Age-Related Macular Degeneration
– Potential Roles for Nutraceuticals in its Prevention and Control

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Abstract
Many lines of evidence point to oxidative stress as a key pathogenic factor in age-related macular degeneration (AMD); the modestly positive results of the AREDS1 study – which evaluated supplemental zinc, vitamin E, vitamin C, and beta carotene in AMD patients - provide confirmation for this view, while also pointing to a potential role for nutraceuticals in prevention and management of this syndrome. Xanthophyll carotenoids, the key components of macular pigment, appear to play a crucial physiological role in protecting the macula from light-induced oxidative damage, and docosahexaenoic acid (DHA), a major constituent of photoreceptor outer segments, may be needed for optimally efficient rhodopsin function and photoreceptor survival. Epidemiological studies demonstrate reduced risk for AMD in people with increased dietary intakes and tissue levels of long-chain omega-3s and of the xanthophylls, and the ongoing AREDS2 study will determine whether supplementation with these agents can aid AMD control. Ancillary antioxidant measures may also prove useful in this regard. Phase 2 inducers (including lipoic acid and a wide range of phytochemicals), melatonin, and N-acetylcysteine, may have the potential to boost retinal expression of glutathione and of a number of antioxidant enzymes. In light of the recent discovery that the chief phytochemical in spirulina can inhibit NADPH oxidase – a likely source of retinal oxidative stress – spirulina supplementation may merit consideration in AMD management. The recent WAFACS study demonstrates important primary prevention of AMD with high doses of the B vitamins folate, pyridoxine, and cyanocobalamin; conceivably, this reflects the ability of high-dose folate to boost nitric oxide production by oxidatively-stress choroidal vessels. Suggestive evidence that estrogen may reduce risk for wet AMD raises the possibility that soy isoflavones could likewise provide benefit in this regard. It is not unreasonable to hope that comprehensive nutraceutical strategies that are rational and complementary will someday demonstrate very considerable efficacy in the prevention and control of AMD. Moreover, there is reason to suspect that lutein and zeaxanthin may aid prevention of cataract (as they provide antioxidant protection for the lens) while helping to combat visual fatigue and glare.

Pathophysiology of AMD
Age-related macular degeneration (ARMD, AMD, ARED), or aging macula disease, is the leading cause of vision loss and partial blindness among elderly Americans 60 and older.¹ This group represents an increasingly larger percentage of the general population; hence vision loss from macular degeneration is a growing problem. Men and women are about equally affected, and the incidence of AMD in blacks is lower than it is in whites. AMD is the most common cause of visual impairment in the developed world.
AMD is a degenerative disease of the macula, which is the part of the retina responsible for the sharp central vision needed to read or drive; central vision loss is likely to occur. The National Eye Institute estimates that there are presently about 1.75 million people in the U.S. with advanced AMD and associated vision loss. The number is expected to grow to approximately 3 million by 2020.

AMD may be manifested as a sudden worsening and distortion of central vision and may progress rapidly with a course of only weeks or months. It is characterized by abnormalities in the macula area. The central area or fovea of the macula contains the highest density of cone photoreceptors in the retina and mediates high-acuity vision. Typically, AMD has a preclinical, asymptomatic phase, in which extracellular waste material accumulates in the space between the basement membrane—Bruch’s membrane—and the pigmented epithelial layer (comprised of retinal pigmented epithelial cells – RPE) which overlies it, forming yellow-white spots known as drusen.

RPE cells are remarkably versatile in their contribution to healthful retinal function, and it is generally believed that dysfunction or death of these cells is crucial to AMD pathogenesis. The retinal pigmented epithelium serves as the blood-retinal barrier, and regulates the transport of oxygen, substrates and waste products between the choroidal vasculature and the retina. Loss of RPE tight junctions – which evidently compromises this barrier function – is a hallmark of AMD. RPE cells also generate growth factors that aid the survival of retinal photoreceptors and other cellular constituents of the inner retina, while suppressing pathological choroidal neovascularization. The most curious function of RPE cells is to phagocytize photoreceptor outer segments (POS). While doing this, they isomerize all-trans retinyl esters derived from POS back to 11-cis-retinal, which is then returned to the photoreceptors for use in rhodopsin synthesis; this is a crucial function, as photoreceptors are incapable of performing this isomerization themselves. The phagocytic function of RPE cells entails continual lysosomal activity; when this activity is sub-optimally efficient, as it appears to be in AMD, lipofuscin deposits accumulate in these cells. Lipofuscin has photosensitizing activity, and components of lipofuscin – notably A2-E, a retinoid metabolite – can impair lysosomal function, in part by blocking the proton pump that acidifies lysosomes. (This raises the specter of a vicious cycle in which lipofuscin accumulation encourages further accumulation of lipofuscin). Proteomic studies indicate that the drusen that characterizes AMD is derived largely from inadequately degraded POS components as well as lysosomal proteins – suggesting that stressed RPE cells are disgorging half-digested lysosomal contents into the sub-epithelial space. Drusen is also rich in complement metabolites; this accords well with the discovery that AMD is more common in individuals who carry reduced-function alleles of key proteins that regulate the complement cascades. Degeneration of the RPE is considered the most important hallmark of AMD. This is characterized by pigment mottling, accumulation of intercellular lysosomal lipofuscin and extracellular drusen, loss of tight junctions, and apoptotic cell death. As discussed below, chronic oxidative stress and inflammation are strongly linked to RPE senescence and the pathogenesis of AMD.

Advanced forms of AMD include both dry and wet (or neovascular) AMD. The dry form of AMD is far more common, but the wet form occurs together with the dry form in about 15% of cases. Dry AMD is characterized by progressive apoptosis (programmed cell death) of the light-sensitive macula part of the retina, required for fine vision; progressive apoptotic loss of photoreceptors, RPE cells, and the underlying cells in the choroidal capillary layer is noted. Wet AMD is characterized by choroidal
neovascularization with vascular leakage into subretinal spaces, and can lead to sudden, catastrophic, and permanent visual loss.

AMD impairs central vision. Neurosensory detachment, retinal hemorrhages, retinal scarring, and photoreceptor apoptosis impair the ability of photoreceptors to mediate central vision, resulting in legal blindness, with preservation of peripheral vision. AMD is the most common cause of blindness among the elderly. Subjects with a family history of AMD, smokers, and people who are obese or sedentary are also at increased risk for this syndrome. People who are genetically prone to increased activation of the alternative complement pathway – such as those carrying certain alleles of the gene for complement factor H – are at greatly increased risk, perhaps reflecting a role for complement in drusen formation.

Contributing factors for AMD, include light-induced toxic effects, especially from blue light and ultraviolet light and the exogenous chromophores—amiodarone, chloroquine, phenothiazines, lithium and herbs such as St. John's wort. These exogenous chromophores can increase susceptibility to light-induced toxic effects.

