proven an efficiency in a randomized multicentric study lasting for several years.

These supplementations are of course compatible with simple food advice and with advice about protection from general oxidative stress factors (smoking, sun light exposure). A better formulation and an optimization of the amount of the supplements currently proposed could increase their interest. New research and new clinical studies such as the AREDS II are ongoing in order to validate these formulations. A better validation could give these supplementations the status of drugs involved in an "evidence based medicine".

Introduction

The functional repercussion and the frequency of AMD have placed this disease at the rank of a public health problem (1). Moreover, the incidence and the prevalence of AMD increases with the ageing of the population and the improvement of the diagnostic tools (2). The AREDS and LAST studies have shown the involvement of oxidative stress and the importance of nutrition factors, especially at the earliest stages of the disease (3, 4). These studies also illustrated the possibility of delaying or of decreasing the incidence of the vision threatening complications of AMD. They have led to a modification of the rules of management of the early stages of AMD (5, 6). Taking into account the incidence of AMD, the simple fact of delaying the complications would greatly lower the visual impairment and the cost related to the disease.

More recently the publication of two meta-analyses (7-9), together with the results of the *Supplementation en Vitamines et Mineraux Antioxydants* (SU.VI.MAX) study (10) have questioned the interest and the safety of high amounts of oral antioxidants. Besides, other intervention studies involving supplementation with Omega 3 PUFAs and an AREDS II study testing different type of micronutrition supplementation are in process (11, 12).

For all these reasons, it seemed useful to give here a report about the nutrition factors involved in AMD.

Classification of AMD

Various nomenclatures of AMD have been proposed. The epidemiologists have developed a common classification and grading system (13). The term "early AMD" or Age related Maculopathy (ARM) is often used for the early stages of the disease, including soft drusen and pigment changes. Geographic atrophy and neovascular or exudative AMD are the endstages of the disease and often called AMD or "Late AMD". Table 1 describes the different stages of the international classification of ARM and AMD (14).

Figure 1 and 2 illustrates different lesions and stages described in table 1.

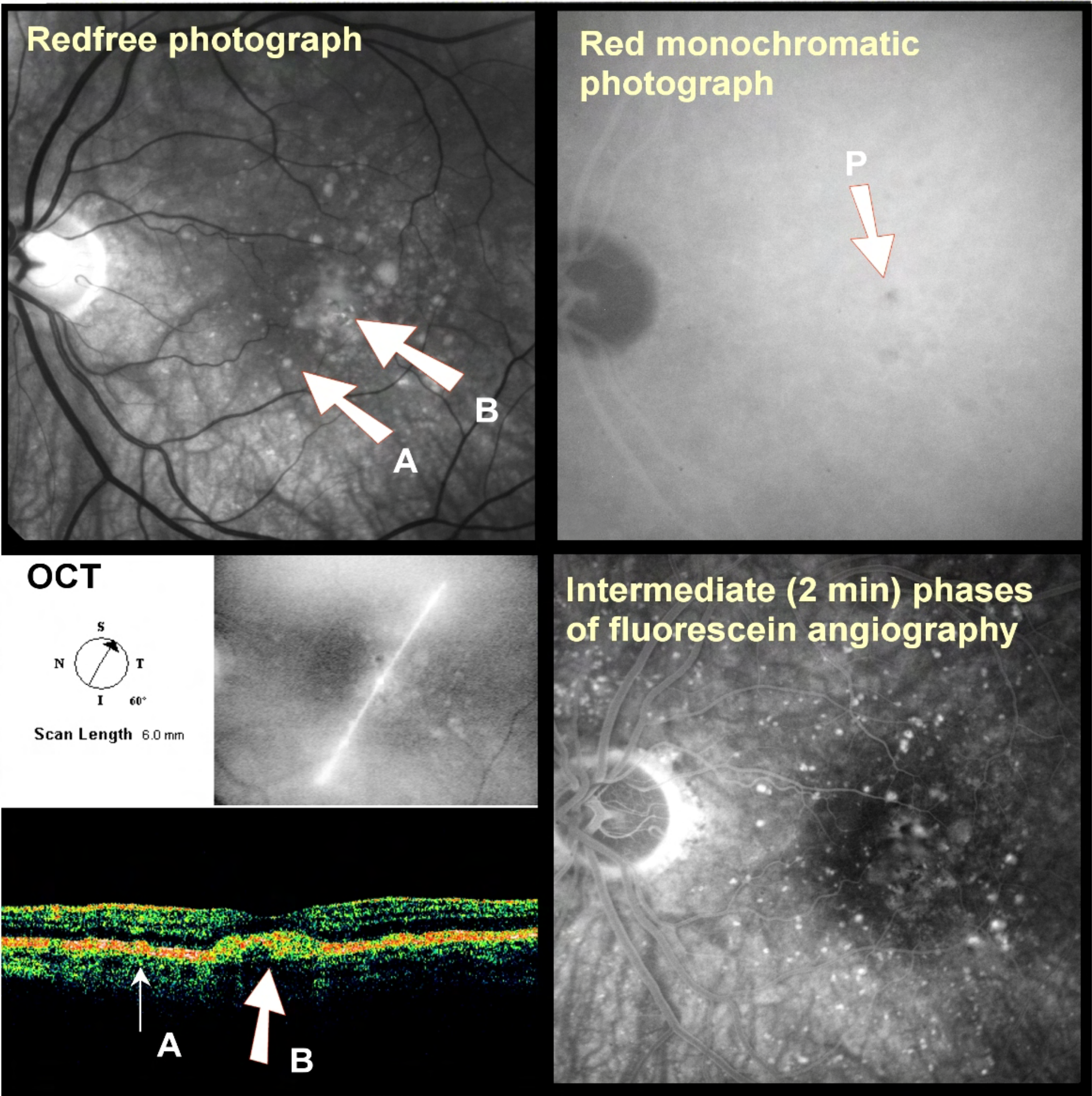


Figure 1. Stage III of AMD with different types of drusen associated with pigment changes. On the redfree photograph arrow A corresponds to a hard drusen (<63µm), while arrow B corresponds to confluent soft drusen. The red monochromatic photograph shows pigment changes (P). the OCT hardly discloses a hard drusen (A), and shows the absence of retinal thickening over the confluent soft drusen (B). On fluorescein angiographie the material of drusen is progressively colored without leakage.

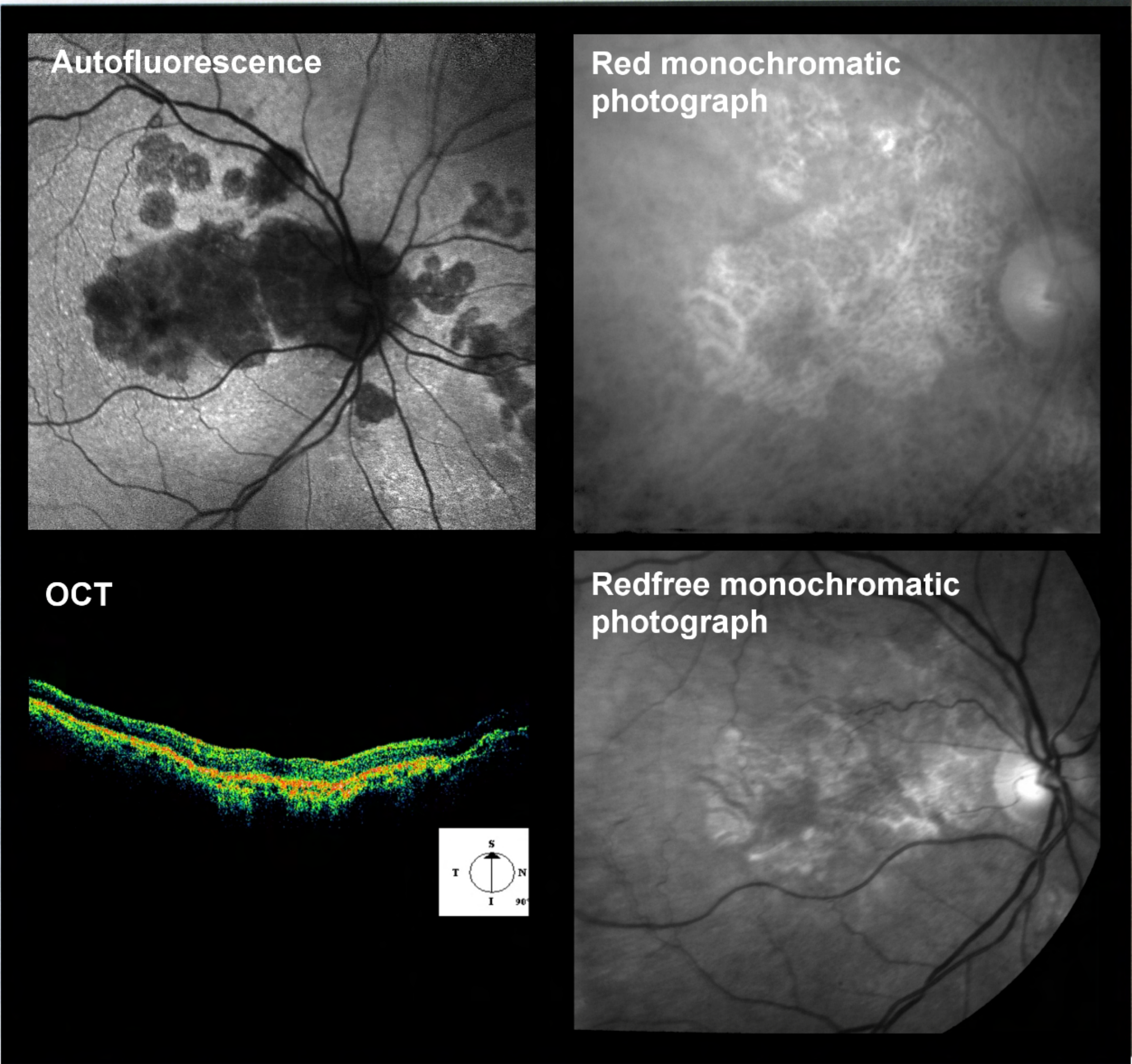


Figure 2. Stage IV. advanced atrophic AMD. The autofluorescence, the red monochromatic and the red free photographs show the areas with pigmentary epithelium atrophy. Some sparing of the foveal zone is although disclosed. The OCT better shows the persistance of a central zone of pigmentary epithelium.

Table 1. Classification of mutually Exclusive Stages of Age Related Maculopathy (ARM) and Age Related Macular Degeneration (AMD).