However, subjects who have a favorable risk profile may also develop AMD. Clearly, the most important risk factor for AMD is simply getting older.

The Ocular Pigments – Mr. Soemmering’s curious discovery of a yellow spot

In 1798, Everard Home, Esq, FRS, presented to the Royal Society of London a curious discovery made by a Professor Soemmering in 1795. Soemmering, a respected anatomist, carefully examined the retina of a young man who had drowned, and observed a yellow spot with, what he thought, was a small hole in its middle. In further studies, he noted that this yellow spot was pale in the young and old, and brighter in young adults. The yellow spot is of course the macula lutea (from Latin macula, “spot”, and lutea, “yellow.”), also known as macular yellow. And, to be historically correct, the first recorded observation of macular yellow was made by F. Buzzi in 1782.

It was years later (1945) that the Nobel Laureate George Wald identified the yellow spot as a macular yellow pigment. He extracted a small number of maculas with benzene or chloroform, and found the yellow pigment to be a hydroxy-carotenoid or xanthophyll; he thought that in all probability it was comprised of the dietary xanthophylls lutein and zeaxanthin. In the 1980s, R.A. Bone and his colleagues confirmed that Wald’s hunch had been correct.

Lutein and zeaxanthin belong to the the xanthophyll class of carotenoids, also known as hydroxy-carotenoids. They are natural fat-soluble yellowish pigments found in some plants, most notably green leafy vegetables (spinach, collard greens, etc.), algae and photosynthetic bacteria. Humans are unable to synthesize these substances and must obtain them from their diets; hence, they are essential nutrients. In the organisms which make them, they serve as accessory light-gathering pigments, and provide protection from the harmful impact of ultra-violet radiation and the reactive oxygen species which it gives rise to. The light energy which they collect is used to drive photosynthesis; in this respect their function is similar to that of chlorophyll. In humans, these pigments are found in extraordinarily high concentrations in the macula; they are also found in the crystalline lens. Their physiological location, as well as their ability to absorb blue light and quench dangerous singlet oxygen, suggest that they function to protect the macula
and the lens from light-induced oxidative stress. Indeed, it is now strongly suspected that they play an important role in providing protection against age-related macular degeneration (AMD) and possibly cataracts as well – a conclusion that is concordant with reports that macular pigment density tends to be lower in patients with AMD. (Lower pigment density in AMD does not appear to be a trivial consequence of photoreceptor loss, since pigment density in AMD eyes also is decreased in peripheral retinal areas not affected by such loss.) Macular pigment also functions to reduce the impact of light scatter and chromatic aberration on visual performance.

A number of studies have demonstrated that the amount of the macular pigment can be increased by dietary supplementation with lutein or zeaxanthin or by ingestion of xanthophyll-rich foods. Lutein and zeaxanthin, as well as their esters, are efficiently absorbed – more avidly so than beta-carotene - and ultimately wind up in the macula; their trans isomers are most available. In foods and in supplements, lutein and zeaxanthin are found mainly in their ester forms, which undergo intestinal hydrolysis to their free forms before being absorbed and ultimately incorporated into the macula. Xanthophyll uptake by RPE cells – which constitute the blood-retinal barrier, and hence regulates the availability of xanthophylls to photoreceptors – is mediated by scavenger receptor class B type I, which has relatively low affinity for beta-carotene. The characteristically high concentration of xanthophylls in the macula may also reflect the presence of a xanthophyll binding protein (XBP); this protein protects xanthophylls from degradation by reactive oxygen species, and the XBP-xanthophyll complex may have better antioxidant activity than the free xanthophyll. A retinal protein which specifically binds lutein has also been characterized recently. Macular pigment also contains meso-zeaxanthin, which is derived from isomerization of lutein within the macula. Although little meso-zeaxanthin is found in natural diets (it is found in seafood, but not in vegetables) or in plasma, supplementation studies show that orally administered meso-zeaxanthin is well absorbed and can improve macular pigment density. Another intriguing natural xanthophyll is astaxanthin, produced by certain types of algae; this is what makes flamingoes pink! Astaxanthin has more versatile antioxidant activity than any of the other xanthophylls, but it is not a significant component of most human diets, and its impact on macular pigment or retinal function remains to be assessed.

It should be noted that, unlike beta-carotene, the xanthophyll carotenoids lack pro-vitamin A activity. In other words, they cannot give rise to the 11-cis-retinal required for rhodopsin synthesis; dietary carotene or pre-formed retinol is required for this purpose. In light of epidemiological evidence that dietary pre-formed retinol has the potential to compromise bone density, modest intakes of dietary beta-carotene, from foods or supplements, may be the most prudent way to meet the retina’s requirement for retinal. However, there is no evidence that beta-carotene contributes notably to antioxidant protection of the retina.

In 1994, Dr. Johanna Seddon and co-workers at Harvard published a case-control study which linked a high intake of xanthophyll-rich food, particularly dark green leafy vegetables such as spinach, with notably reduced risk for AMD. (The odds ratio for consuming spinach or collard greens 5-6 times weekly was found to be 0.14 [0.01-1.2].) This finding accorded well with the recent discovery that xanthophylls constitute the macular pigment, and triggered tremendous interest in the potential of supplemental or dietary xanthophylls to protect the retina. In a number of subsequent studies, oral administration of xanthophylls for three months or longer, either in supplements or natural foods, was shown to increase macular pigment density. However, the response of macular pigment to
xanthophylls intake was quite variable from person to person. In particular, a lesser response was noted in overweight subjects; baseline body fat also tends to correlate negatively with macular pigment density, and perhaps an adverse effect of obesity on macular xanthophyll uptake is at least partially responsible for the increase in AMD risk associated with obesity noted in some epidemiological studies. Further research is needed to gain better insight into the range of variable that can influence response to dietary xanthophylls. Most of the xanthophyll dietary supplements currently being marketed are derived from the petals of the marigold plant, which is rich in both lutein and zeaxanthin. Dietary meso-zeaxanthin is found mainly in seafood.

Measurement of Macular Pigments In Vivo

In light of the facts that macular pigment plays a key role in preserving retinal health and optimizing retinal function, and that response of this pigment to dietary xanthophylls varies from person to person, convenient and accurate techniques for assessing macular pigment density are clearly needed.

A number of technologies exist for the non-invasive measurement of the retinal pigments (lutein, zeaxanthin, meso-zeaxanthin) in the macula of the living eye, including heterochromatic flicker photometry, Raman spectroscopy and fundus autofluorescence imaging. Presently, heterochromatic flicker photometry, a psychophysical test, is the method of choice. Instruments based on flicker photometry are currently available to the general medical community. They are easy and inexpensive to use, fast, and accurate; most importantly, they are able to detect low levels of macular pigment. Arguably, flicker photometry is the best validated method for measuring macular pigment density.