Stages of AMD		Definition
0	0a	No signs of Age Related Maculopathy
	0b	Hard drusen (< 63µm) only
1	1a	Soft drusen (≥ 63µm) only
	1b	Pigmentary abnormalities only, no soft drusen (≥ 63µm)
2	2a	Soft indistinct drusen (≥ 125µm) or reticular drusen only
	2b	Soft distinct drusen (≥ 63µm) with pigmentary abnormalities
3	3	Soft indistinct (≥ 125µm) or reticular drusen with pigmentary abnormalities
4	4	Atrophic or neovascular Age Related Macular Degeneration (AMD)



Figure 3. Stage IV: Neovascular form of AMD with classic subfoveal choroidal neovascularization.

Risk factors of AMD

The risk factors of AMD are often related to oxidative stress and / or to the metabolism of lipids. Therefore it is valuable to have here a short review of the main factors involved.

Genetic factors

The genetic factors of AMD involve several compounds. Among them, the polymorphism of complement factor H is the most recently discovered.

The polymorphism of the apolipoprotein E (ApoE) which is presented under three major alleles in the general population (e3, e2, e4) plays a role in the pathogenesis of AMD. The ApoE allows the reorganization of injured neuronal membranes in bringing constitutive lipids. Some authors have shown that allele e4 is more frequent among Alzheimer patients, perhaps because the ApoE4 would be less efficient than the other subtypes (15). On the contrary the patients with the APOE epsilon4 allele have been associated with a decreased risk of AMD (16-18). The epsilon 2 allele has conversly been associated with a slightly increased risk of AMD (16).

A part from being involved with the Stargardt disease (19), the ABCR gene could be involved in some atrophic or exsudative forms of AMD (20-22). Some authors have proposed that this gene would not be a factor of a major importance (23).

It has been shown recently that the polymorphism of factor H of the complement cascade could represent a risk for AMD (24-26). The factor H is a protein modulating the activation of complement. It can also modulate or

inactivate some components interacting with the complement cascade such as the C reactive protein. Variations of factor H would then lead to a chronic increase of some inflammatory reactions. Chronic inflammatory factors can start the activation of angiogenic factors (27). Interestingly, soft drusen representing an early stage of AMD contain some factors of the complement (28, 29).

Constitutional factors

Cardiovascular pathologies, arterial hypertension have been correlated to AMD (30-32). Some biological markers related to cardiovascular diseases have also been linked to AMD : the risk of presenting soft drusen which are markers of the early stage of the disease has been shown to increase with increasing levels of HDL-cholesterol (33). More recently, baseline cardiovascular disease biomarkers such as C reactive protein and Interleukine 6 have also been associated with an increased risk for progression of AMD (34).

Obesity was recently added to these constitutional factors (35, 36).

Clear iris has been correlated to the risk of AMD (37-41) although the importance of this factor has been a matter of debate.

Lastly plasma levels of carotenoids such as lutein have been linked to AMD (42, 43). This notion is coherent with the involvement of oxidative stress and nutritional factors in the pathogenesis of the disease (43-45).

Environment factors

Most of the environment risk factors of AMD are linked to oxidative stress. Among them, smoking is the strongest (46-51). An increase of the risk of AMD among smokers had initially been noted by Paekau in 1978 (52). Many studies then confirmed the association between cigarette smoking and AMD (42, 44, 47-49, 53-56). This association is particularly strong for the exsudatives forms of the disease (55) with a proportional increase of the relative risk depending on the amount of cigarettes a day (49). Two studies showed a reduction of the risk according to the duration after the cessation of smoking (55, 57). The importance of the association between cigarette smoking and AMD suggest a causal link (2).

Chronic exposure to the blue light has also been advocated (58-60) although the importance of this factor is still discussed (61, 62).

The deficiencies in vitamins (43, 63-66) and trace minerals (42, 64, 67, 68) have been recently proven to be risk factors of AMD. However, at first the studies evaluating the interest of vitamins or trace minerals prescribed separately did not showed any protective effect (67, 69). The probable involvement of oxidative stress in the pathogenesis of AMD (43, 58), the results of intervention studies including diets rich in carotenoids or other

antioxidants (63) led to undertake further studies such as the AREDS which illustrated a protective effect from a high dosage multivitamins cocktail (4).

More recently imbalances in the diet of fatty acids have also been shown to be risk factors of AMD (70-73). As pointed out by Cho et al. the total fat intake is related to the risk of AMD and this can be due to intakes of individual fatty acids, such as linolenic acid, rather than to total fat intakes (74). Higher intake of specific types of fat, including vegetable oil, monounsaturated, and polyunsaturated fats and linoleic acid rather than total fat intake has also be associated with a greater risk of AMD by Seddon (75). On the contrary, diets high in omega-3 fatty acids and fish have been inversely associated with the risk of AMD (74, 75). Higher intakes of omega-3 long chain polyunsaturated fatty acids (LCPUFAs) have also been associated with a decreased risk of neovascular AMD (11)

Lastly, another environment factor - cataract surgery - has been pointed out as a risk factor for AMD (76). However the presence of a cataract itself can be related to oxidative stress which can also be a factor of AMD (77).

Mechanisms

Early stages

Drusen are extracellular deposits that accumulate below the retinal pigment epithelium, on Bruch's membrane, and are risk factors for developing AMD (78). The early stages of the disease gathers the association of serous drusen and / or pigment changes. According to the size and the coalescence of drusen and the presence or absence of pigment changes various stages of the early stages of AMD are defined in table 1.

The foveal retina receives nutrients and oxygen from the inner layers, i. e. the choriocapillaris. These nutrients cross the Bruch membrane and then the pigmentary epithelium before reaching the photoreceptors. Besides, the foveal retina disposes waste material and fluids through the same elements toward the choriocapillaris. There is a strong interdependance between choriocapillaris, Bruch membrane, pigmentary epithelium and the retina cells.

With age, the permeability of Bruch membrane decreases (79) and the exchanges between choriocapillaris and the retina slowly decrease. Histology studies have shown that the first stage of the disease consist of an accumulation of basal laminar deposits on Bruch's membrane (80). Thereafter, the formation of the serous drusen on the inner face of Bruch's membrane represents a step towards the development of the disease (81). These drusen further decrease the exchanges between choriocapillaris and the retina.

With age, the concentration of lipofuscine which is a group of complex autofluorescent lipid/protein aggregates rises in the pigmentary epithelium cells (59). This has been correlated with a decrease of the capacity for

phagocytosis from the pigmentary epithelium. This reduction of waste phagocytosis leads to a further accumulation of waste materials on the inner part of Bruch Membrane (82-84). Further on, the confluence of drusen can lead to the formation of a drusenoid detachment of pigmentary epithelium that can evolve towards an exsudative or an atrophic form of the disease.

Oxidative stress

During the cellular metabolism, the consumption of oxygen and glucose for the production of TPA leads to the formation of reactive oxygen species (ROS) (85). These chemical species have a great reactivity and can cause significant molecular disorders related to the oxidation of the lipids, proteins, ADN leading to cellular lesions (86, 87).

The high reactivity of the ROS is normally compensated by many antioxidant systems (59, 88). As further developed in another chapter of this book, oxidative stress is an imbalance between the production of RLO and the antioxidant systems (85).

Antioxidants systems and retina

Several systems have a protective role against oxidative stress on the retina and in particular on the macular retina. The great amount of polyunsaturated lipids within the cells' membrane and the membranes of the disks of the photoreceptors, together with the high level of oxygen and the high metabolism of retina make this tissue particularly sensible to oxidation.

Non specific systems

The principal system of defense relies on **circulating antioxidants** with in first line specific enzymes (catalase, superoxyde dismutase, glutathione peroxydase), whose majority require the presence of metal cofactors (zinc, copper, selenium, manganese) (30-32, 89, 90). In second lines the antioxidant defense rely on the free radicals scavengers such as the vitamins E, C, A, (58, 59).

Heat shock proteins (HSP) can act as a non specific tissue response to oxidative stress. The function of these proteins is to maintain basic cellular functions both under basal condition and after cellular stress (91). Stress-related functions of HSP include the refolding, translocation and degradation of proteins. In this way, they help maintain the cytoskeletal integrity and metabolic homeostasis of cells (92-94). HSP hyperexpression has been shown after oxidative stress in ophtalmology (95-98).

Retina specific systems

In a more specific way in ophthalmology, **macular pigment**, mainly composed of two carotenoids, Lutein and Zeaxanthin acts as an antioxidant system. The macular yellow pigment has an absorption between 400 and

550nm with a peak at 460nm making it possible to play a filter role for the foveal photoreceptors (43, 99, 100). As the blue photons are the most energized, they can trigger the lipofuscin to generate ROS (101-104). Besides, a role in optimizing foveal vision by filtering blue light has been shown (105, 106). Further on, Lutein and Zeaxanthine can act as free radicals scavengers (43, 58, 107, 108). Lastly, an antiapoptotic role has been described (109). Then, the macular pigment has a protective role against oxidative stress, especially when induced by light.

The continual **replacement of the discs** of the outer segments of represent a second specific antioxidant system for the retina. The most recent discs, synthesized by the photoreceptor are located at the inner part of the stack and the oldest discs are located at the external part of it, beneath the pigmentary epithelium (EP) (110, 111). These old discs are regularly phagocytosed by the RPE and the external segment of each photoreceptor is renewed in approximately 10 days. Some waste products are recycled, but the majority of them are eliminated through the choroidal circulation (81, 112). Thus, the integrity of retina partly relies on the capacity of the RPE to digest the external segments of the photoreceptors, and on the capacity to eliminate waste towards circulation (43, 113).

Aging of retina and choroid favors oxidative stress

With age, the accumulation of lipofuscin in the RPE cells leads toward a decrease of phagocytosis and toward a blue light susceptibility (59).

The decrease of phagocytosis leads to an accumulation of materials under the RPE cells that decreases the intake of circulating antioxidants. Blue light triggers the production of ROS, also generating an oxidative damage .

Among patients with AMD these phenomena would be earlier than among other subjects, starting near the parafoveal rods (114). During the disease, the mechanisms of defense from oxidative damage would be decreased and the production of free radicals would be increased.

Complex mechanisms are involved in the pathogenesis of AMD. All of them are not directly related to oxidative stress. The onset of choroidal neovascularization (CNV) for example is probably related to a change in the balance of growth factors derived from the RPE (80).

Lipids

In cardiology, epidemiological studies have demonstrated a strong correlation between omega-3 Long Chain PUFAs intakes and a reduction of sudden death from myocardial infarction (115, 116). This benefit has been linked to the presence of docosahexaenoic acid (22: 6 n-3) (DHA). DHA consumption reduces plasma triglycerides and the risk of thrombosis. It also

prevents cardiac arrhythmias (117, 118). Other benefits of DHA have been reported, for inflammatory autoimmune diseases (119) or for the development of the nervous system, including the retina (72, 120-122) suggesting a role in ophthalmology.