Heterochromatic flicker photometry is performed by viewing a small circular stimulus that alternates between a test wave that is not absorbed by the macular pigment and a reference wavelength that is absorbed. The absorbed wavelength is blue (460 nm), and the nonabsorbed wavelength is green (540 nm). The distribution of macular pigment optical density (MPOD) in the retina can be determined with heterochromatic flicker photometry using a series of annular stimuli of different diameters.

The noted biophysicist Richard A. Bone, Professor of Physics at Florida International University, has much experience in the use of heterochromatic flicker photometry to study macular pigments in vivo. He says, “We can definitely track changes in macular pigments resulting from supplementation. The risk of AMD appears to be reduced by diets rich in xanthophylls, for those with high serum levels of lutein and zeaxanthin, and those with high macular pigment levels. Macular carotenoid levels increase from supplementation with lutein and zeaxanthin. Patients with particularly low macular pigment can change their diet or add a supplement to encourage an increase in macular pigment.”

Oxidative Stress Plays a Key Role in the Pathogenesis of AMD

Although the pathogenesis of AMD remains murky, there is a growing consensus that oxidative stress plays a key role in driving this syndrome. Chemical markers of oxidative stress have consistently been found to be elevated in the maculas of patients with AMD. In cell culture studies, exposure of RPE cells to oxidative stress has been shown to impair the function and survival of these cells – inducing a loss of tight junctions, impairing production of pigmented epithelium-derived factor (PEDF – a growth
factor that provides trophic support for photoreceptors while inhibiting choroidal neovascularization) and of complement factor H (impaired function of which has been linked to increased AMD risk\(^3\), \(^4\)), and promoting apoptosis – effects that are all consistent with the pathology of AMD.\(^{44-47}\) Moreover, oxidative stress can also induce apoptosis in photoreceptors, an effect which seems likely to contribute to the photoreceptor loss characteristic of dry AMD.\(^{48}\) And the most well characterized risk factor for AMD is cigarette smoking, which is well known to promote oxidative stress in tissues;\(^{49-52}\) in mice exposed chronically to cigarette smoke, increased oxidative stress and apoptosis is noted in RPE cells, accompanied by thickening of Bruch’s membrane.\(^{52}\)

Exposure to oxidative stress in the macula is high, for a number of reasons. The rate of oxygen uptake by the retina is higher than in any other of the body’s tissues. High mitochondrial respiration implies high potential for mitochondrial superoxide generation. RPE cells are phagocytic, and like other phagocytes, possess NAPDH oxidase that generates superoxide during phagocytosis.\(^{53-55}\) And, most obviously, the macula is exposed to blue light that generates singlet oxygen and other reactive oxygen species when it interacts with photosensitizing chemicals; the lipofuscin that accumulates in stressed RPE cells is rich in such photosensitizers, some of which, such as A2-E, are derived from all-trans-retinal.\(^{56-58}\)

Photoreceptors are exceptionally susceptible to oxidative damage, as their membranes are extremely rich in the long-chain omega-3 polyunsaturated docosahexaenoic acid (DHA); DHA can constitute up to half of the fatty acids in photoreceptor outer segments (POS).\(^{59}\) This presumably explains why POS membranes are also unusually rich in xanthophyll carotenoids capable of quenching the singlet oxygen that otherwise might peroxidize DHA.\(^{48,60}\) These xanthophylls also are found in photoreceptor axons, where their presumed key function is to absorb blue light before it can penetrate to deeper levels of the macula and generate oxidants. The fact that the xanthophyll content of the macula is subnormal in patients with AMD might in some measure be a consequence of the disease, but it is also reasonable to suspect that this deficit contributes to disease progression by lessening the macula’s antioxidant protection.\(^{14-16}\)

A further line of evidence implicating oxidative stress in AMD is the fact that transgenic mice lacking superoxide dismutase activity have been shown to develop a syndrome very analogous to AMD as they age; drusen, thickened Bruch’s membrane, and choroidal neovascularization are all observed, and the junctions linking retinal pigment endothelium are disrupted – features characteristic of human AMD.\(^{61}\) Increased light exposure accelerates accumulation of drusen in these mice, presumably because light promotes retinal oxidative stress.

Recent research may help to rationalize the high risk for AMD in people who express alleles of genes for complement factors or complement regulatory factors that would tend to up-regulate activation of the alternative complement pathway.\(^3\) Oxidative stress in RPE cells suppresses their production of cell-surface complement inhibitors, increasing risk for alternative pathway activation on the membranes of these cells;\(^{62}\) such activation should be most intense in those with low activity alleles of complement factor H, an inhibitor of the alternative pathway. The resulting complement cascade could explain why complement metabolites are found in drusen.\(^3\) Moreover, ARPE-19 cells have been found to express receptors for the pro-inflammatory complement metabolites C3a and C5a; activation of the C5a receptor promotes production of the key angiogenic factor VEGF.\(^{63}\) When RPE cells are exposed simultaneously to oxidative stress and serum, they produce VEGF; this effect is blocked if the cells are concurrently
exposed to an alternative pathway inhibitor. Exposure of oxidatively stressed RPE cells to serum likewise potentiates their loss of tight junctions – an effect that again is suppressed by an alternative pathway inhibitor. The picture that emerges is that, by up-regulating cell-surface complement activation in RPE cells, oxidative stress promotes drusen formation while inducing a pro-inflammatory, pro-angiogenic phenotype; this mechanism should be all the more intense in those who are genetically prone to overactivation of the alternative complement pathway.

AREDS1 (Age-Related Eye Disease Study-1)

In light of the many lines of evidence implicating oxidative stress in the pathogenesis of AMD, a multi-center study was performed to evaluate the impact of antioxidant supplementation in this syndrome. The first AREDS trial was supported by the National Eye Institute and Bausch & Lomb and recruited 3,640 subjects, ages 55 to 80 years who had been diagnosed with AMD (Age-related macular degeneration) at various stages. Participants were placed in one of four categories:

Category 1; No AMD, a few small drusen in either eye or no drusen:

Category 2; Early Stage AMD, several small drusen or one or both eyes:

Category 3; Intermediate AMD; Many medium-sized drusen or one or more large drusen in one or both eyes:

Category 4; Advanced AMD; In one eye only, either a break-down of light-sensitive cells and supporting tissue in the central retinal area (advanced dry form), or abnormal and fragile blood vessels under the retina (wet form).

The participants in each category were randomly selected to receive daily tablets for one of four treatments: 1) Zinc alone—80mg of zinc as zinc oxide (plus 2 mg copper, as copper oxide); 2) Antioxidants alone—vitamin C, 500mg; vitamin E, 400 IU; beta-carotene, 25,000 IU; 3) A combination of antioxidants plus zinc and copper—vitamin C, 500 mg; vitamin E, 400 IU; beta-carotene, 15mg; zinc, 80mg (as zinc oxide); copper, 2mg (as copper oxide) 4) Placebo.