For AMD, several epidemiological arguments suggest a important role of the balance between saturated and unsaturated fatty acids in the pathogenesis of the early stages of the disease. Moreover, among the unsaturated fatty acids, the balance between omega-3 and omega-6 PUFAs also seems involved with a special protective role of the omega-3 PUFAs (12).

Epidemiological studies

Higher vegetable fat consumption has been associated with an elevated risk for AMD (75). In a study involving 349 subjects, the risk for AMD was significantly elevated for the highest vs lowest quintiles of intake of monounsaturated and polyunsaturated fats. On the contrary, a high intake of omega-3 fatty acids has been associated with a lower risk for AMD among individuals consuming diets low in linoleic acid (an omega-6 fatty acid) (75). The protective role of omega-3 fatty acids and especially of Docosahexaenoic acid (DHA) has been further described (11). This omega-3 Long Chain PUFA is present in fatty fishes (salmon, tuna, mackerel) and mother's milk. DHA is also present at low levels in meat and eggs (117). EPA, another long-chain n-3 fatty acid, is also present in fatty fishes.

Roles of DHA

DHA is the most abundant fatty acid of membranes in photoreceptors. There, it contributes to membrane fluidity (123) and influences functional responses in the retina (124). ERG measures realized among X linked Retinis Pigmentosa patients, suggest a beneficial role for long-term nutritional supplementation in retarding the progression of this disease (124).

DHA enhances the lysosomal acid lipase activity of the RPE, providing a more efficient hydrolysis of intralysosomal RPE lipids components, including lipofuscin (125). Thus, diets rich in fish oil-derived omega-3 fatty acids may reduce RPE lipofuscin accumulation and the development of AMD (125).

One of the roles of DHA is also to provide EPA by retroconversion which, in turn, will be oxygenated to biologically active metabolites that may affect other eicosanoids (123). Some authors have shown that DHA has a high affinity for the interphotoreceptor retinoid-binding protein (IRBP), a predominant protein in the interphotoreceptor matrix of the retina implicated in transfer of retinoids between retinal pigment epithelium and photoreceptor cells (126).

Lastly, an *in vitro* study has shown that DHA can activate intracellular mechanisms modulating the levels of pro- and anti-apoptotic proteins (Bcl-2 family) selectively protecting photoreceptors from oxidative stress (127).

Although several authors have questioned a pro-oxidant role on the retina induced by high amounts of DHA (128-130) no side effect has been reported in the intervention studies (11, 124, 131).

Vitamins and trace minerals

Vitamin C is an antioxidant able to reduce the oxidized vitamin E. The antioxidant properties of this molecule find a logical role in the lens because of its concentration in this tissue. On the contrary, the concentration in Vitamin C is not substantial among the macular retina. Two pilot studies (132, 133) and two intervention studies (134, 135) did not show any significant link between the plasma rates of vitamin C and the risk of AMD. Other studies showed either a non significant or a slightly protective effect of Vitamin C for the prevention of AMD (63, 132). Then, the interest of a supplementation with Vitamin C alone for the prevention of AMD in an attempt of reducing oxidative damage involved in the disease is still a matter of debate. However, the report # 8 of the AREDS study showed a significant result for the prevention of secondary complications of AMD by a formulation of micronutriments with high amounts of vitamin C (4 32, 32).

Vitamin E, is also a strong antioxidant. Vitamin E consumption was not associated with a statistically significant reduced risk for AMD in the Eye Disease Case-Control Study (63). In the Baltimore longitudinal study of aging the data could suggest a protective effect for AMD of high plasma rates of Vitamin E but the authors concluded that the use of vitamin supplements to prevent AMD is not supported by their data, which showed no protective effect of vitamin use (69). On the contrary, in the POLA study, a significant reduction of AMD has been shown among the subjects having the highest plasma concentrations of vitamin E (133). The metabolism of the vitamin E is complex, interacting with the metabolism of lipids and involving a specific protein of transport (136). Moreover the tissue level of the vitamin E is not strongly related with the food intakes (137-139). Here too, the AREDS formulation contained high amounts of vitamin E.

Betacaroten or provitamin A is a carotenoid having significant antioxidant properties. This molecule is not found with high concentration in the retina (conversely to other carotenoids such as Lutein or Zeaxanthin in the macular pigment with a protective role arising logically from their properties and localization). The protective effect for macular oxidative stress of this molecule is not definitively established. Potential side effects among the smokers or former smokers had been highlighted for supplementations at amounts higher than the recommended dietary allowances (RDA) (140-144).

The USA Food and Nutrition Board currently recommends contributions from 3 to 6 mg/j (145). Recently, a meta-analysis taking into account the data of 14 trials with antioxidant vitamin cocktails questioned the safety of high amounts of betacaroten (7, 8).

Zinc is a cofactor of the catalase and of the superoxide dismutase which acts as a free radicals scavenger (89, 90). Zinc is also a cofactor the retinol deshydrogenase which plays a role in the metabolism of the visual pigments and in the metabolism of the retinol binding protein transporting vitamin A. Zinc concentration is high in the retina suggesting a protective role. Only one study had shown a protective effect of zinc for AMD (90). Other studies did not shown any reduction in the relative risk of AMD (74, 132, 134).

Selenium is a cofactor of the glutathione peroxidase (146-149). This enzyme is able to reduce lipidic peroxides and thus plays a role in the control of the oxidative stress. Some authors showed a reduction in the selenium rates among patients presenting an AMD (149).

AREDS

Design and results

The Age-related Eye Disease Study (AREDS) is a multicentric controlled randomized study carried out as a clinical trial of phase III for a drug testing the interest of a multivitamin antioxidants micronutriments. The patients were included until 1998 (4) and the study will close in December 2005. A total of 3 557 patients between 55 and 80 years old were included. The mean follow-up was 6.3 years. The patients were divided into 4 stages according to the gradation of the lesions of the fundus. In each stage, the patients were randomly assigned in one of the 4 groups of treatment: placebo, antioxidants, zinc, antioxidants and zinc. The amounts of vitamin C, vitamin E and of zinc of the study often largely exceeded the recommended dietary allowances (table 2). The administered doses were 3 to 10 times higher than the recommended dietary allowances (RDA) for betacaroten or the vitamin C and up to 60 times for vitamin E.

Table 2. Areds doses and Recommended Dietary Allowances from (171) and (172)M : Male ; F : Female).

	AREDS	RDA	
		FRANCE	USA
Vitamin C (mg)	500	110	75 (F) to 90 (M)
Vitamin E (mg)	268 (400UI)	12	15
Betacarotene (mg)	15	2.1	4.2 (F) - 5.4 (F)
Zinc (mg)	80	10 (F) to 12 (M)	8 (F) to 11 (M)
Cu (2,5mg)	2	1.5 (F) to 2 (M)	0.9

Besides, two thirds of the participants of the study took vitamin supplements corresponding to approximately 100% of the recommended dietary allowances. At year 1 and at year 5 the participants had the increase of the serum rates of vitamin C, vitamin E, betacarotene and zinc which could be awaited according to the supplemented groups considered. A reduction of the plasma rates of lutein and zeaxanthin was also observed (reduction of approximately 22% in the antioxidant groups compared to 7% in the placebo group). The two main endpoints were the progression of the disease (occurrence of a complication or progression) and vision (evaluated once a year).

The main result of the study was a reduction of 25% of the risk of progression of the disease in the group receiving zinc and antioxidants for patients with drusen of intermediate size or large the drusen (stage 3) or an advanced AMD on the "first eye" (stage 4) (the risk of progression was decreased from 16% to 12 % at 5 years). Among these patients, the risk of vision loss was also decreased (reduction of 27% of the risk of loss of 15 letters on an ETDRS chart). On the basis of these results, the authors concluded to the interest of a supplementation with vitamins and trace minerals among patients presenting serous drusen whose size is higher than 125 μ m or whose total surface represents more than 1/5th of the optic disk area and among patients presenting a unilateral advanced AMD or an atrophy.

Comments

The report # 8 of the AREDS represents a significant step forward showing the role of oxidative stress in the pathogenesis of AMD. This study has provided a strong basis to propose a supplementation with antioxidants micronutriments to the patients on stage 3 and 4 of AMD.

However, the duration of this study and the improvement of knowledges about the pathogenesis of AMD since its onset give way to some remarks concerning the design and the conclusions of the study.

The publication of the study L.A.S.T. (Lutein Antioxidant Supplementation Trial) reporting an interest of lutein supplements for atrophic forms of AMD in 2004 (3) is largely posterior to the AREDS. Thus, when the AREDS began, the knowledges about macular pigment were recent and no supplement of Lutein nor Zeaxanthin were available (150). For this reason, several authors pointed out that the AREDS formulation comprises neither lutein (L) nor zeaxanthin (Z) (150, 151). The plasmatic reduction of the L and Z rates observed in the treated groups of the AREDS even makes it possible that the betacaroten supplementation decreased the intestinal absorption of L and Z by a competition of absorption (152). The choice of betacaroten as a provitamin A carotenoid having antioxidant properties was not justified in the AREDS, nor was the high dosage of this supplement. Some authors have

pointed out the interest of another carotenoid, lycopene which has 11 double bonds leading to significant antioxidant properties (153-155)

Some authors have noticed that the zinc oxide used in study AREDS has a reduced biodisponibility (156). Then both the interest and the risk of a toxicity from zinc would have probably been more significant if zinc sulphate or gluconate had been used (151). In the same way, the copper oxide added to the vitamin cocktail in prevention of an anaemia is often regarded as inert, not absorbed by the nonruminant animals (156). For some authors, it is probable that the copper oxide used in the study had no great effect (151).

The statistical design of the AREDS carried out in a prospective randomized way like a clinical trial for a drug is rigourous. However subgroups have been separated a *posteriori* undermeaning the significancy of the results. Thus the analysis of report 8 of the AREDS tends to be a retrospective analysis of an initially prospective study.

After the AREDS

In November 2004, the results of two meta analyzis have questioned the harmlessness of high antioxidant amounts of micronutriments close to those of the AREDS. The first study published by Bjelakovic (7, 8) questions the role of Betacaroten. In the study published by Miller, high amounts of vitamin E are questioned (9).