(The inclusion of zinc in this protocol presumably reflects the fact that, whereas zinc is usually thought of as an anabolic mineral, increased cellular levels of zinc are known to protect protein sulfhydryl groups from oxidation; copper was included because high zinc intakes can reduce the efficiency of copper absorption.)

After five years of treatment, no benefit was seen in 1,063 participants who joined the study with only multiple small or a few medium drusen and good vision in the eye with more advanced disease. When the participants who had a low rate of progression to advanced AMD were excluded from the analysis the estimated probability of progression to advanced AMD among the remaining participants was 28%, with placebo, 23% with antioxidants alone, 22% with zinc (and copper) alone, and 20% with both zinc (and copper) and antioxidants. The estimated probability that at least three lines of vision would be lost at five years in at least one eye was 29% with placebo, 26%, with antioxidants, 25% with zinc (and copper) and 23% with both zinc (and copper) and antioxidants. Only the combination of zinc (and copper) and antioxidants had a statistically significant treatment effect compared with placebo. It can be concluded
that the trial was positive only in participants who already had moderate or advanced AMD. No cataract benefits were observed in any of the participants who had cataracts. Further, even though AREDS1 was the first study demonstrating benefit against AMD, it failed to demonstrate improvement in vision.

The outcome of AREDS1, while providing some support for the role of oxidants in AMD pathogenesis, was not as definitive or as striking as might have been hoped. This could however have reflected the inherent limitations of the antioxidants employed in this study. Most notably, AREDS1 did not evaluate the xanthophyll carotenoids that appear to contribute importantly to macular antioxidant protection. Vitamin E, while protecting membranes from peroxidation, has little impact on other key downstream consequences of oxidative stress that influence cellular function. Except in individuals with poor baseline vitamin C status, supplemental vitamin C is unlikely to notably increase intracellular vitamin C levels, owing to saturation of intracellular uptake. And there is little reason to believe that beta-carotene contributes meaningfully to antioxidant protection in the retina. The fact that AREDS1 did have a favorable outcome, albeit less than stellar, encourages the hope that more aggressive and rational antioxidant strategies, employed in conjunction with other nutraceuticals potentially beneficial to retinal health, can achieve a more definitive benefit.

**Long-Chain Omega-3s**

The brain is rich in long-chain omega-3 fatty acids – most notably docosahexaenoic acid (DHA) – and the retina has the highest omega-3 content in the body – DHA can constitute up to 50% of the fatty acids in rod photoreceptor outer segments. This evidently bespeaks a vital structural or functional role for DHA in retinal neurons, and indeed, the DHA-rich phospholipids in outer segment membranes appear to be crucial for efficient function of the rhodopsin that resides in those membranes. Moreover, in vitro, rat photoreceptor neurons undergo apoptosis if deprived of DHA. With respect to DHA’s biosynthetic precursor, eicosapentaenoic acid (EPA), while it is a less prominent structural component of the retina, it has important anti-inflammatory potential owing to its ability to competitively inhibit production of arachidonate-derived pro-inflammatory prostanoids, and to give rise to other mediators (resolwins, lipoxins) which play a role in the resolution of inflammation. Furthermore, EPA has anti-angiogenic potential reflecting decreased endothelial expression of Flk-1, a key receptor for vascular endothelial growth factor; DHA likewise can suppress endothelial VEGF signaling. Long-chain omega-3s are highly susceptible to oxidative damage; one of the key roles of retinal xanthophyll carotenoids, present in high concentrations in photoreceptor outer segments, may be to preserve the native structure of DHA in these photoreceptors.

Moreover, recent studies demonstrate that, within RPE cells, DHA can be converted via 15-lipoxygenase activity to neuroprotectin D1 (NPD1), a factor which interacts with a specific receptor to exert an autocrine anti-inflammatory and anti-apoptotic effect on these cells. This conversion of DHA to NPD1 is accelerated by oxidative stress or the unfolded protein response, and is also up-regulated by autocrine PEDF activity; hence, NPD1 provides feedback protection to RPE cells undergoing stress. In *vitro*, loss of 15-lipoxygenase activity renders RPE cells more vulnerable to oxidative stress-induced apoptosis. The DHA for NPD1 production is highly available to RPE cells, as they are continuously phagocytizing photoreceptor outer segments rich in this fatty acid.
Thus, it is not surprising that arguably the best-established association between diet and AMD risk is the finding that those who consume diets high in EPA/DHA or in oily fish (the richest food source of these fatty acids) are at decreased risk for both geographic atrophy (dry) and neovascular (wet) AMD. In the AREDS cohort, after adjustment for appropriate covariants, those in the upper quintile of DHA+EPA intake (averaging 0.11% of total dietary energy) had an odds ratio of 0.65 and 0.68 for progression to geographic atrophy or neovascular AMD, respectively, over 12 years of follow-up. Note that, in an individual consuming 2400 kcals daily, this intake of long-chain omega-3 amounts to about 300 mg daily, suggesting that the protective impact of these fatty acids is quite powerful and achievable with modest intakes. In the cross-sectional EUREYE study, those who consumed oily fish at least once per week, as contrasted to those who ate oily fish less frequently, were at only half the risk for neovascular AMD.

In a recent study involving a murine model for AMD (Ccl2−/−, Cx3cr1−/−), a diet enriched in EPA/DHA was associated with a slower progression of retinal lesions and even a degree of lesion reversion; markers of retinal inflammation were substantially reduced in the omega-3-fed mice. Although no completed clinical studies have yet evaluated the impact of omega-3 supplementation alone in AMD, an intriguing double-blind study by Feher et al. examined a regimen providing daily intakes of 100 mg acetyl-L-carnitine, 530 mg of long-chain omega-3s, and 10 mg of coenzyme Q10 in patients with early AMD over 12 months. In the three measures of visual function employed – visual field mean defect, visual acuity, and foveal sensitivity – changes in the treated group were significantly better than those in the placebo group, and on average reflected net improvement. Active treatment also performed better than placebo with respect to fundus alterations, with the treated group achieving a statistically significant reduction in the drusen-covered area. Although the authors attributed the improvements in the treated group to an allegedly favorable impact of the supplement on retinal mitochondrial function (they refer to the supplement as “mitotropic”), the doses of coenzyme Q10 and acetyl-L-carnitine were so low relative to dose ranges generally thought to be clinically relevant, that it is reasonable to conclude that the chief benefits of the supplementation were conferred by the omega-3 component (well within the dose range associated with marked protection in epidemiological studies). If this analysis is correct, it bodes well for the ultimate results of the ongoing AREDS2 study, and moreover suggests that good omega-3 nutrition is likely to have a favorable impact on early AMD.

AREDS2 (Age-Related Eye Disease 2)

AREDS2 is a multi-center, randomized trial of lutein, zeaxanthin, and omega-3 long-chain polyunsaturated fatty acids (docosahexaenoic Acid [DHA] and eicosapentaenoic Acid [EPA]) in AMD and cataracts, sponsored by the National Eye Institute. It is a 2X2 factorial placebo-controlled study in which one quarter of the cohort are receiving placebo, one quarter lutein (10 mg) and zeaxanthin (2), one quarter omega-3 fatty acids (EPA 650 mg, DHA 350 mg) and one quarter a combination of omega-3, lutein and zeaxanthin. Participants will be offered the option of concurrently taking an AREDS1-like supplement, Those that choose to do so will be randomized to receive one of four versions of this supplement; in some of these versions, beta carotene will be omitted, and/or the zinc dose will be a more moderate 25 mg.

Recruitment for the trial began in 2006 and enrollment concluded in June, 2008. There are approximately 4,000 participants aged 50-85 years who at the time of enrollment have either—1) bilateral large drusen or
2) large drusen in one eye and advanced AMD (neovascular AMD or central geographic atrophy) in the fellow eye. The trial is scheduled to conclude in 2013.

The objectives of AREDS2 are to:

1) Study the effects of high supplemental doses of the xanthophylls (lutein and zeaxanthin) and omega-3 long chain polyunsaturated acids on the development of advanced AMD.

2) Study the effects of these supplements on cataracts and moderate vision loss (doubling of the visual angle or the loss of 15 or more letters on the ETDRS chart). (The ETDRS is a visual acuity chart used in eye research. Originally, the chart was used in the Early Treatment of Diabetic Retinopathy Study.)

3) Study the effects of eliminating beta-carotene in the AREDS1 formulation on the development and progression of AMD.

4) Study the effects of reducing zinc in the original AREDS1 formulation on the development and progression of AMD.

5) Validate the fundus photographic AMD scale developed from the Age-Related Eye Disease Study.

6) Enrollment concluded in June, 2008 and participants will be followed between five and six years.

The ratio of EPA/DHA employed in AREDS2 presumably reflects the predominance of EPA in most natural fish oils, and may have been chosen for reasons of cost and convenience. It might reasonably be argued that, owing to the prominent and essential structural role of DHA in retinal phospholipids, a supplement placing greater emphasis on DHA might be warranted. A counterargument might be that EPA has greater anti-inflammatory potential than DHA, owing to its antagonism of arachidonate-derived prostanoid production – and that in any case EPA can be converted to DHA in vivo; but a 650 mg daily dose of EPA is considerably lower than the intakes which have shown useful systemic anti-inflammatory effects in other clinical disorders.91 Perhaps there will be a two-tiered impact of omega-3s on AMD control – relatively modest intakes such as those employed in AREDS2, or that have been evaluated in AMD epidemiology, may satisfy the structural needs of the retina, whereas higher intakes – perhaps in conjunction with restriction of dietary omega6s91 - might be required for optimal anti-inflammatory/anti-angiogenic effects that arguably would be beneficial for preventing the progression of neovascular AMD.

Ancillary Antioxidant Protection – Phase 2 Inducers

A rational consideration of the molecular biology of antioxidant protection suggests, that in addition to xanthophylls and the nutrients evaluated in AREDS1, additional antioxidants may have potential for protecting the retina and slowing the progression of AMD.

A very wide range of dietary phytochemicals can act directly as oxidant scavengers. But far more potent antioxidant protection can be achieved with phytochemicals which also can stimulate a so-called “phase 2” response.92, 93 This response entails increased transcription of genes which code for a range of antioxidant and anticarcinogenic enzymes, as well as of the gene for the enzyme – gamma-glutamylcysteine synthetase – that is rate-limiting for production of the prominent intracellular scavenger glutathione. Phytochemicals which trigger this response do so by activating the transcription factor Nrf2, which binds to the promoter region of these genes and promotes their efficient transcription. This activation, in turn, reflects the fact that certain metabolites of these phytochemicals can interact covalently with the Keap1 protein, thereby preventing it from binding to Nrf2 and retaining it in the cytoplasm.94
The impact of phase 2 inducers on glutathione levels is of particular importance, since glutathione not only scavenges many oxidants directly, but it also works with certain enzymes to restore the natural structure of proteins in which oxidants have induced disulfide formation.

Phase 2-inducing phytochemicals are prominent components of common foods such as green tea, allium vegetables (garlic, onions), and cruciferous vegetables (broccoli, cabbage, cauliflower, kale, etc.). They are also found in many commonly used herbal preparations, including some that have traditionally been used for ocular protection (e.g. extracts of bilberry, grape seed, and green tea). The phase 2-inducing components of some of these foods or herbs have shown protective antioxidant activity in retinal pigmented epithelial cells in vitro, and oral administration of green tea polyphenols to albino rats prevents light-induced damage to photoreceptors. In light of the heavy consumption of green tea in East Asia, it is striking that so far no published epidemiological studies have attempted to correlate habitual green tea intake with AMD risk or prognosis. However, the dose-dependency of the clinical phase 2 inducing effect achieved by ingestion of these foods or herbs requires further clarification, and the concentration of the active inductive components is likely to vary as a function of cultivar and method of preparation.

Alpha-Lipoic acid, an endogenously synthesized cofactor for several alpha-keto decarboxylase enzymes, has excellent antioxidant activity in many rodent studies, although its direct oxidant scavenging activity is versatile, it also has been shown to be a phase 2 inducer. Lipoic acid may have good potential for use in AMD, as in rodent studies it has shown central neuroprotective effects – suggesting that it has good access to the brain parenchyma (and likely the retina as well). Moreover, in an oral dose of 600 mg 1-3 times daily, lipoic acid has been shown to be therapeutically useful in diabetic neuropathy; hence, clinical doses within this range may have potential for protecting the retina. Ames and colleagues have shown that, at concentrations of 0.2-0.5 mM, lipoic acid moderates oxidative stress and prevents apoptosis in human fetal RPE cells exposed to 0.8 mM of the potent oxidant tert-butylhydroperoxide. The lipoic acid also up-regulated expression of mRNA for gamma-glutamylcysteine synthetase and increased intracellular glutathione levels – demonstrating that in these levels lipoic acid can indeed induce a phase 2 response in RPE cells. Whether the lower physiological levels achievable through supplementation can likewise provide protection to the retina in vivo, is not yet clear, but the utility of oral lipoic acid in the management of diabetic neuropathy suggests that this may be a reasonable possibility. To date, there do not appear to be any published clinical studies in which the impact of lipoic acid on AMD progression has been assessed.