The study of Bjelakovic, gathers the data of 14 randomized tests with 170 525 subjects and evaluating the interest of supplements with antioxidant vitamins (A, C, E, betacaroten, Selenium) for the prevention of digestive cancers were analyzed. In this meta analyzis, not only the antioxidants, (alone or in combination) have not showed a significant interest on the prevention of digestive cancers but the mortality of the subjects receiving the supplements was increased. The authors calculated that according to the analyzed data, given a risk of mortality of 6,7% in the placebo group and a relative risk of surmortality of 1,06 in the treated group, each time 112 subjects are supplemented, 1 additional death is registered in the treated group.

In the study published by Miller, 135 967 participants from 19 clinical trials were analyzed. Vitamin E was used alone or in association and the amounts ranged between 16.5 and 2000 UI per day. The analysis showed a statistically significant relation between the amount of vitamin E and mortality (whatever its cause) of the subjects, with an increased risk for the amounts higher than 150 UI per day. In this analysis, high amounts of Vit E (400 UI/J) were often related to small samples with patients with chronic diseases. The generalization of the results to the adults in good health thus remains troublesome. In the same way, the precise evaluation of a threshold from which the risk increases is difficult. The authors conclude that the supplementations with high amounts of vitamin E (400 UI/J) can increase the

mortality of the subjects and should be avoided. These data do not question the role of oxidative stress in AMD nor the interest of nutritional supplementations moderately higher than the RDA, such as in the study SU.VI.MAX. whose results were recently published (10).

Age-related eye disease study II (AREDS II)

Since a number of scientific questions remain unanswered while the AREDS is closing in December 2005, the National Eye Institute will undertake an AREDS II with the primary objective to determine whether oral supplementation with macular xanthophylls (lutein at 10mg/d and zeaxanthin at 2mg/d) and/or omega-3 long-chain polyunsaturated fatty acids (DHA + EPA at 1g/d) will decrease the risk of progression to advanced AMD, as compared to placebo. AREDS II will include around 4000 participants with moderate risk of AMD (stage III or stage IV in one eye).

Macular pigment

The term of Macula lutea initially refers to the reflection related to the zone of accumulation of the yellow (or xanthophyll) pigment in the center of the foveal depression (99, 100). This term macula lutea gradually shortened to "macula" whereas the concept on the contrary widened to indicate the zone from the 3 to 4 mm around the foveola (100). In 1945 G. Wald identified the macular pigment as a carotenoid found in green leaves (157). In 1985 Bone showed that the macular pigment was a mixture of Lutein and Zeaxanthin (158).

Physico-chemical structure

Lutein and zeaxanthin with a third component, mesozeaxanthin (in lower proportion) were characterized during the years 1980'. Lutein and zeaxanthin are two hydroxycarotenoids isomers of betacarotene without vitamine A activity (158). These molecules are of dietary origin and not synthesized by humans although zeaxanthin is a metabolite of lutein.

The antioxidant activity of lutein and zeaxanthin and their protective role from macular oxidative stress were mostly evaluated during the years 1990' (43, 153, 159, 160). The density of macular pigment varies in the 2 central mm. In the center of the foveal zone, 50 to 94% of the incidental light can be absorbed by this pigment. The peak of absorption to 460nm corresponds to the peak of absorption of the cones S, providing a quasi specific protection of these cones by the macular pigment (106, 114, 161). Lutein and zeaxanthin are localised in the layer of fibres of Henle in the center of the foveola (43, 99, 100). In the juxtafoveal zone the pigments are partly localised in the plexiform internal layer. In addition, the respective concentrations of lutein and

zeaxanthin vary between the center of the foveola and the juxtafoveal zone. Zeaxanthin is predominant near the foveola whereas Lutein is the main pigment in the juxtafoveal zone. Lutein and zeaxanthin are also found in the axons of photoreceptors and in their outer segment. They can be found in the pigmentary epithelium cells (43 294).

Role of lutein and zeaxanthin

The relative sparing of the foveal zone at the early stages of the atrophic forms of AMD can be attributed to the protective effect of macular pigment. In the same way, drusen are for a long time coalescent within the temporal part of macula before reaching the foveal zone (162, 163).

This assumption about the protection by macular pigment is congruent with the concept according to which the first photoreceptors damaged in the AMD are the rods. It is considered that during a 90 years life, approximately 30% of the rods within the 4 mm around the center of the fovea are lost and that the percentage of rod loss is most significant within a ring between 0.5 and 3 mm starting from the center (114, 164, 165). The lesions of AMD innitally appear within this ring (166).

The antioxidant properties of Lutein and Zeaxanthin find here a protective role for the neuroretina and pigmentary epithelium (43, 167). Conversely to the other carotenoids such as Betacarotene which are abundant in blood, only lutein and zeaxanthin are present at high concentration in the macular retina.

The presence of the hydroxyles groups on the hydrophobic cycles at the ends of these molecules could play a role to explain this selective concentration. These hydroxyles groups miss on the Betacaroten molecule. They could play a role for a binding to phospholipids of the wall of the cones and rods allowing an appropriate space orientation (168).

Supplementation

The protective role of macular pigment from oxidative stress led to an evaluation of the variations of its density among patients with or without AMD. Some authors have showed that age and an advanced AMD in the fellow eye (two most important risk factors for AMD) are associated with a relative absence of MP (167).

Then, some studies aimed to determine whether MP density in the retina could be raised by increasing dietary intake of carotenoids from foods. Indeed, among dietary carotenoids routinely detected in human plasma, only lutein and zeaxanthin appear to accumulate in the human retina at much higher concentration than other equally prominent carotenoids such as lycopene, alphacarotene and betacarotene (160).