**Melatonin**

The small tryptophan-derived hormone melatonin is synthesized primarily within the pineal gland, though it is produced in more minor quantities by other tissues, including the retina. The nocturnal burst of melatonin secretion, regulated in part by retinal light exposure, plays a role in promoting appropriate circadian physiological rhythms. However, acting via membrane as well as nuclear receptors, melatonin also exerts potent antioxidant effects on many tissues, via up-regulated expression of several key antioxidant enzymes (superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase) as well as of gamma-glutamylcysteine synthetase. The mechanism of this up-regulation is not as well defined as that of phase 2 induction, but there is no evidence that it involves Nfr2, and it is thus quite conceivable that the effects of phase 2 inducers and of melatonin on expression of these enzymes will be
complementary. These inductive effects of melatonin are prominent in the CNS of rodents, and hence likely play a role in the versatile neuroprotective properties of supplemental melatonin observed in rodent studies.\textsuperscript{110, 111} Many types of cells in the retina – including RPE, photoreceptors, and ganglion cells – have been shown to express melatonin receptors.\textsuperscript{112}

Diurnal exposure of ARPE-19 cells to melatonin for 3 days was noted to blunt the impact of subsequent hydrogen peroxide exposure on cell death and mitochondrial DNA damage; hence, the RPE appears to be responsive to the antioxidant hormonal activity of melatonin.\textsuperscript{113} This finding encouraged an open study in which patients at various stages of AMD were treated with 3 mg melatonin nightly for 6-24 months.\textsuperscript{114} 55 patients received melatonin for at least 6 months. The authors noted that visual acuity remained stable in the large majority of patients; they also noted that “change in the fundus picture is also promising…. Only 8 eyes showed an increase in retinal blood and 6 eyes showed an increase in retinal exudates. The majority had a reduction compared to pathologic changes before treatment in these patients, although atrophy was slightly increased.” The authors conclude that, relative to the clinical course that could be expected based on literature reports, the response of their patients was favorable, and that melatonin merits more formal study in the prevention and management of AMD.

A more recent study has attempted to assess nocturnal melatonin production in AMD patients by measuring the levels of the chief melatonin metabolite 6-sulfatoxymelatonin in morning urine (expressed as its ratio to creatinine).\textsuperscript{115} This ratio was found to be about 40% lower in AMD patients than in age- and gender-matched controls. Whether this striking disparity is a cause or an effect of AMD – bearing in mind that light reception regulates pineal melatonin release – is open to question, although the authors note that, among their patients with AMD, sulfatoxymelatonin levels were no lower in those with poor visual acuity than in those with better acuity. In any case, even if the pathologic process in AMD does somehow decrease pineal melatonin production, it could be argued that, owing to melatonin’s key physiological/antioxidant roles, this decline should be compensated by supplementation. Moreover pineal melatonin production declines during healthy aging\textsuperscript{116} and it is conceivable that this phenomenon contributes to the higher risk for AMD in the elderly.

\textbf{N-Acetylcysteine – Substrate Support for Glutathione Synthesis}

Whereas gamma-glutamylcysteine synthetase is the rate-limiting enzyme for glutathione synthesis, intracellular cysteine is the rate-limiting substrate for this process. The antioxidant effects of N-acetylcysteine (NAC) supplementation have been traced to its ability to boost intracellular levels of free cysteine, and thereby boost cellular glutathione production.\textsuperscript{117-119} These considerations suggest that concurrent supplementation with NAC might amplify the ability of phase 2 inducers or of melatonin to increase glutathione in RPE cells or retinal photoreceptors. In divided doses providing 600-1800 mg daily, NAC is typically well tolerated, and has shown clinical potential as an antioxidant.\textsuperscript{118, 119} Although there do not appear be any published clinical studies which have evaluated NAC in AMD, the exposure of RPE cells to NAC in vitro has been reported to protect them from apoptosis when they are subjected to hypoxia or toxic carotenoid-derived aldehydes.\textsuperscript{120, 121} Moreover, NAC was found to have a favorable impact on the efficiency of lysosomal function in RPE cells pre-loaded with oxidized photoreceptor outer segments, such that accumulation of lipofuscin was decreased.\textsuperscript{122} In rats subjected to intraocular hypertension, systemic administration of NAC prevented the induced increase in retinal oxidative
stress. These considerations suggest that NAC may have important potential for lessening the contribution of oxidative stress to the pathogenesis of AMD; it is reasonable to expect that this potential will be best actualized if induction of gamma-glutamylcysteine synthetase is achieved concurrently.

**Targeting NADPH Oxidase**

There is some reason to suspect that NADPH oxidase may be a key source of oxidant stress in RPE cells. RPE cells act as phagocytes, engulfing outer segments of retinal rod photoreceptors; like other phagocytes, RPE possess NADPH activity that increases during phagocytosis. More recent work establishes that RPE expresses p22phox, a key membrane component of NADPH oxidase complexes. Intriguingly, viral delivery of small interfering RNA for p22phox to the subretinal space prevents choroidal neovascularization in a mouse model of AMD (involving laser disruption of Bruch’s membrane). The authors conclude that “NADPH oxidase-mediated ROS production in RPE cells may play an important role in the genesis of neovascular AMD, and this pathway may represent a new target for therapeutic intervention in AMD.” In addition, NAPDH oxidase activity is expressed by retinal neurons; this activity increases when growth factor support is withdrawn (as might be expected if death or dysfunction of retinal pigment epithelium impairs its trophic function), and there is suggestive evidence that NADPH oxidase activation may drive the apoptotic death of cone cells in retinitis pigmentosa.

These findings are of particular interest in light of the recent discoveries that intracellular free bilirubin functions intracellularly as an inhibitor of NAPDH oxidase (thereby rationalizing the antioxidant activity of heme oxygenase), and that phycocyanobilin (PhyCB), a bilirubin homolog that functions as a chromophore in spirulina and other microalgae, can mimic this activity. Since orally administered spirulina or phycocyanin – the spirulina protein which contains PhyCB as a chromophore – exerts neuroprotective effects in rodent studies, there is reason to suspect that spirulina or PhyCB-enriched spirulina extracts may have the potential to confer antioxidant protection to the retina. So far, however, no studies have examined the impact of spirulina or phycocyanin on retinal function or pathology. It would be of particular interest to determine whether individuals with Gilbert syndrome, an innocuous genetic variant associated with chronically elevated plasma levels of free bilirubin and reduced risk for cardiovascular disease, are at decreased risk for AMD.

**Coenzyme Q10**

Coenzyme Q10 functions as an obligate mediator of electron transport in mitochondrial respiration; in the mitochondrial inner membrane, it can function as either an antioxidant or a pro-oxidant, depending on the circumstances. A recent study concludes that coenzyme Q10 content of the human retina and choroid decline by about 40% during the aging process; the authors suggest that this may compromise the bioenergetics and antioxidant protection of the aging retina, possibly contributing to risk for AMD. Moreover, plasma levels of coenzyme Q10 were found to be lower in patients with wet AMD that in age-matched controls. Several studies demonstrate that pre-administration of coenzyme Q10 to rats protects the retina from excitotoxicity induced by transient ischemia or NMDA administration, and also can protect retinal ganglion cells for hydrogen peroxide in vitro. These considerations suggest that coenzyme Q supplementation may provide worthwhile antioxidant protection for the retina, and merits evaluation for control of AMD. Unfortunately, the only published clinical study to assess a regimen containing coenzyme Q10 in patients with AMD provided such a low dose (10 mg daily) that it...
Searching for Ancillary Strategies – High-Dose Folate and the WAFACS Study

The foregoing discussion strongly suggests that supplementation with xanthophyll carotenoids, long-chain omega-3 fatty acids, and various adjunctive antioxidants may have important potential for preventing and controlling AMD; the on-going AREDS2 study, as well as other studies in progress or in planning, should provide more definitive information in this regard. Nonetheless, the pathogenesis of AMD remains poorly understood, and it seems likely that a point of diminishing returns will be reached as antioxidant measures are piled atop each other. Hence, it may be worthwhile to identify additional targets that can be addressed in AMD management – strategies with potential for complementing the efficacy of antioxidants and omega-3s. The recent WAFACS study may provide a promising lead in this regard.

The Women’s Antioxidant and Folic Acid Cardiovascular Study (WAFACS) randomized 5,442 women at high cardiovascular risk (with pre-existing cardiovascular disease or with 3 or more cardiovascular risk factors) to receive a supplement providing 2.5 mg folic acid, 50 mg pyridoxine, and 1 mg cyanocobalamin, or matching placebo.141 5,205 of these women did not have a diagnosis of AMD at baseline. Although the primary intent of this study was to examine the impact of homocysteine-lowering supplementation on cardiovascular risk, the authors prospectively decided to include assessment of new AMD incidence in their protocol – and indeed several subsequent reports have associating elevated homocysteine with increased risk for wet or total AMD.142-146 During an average follow-up of 7.3 years, the participants reported 55 new cases of AMD in the supplemented group and 82 in the placebo group (RR 0.66; P=0.02); visually significant new AMD was reported by 26 subjects in the treated group, vs. 44 in the placebo group (RR 0.59; P=0.3). Thus, one or more of these high-dose vitamins may have important potential for primary prevention of AMD.

A sub-group analysis indicated that homocysteine levels fell by an average of 18% in the treated group, and the authors suggested that this may have contributed to the observed benefit. However, prospective supplementation studies (including WAFACS) are concluding that moderately elevated homocysteine is unlikely to be a true mediator of cardiovascular disease, but rather may be serving as a marker for other factors which are mediators (such as systemic inflammation).147-149 this is consistent with the fact that few if any in vitro studies demonstrate that homocysteine concentrations within the normal physiological range (as opposed to the much higher levels associated with genetic hyperhomocysteinemia) exert significant effects on cells. Also, it is hard to believe that a reduction of only 18% in moderately elevated homocysteine could be primarily responsible for the large protective effect on AMD observed in the WAFACS study.

However, as the authors also note, folate intakes an order of magnitude higher than the nutritional range have shown favorable effects on dysfunctional vascular endothelia that are clearly independent of homocysteine modulation.150-153 These effects may be mediated by high intracellular concentrations of reduced folates that have potent and versatile antioxidant activity, including the ability to scavenge peroxynitrite-derived oxidants.154-156 Indeed, high-dose folate or its reduced metabolites have shown the
ability to prevent the uncoupling of the endothelial nitric oxide synthase in oxidatively–stressed endothelial cells that results from peroxynitrite-mediated oxidation of eNOS’s essential cofactor, tetrahydrobiopterin.\textsuperscript{154, 157}

In light of several reports correlating disorders characterized by endothelial dysfunction with AMD risk (such as atherosclerosis, hypertension, and obesity\textsuperscript{158-164}), the following interpretation seems plausible: NO produced by the eNOS of choroidal vessels plays a role in preventing the onset of inflammation and oxidative stress in RPE cells – either by a direct impact on these cells, or by sustaining efficient choroidal perfusion. In some individuals, oxidative stress in these vessels compromises this protective NO production by uncoupling eNOS – but these vessels are exquisitely sensitive to the antioxidant benefit of a daily folate dose as low as 2.5 mg (too low to be significantly protective in systemic vascular disease). This hypothesis is highly speculative, since few if any studies have evaluated the impact of moderate physiological levels of NO and/or cGMP on pro-inflammatory processes in RPE cells; nor is there evidence that relative hypoxia promotes these processes. It is however seemingly consistent with a reports that eNOS expression is decreased in the choroidal vessels of AMD patients, and that choroidal blood flow is decreased in dry AMD;\textsuperscript{165-167} it also might help to rationalize the favorable impact of exercise - which has an inductive impact on endothelial eNOS expression\textsuperscript{168} - on AMD risk (as discussed below). The alternative hypothesis that high-dose folate provides direct antioxidant protection to RPE cells appears less likely, in light of evidence that basal uptake of folate by RPE cells is mediated by a high affinity receptor (folate receptor alpha) whose capacity is almost saturated at normal physiological concentrations of folate.\textsuperscript{169, 170}

An intriguing aspect of the WAFACS data is that no clear benefit was noted in the first year – with substantial benefit accruing thereafter. This pattern might be expected if NO helps to prevent the establishment of an inflammatory, pro-oxidant phenotype in RPE cells, but has a lesser impact once such a phenotype has been established. Indeed, once RPE superoxide production increases, quenching of NO by this superoxide would presumably lessen any protective impact of NO. Hence, high-dose folate might prove to more useful for primary prevention of AMD, than for control of the progressing syndrome.

It is of course conceivable that high doses of pyridoxine or of vitamin B12 were responsible for the AMD prevention observed in the WAFACS trial – or that the impressive results are simply a non-confirmable fluke. With respect to vitamin B12, reduced serum levels were correlated with increased AMD risk in the Blue Mountain Study,\textsuperscript{146} whereas in other studies no correlation was noted, or only a correlation of low B12 with wet AMD.\textsuperscript{142, 171, 172} A literature search turns up nothing of interest with respect to impact of pyridoxine on AMD. Hence, the possibility that high-dose folate favorably influences this syndrome, perhaps by sustaining choroidal NO generation, is worthy of further consideration. (The concern that high-dose folate might mask the early symptoms of B12 deficiency in pernicious anemia – the reason why the U.S. Food and Drug Administration bans high-dose folate supplements – has no logical force if a sufficiently high oral dose of B12 is co-administered, as it was in the WAFACS trial; daily oral intakes of 400 mcg B12 or more have been shown to correct B12 deficiency in patients with pernicious anemia, owing to a mass action effect on B12 absorption.\textsuperscript{173, 174})