In a series of 13 subjects, an increase in MP density was obtained within 4 weeks of dietary modification for most, but not all, subjects. When MP density

increased with dietary modification, it remained elevated for at least several months after resuming an unmodified diet. Augmentation of MP for both experimental and clinical investigation appears to be feasible for many persons (159).

Another study involving 8 subjects, showed that supplementation with lutein can significantly increase the density of the MP (169).

The response to dietary lutein and zeaxanthin in macula varies among individuals with "responders" subjects and "not responders" (159). Adipose tissue is a major storage organ for carotenoids (170). Serum concentration of carotenoids seem to correlate to the recent intakes of carotenoids, whereas adipose tissue concentration of carotenoids would be a better indicator of long term intakes (159). Then given the large range among people in the amount of body fat, adipose tissue and body composition may have strong influence on the distribution of carotenoids within the body.

The LAST study involved a moderate dose of Lutein supplementation and showed an interest for the management of atrophic forms of the AMD (3). The study was designed as a randomized double blind trial with control over 12 months among American veterans between August 1999 and May 2001. Ninety patients with an atrophic AMD were referred by ophthalmologists towards two medical centres.

Patients of group 1 (L) received Lutein 10mg/J; in group 2, Lutein 10mg was associated a cocktail of antioxidant supplementation with significant amounts (vitamin A, vitamins of group B, vitamin C, vitamin D, vitamin E, glutathione, zinc, selenium etc..) ; in group 3, a placebo of maltodextrine was used. In groups 1 L and 2 L/A, the optical density of macular pigment increased (roughly of 0,09 units log compared to the base line). Vision improved of 5,4 ETDRS letters in group 1 (L) and of 3,5 letters for the group 2 (L/A), and the contrast sensitivity also improved. There were also a clear subjective improvement of the metamorphopsia and/or scotomas evaluated with an Amsler grid, in group 1 (L).

Among patients with the placebo (group 3) no significant change of these parameters was observed. Thus, although the follow up is relatively short and the endpoints were often subjective, a certain number of parameters were improved during the study. As pointed out by the authors, other studies remain necessary with more patients and a longer follow up to evaluate the long-term effects of Lutein on vision of patients with an atrophic AMD.

Conclusion

The epidemiology of nutrition factors involved in AMD helps to better understand the pathogenesis of the disease. These factors are multiple and intricate, and have a role at different levels.

The role of nutritional factors related to oxidative stress has been illustrated here. Then antioxidant micronutrients, free radicals scavengers or the carotenoids protecting from the blue light such as Lutein, are of importance for a protection from the disease. As mentioned, these nutrition factors are intricated with environment factors such as smoking or chronic exposure to blue light.

In practice, as illustrated on table 3, simple food advice to the patients and cessation or reduction of cigarette smoking would be elementary advice for patients at risk (e.g. with a family history) of AMD or with drusen smaller than 125µm. At these stages, based on pilot studies and while waiting for the results of the AREDS II study, a supplementation with omega-3 fatty acids (DHA) could also be proposed.

For atrophic AMD, the use of an optimized lighting and a magnifying glass for reading and of glasses clips filtering blue light for outdoor can be associated with food advice and / or lutein supplementation on the basis of the

Table 3. Primary prevention and secondary prevention that could be proposed for ARM and AMD. The primary prevention is based on very few monocentric intervention studies. This primary prevention would include food advice and possible DHA supplements.

The secondary prevention is based on epidemiologic studies about micronutrients and on the results of the AREDS and LAST studies. This secondary prevention involves a possible supplementation with a multivitamins cocktail "AREDS type" or with lutein (and zeaxanthin). The results of the AREDS II study will be of great interest to assess the amount of vitamin E and betacaroten in these multivitamin formulations.

ARM or AMD	Type of prevention	Remarks
Advanced AMD : - Atrophic or neovascular Age Related Macular Degeneration (AMD)	Secondary prevention of further complication	Stage 4 of AMD. Prevention is aimed to protect the fellow eye
Mild lesions - Atrophy without involvement of foveal zone - Soft indistinct ($\geq 125\mu\text{m}$) or reticular drusen with pigmentary abnormalities	Secondary prevention of further complication	Stade 3 of AMD. Prevention is aimed to protect the "first" eye with the lesions but also the fellow eye if there are less evolved lesions
Age Related Maculopathy - Soft indistinct drusen ($\geq 125\mu\text{m}$) or reticular drusen only - Soft drusen ($\geq 63\mu\text{m}$) - Pigmentary abnormalities Risk factor of AMD - Family history - Smoking - Hypercholesterolemia - Etc. Patients with a nutrition deficit	Primary prevention aimed to delay or prevent the onset of disease	Stages 1 and 2 of ARM

L.A.S.T. study. The relative short follow up of the study and the need for other intervention studies to confirm the results must be explained to the patients.

For the more advanced stages of the disease, (stages III or IV) an AREDS formulation can be proposed. Here too, an explanation is of utmost importance for the patients. Even if in the AREDS study no severe side effect has been described, the high doses of the cocktail must be balanced with the results of the meta-analysis by Bjelakovic and Miller, especially for younger patients.

In the future, an optimization of the formulation of the supplements currently proposed after the publication of the results of the AREDS II study could increase their interest.

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