If NO derived from choroidal vessels does indeed help to prevent AMD, additional strategies for boosting this NO production can be envisioned. Cocoa flavanols (most notably epicatechin) stimulate endothelial
NO release, and can acutely enhance brain perfusion.\textsuperscript{175-177} The favorable impact of exercise training on eNOS expression has been noted. Uncoupling of eNOS might be minimized not only by high-dose folate, but also by PhyCB, which should lessen endothelial (and possibly RPE) superoxide production. Supplemental citrulline might be employed to optimize substrate availability for eNOS.\textsuperscript{178}

Moreover, nitrate ingested from green leafy vegetables has the potential to boost tissue NO production, independent of NOS activity.\textsuperscript{179-182} Much of this nitrate is secreted into saliva, where it can be reduced to nitrite by commensal oral bacteria. This nitrite can then be absorbed, and further reduced to NO by blood or tissue heme proteins; sufficient NO can be generated in this way to lower systemic blood pressure.\textsuperscript{180, 181, 183} Recall that, in the classic Seddon study, risk for AMD was remarkably low in those claiming to eat spinach or collard greens nearly every day.\textsuperscript{34} Could this apparent protection reflect the joint impact of lutein and of dietary nitrate?

**Further Clues from Epidemiology – Estrogen, Exercise, and Glycemic Index**

The balance of evidence appears to point to hormone-replacement therapy or oral contraceptive use as protective with respect to risk for neovascular AMD;\textsuperscript{184-188} moreover, in women, risk for AMD may be greater in those with early menopause.\textsuperscript{189} Human RPE cells express both isoforms of the estrogen receptor.\textsuperscript{190} In female mice fed a high-fat diet and exposed to blue-green light, ovariectomy increases the development of sub-RPE deposits and the thickening of Bruch’s membrane – an effect which may be attributable in part to loss of matrix metalloproteinase-2 activity in RPE cells.\textsuperscript{191, 192} The effects of ovariectomy in this model are replicated in ERbeta-knockout mice, but not ERalpha-knockout mice, implicating the beta isoform of the estrogen receptor in prevention of sub-RPE deposits.\textsuperscript{193} In vitro, estradiol exerts an anti-inflammatory effect on RPE cells, associated with down-regulation of NF-kappaB activation and IL-6 production, and also protects RPE cells from hydrogen peroxide-mediated cytotoxicity; these latter studies did not clarify the isoform of the estrogen receptor responsible for these protective effects.\textsuperscript{194, 195}

There is reason to suspect that the physiological effects of soy isoflavones reflect selective activation of ERbeta; this may explain why isoflavones can exert various protective estrogenic effects without inducing feminizing effects or increasing cancer risk.\textsuperscript{196} Hence, to the extent that the putatively favorable effects of estrogenic activity on retinal health are mediated by ERbeta, soy isoflavone ingestion may have potential for aiding control of AMD. To date, there are no published epidemiological studies which have evaluated the impact of soy ingestion on AMD risk; studies targeting East Asia may be worthwhile in this regard. Oral administration of soy isoflavones to rats has been reported to decrease the retinal vascular leakage associated with diabetes, and, in young male rats, to increase retinal thickness.\textsuperscript{197, 198} (Retinal studies in which genistein has been employed as a tyrosine kinase inhibitor are not physiologically relevant, as these require grossly supraphysiological concentrations.) Further evaluation of retinal effects of soy isoflavones in rodent models, particularly those pertinent to AMD, may be indicated.

There is accumulating evidence that regular vigorous physical activity may be linked to decreased AMD risk. The recent data on physical activity show an important dose-dependency; in a prospective study focusing on a cohort of runners, those who ran 8 or more km daily experienced less than half as much AMD as those who ran less than 2 km a day.\textsuperscript{199} However, less draconian levels of physical activity, relative to a sedentary lifestyle, emerge as protective for exudative AMD in the Beaver Dam Eye
The evident implication is that patients at risk for, or in the early stages of, AMD should be encouraged to exercise regularly if feasible. However, it could also be of great importance to understand why exercise is protective in this regard, as it might then be possible to suggest nutraceutical or pharmaceutical strategies which could mimic the protective impact of exercise on the retina. Theoretically, improved insulin sensitivity, increased vascular production of nitric oxide, or induction of neurotrophic hormones or other proteins that aid stress resistance – as demonstrated in the brains of rodents allowed to exercise regularly\textsuperscript{201,202} – could be responsible for the retinal protection afforded by exercise.

With respect to diet, recent analyses of the Nurses’ Health Study and AREDS1 indicate that frequent consumption of high-glycemic-index carbohydrates is linked to increased risk for both geographic atrophy and neovascular AMD; total carbohydrate intake does not emerge as a risk factor.\textsuperscript{203-205} The impact of glycemic index on AMD risk might reasonably be attributed to adverse effects of advanced glycation end-products (AGEs), although a the high post-absorptive flux of free fatty acids that follows high-glycemic-index meals could be offered as an alternative or adjunctive explanation.\textsuperscript{206,207} If AGEs do contribute to risk for AMD – not unreasonable in light of their pro-oxidant effects\textsuperscript{208} – then supplemental glycine, shown to decrease AGE formation in diabetics, might be useful in this regard.\textsuperscript{209-211} And feasible measures for lessening the effective glycemic index of meals – concurrent consumption of acarbose, soluble fiber, or vinegar, for example\textsuperscript{212-218} - might also have some modest utility for slowing the onset or progression of AMD.

**Overview**

Although the pathogenesis of AMD remains poorly understood, available evidence suggests that there may be considerable scope for preventing and treating this syndrome with rationally designed nutraceuticals. AREDS1 has established that supplemental zinc, and the combination of vitamin E, vitamin C, and beta carotene, has some modest utility, most notably in later-stage disease. But there is good reason to suspect – as is being assessed in AREDS2 – that long-chain omega-3s and xanthophyll carotenoids may be at least as useful in this regard, and quite likely beneficial for primary prevention. The B vitamins provided in the WAFACS trial – perhaps most notably high-dose folate – may likewise aid primary prevention of AMD. And evidence of varying degrees of cogency suggests that a wide range of additional agents – including phase 2 inducers, melatonin, N-acetylcysteine, spirulina, coenzyme Q10, and soy isoflavones – may merit consideration for AMD prevention and control. In light of current epidemiological findings, it may be appropriate to recommend smoking cessation, regular physical activity – prolonged in duration if feasible – and lower glycemic-index foods for patients at risk for AMD (particularly since these measures are likely to be beneficial for overall health, in any case). As we develop a better understanding of how exercise or glycemic index influence retinal health, it may prove possible to mimic some of these protective effects with certain nutraceuticals or drugs, and to design elaborate regimens which attack the pathogenesis of AMD from a number of complementary angles.
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