Age-related Macular Degeneration Beyond the Age-related Eye Disease Study II

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1These authors contributed equally to this work, at different developmental phases.

**Key points**

- Age-related macular degeneration (AMD) is age dependent with a gradual, insidious onset until the eighth decade of life when there is an exponential increase in the number of patients with this condition.

Continued
INTRODUCTION
Pharmacologic breakthroughs abound for treating acute neovascular age-related macular degeneration (AMD). However, for most patients with atrophic (dry) AMD, or when exudative (wet) AMD is treated with intravitreous anti–vascular endothelial growth factor (VEGF) agents, there is a common experience. That theme is a relentless progressive destruction of the photoreceptor Bruch membrane–retinal pigment epithelium (RPE) complex and neural retina with age. A plethora of emerging investigational, atrophic AMD agents address the compartmental processes of visual cycle modulation, neural growth/viability, inflammation, amyloid accumulation, alternative complement, antioxidants, and stem cells. However, AMD remains, at its essence, a chronic multifactorial disease of aging, under the influence of genes activated by supportive as well as destructive environmental influences.

Underlying retinal health, the supportive vascular choroidal bed thins with age, and much faster in patients with glaucoma or AMD. Oculovascular disease is activated by several repeated and prolonged mechanical, physical, chemical, microbiological, immunologic, and genetic factors causing a protracted host defense response with consequent vascular damage [1]. Protection of peripheral endothelial end-organ capillary beds like the choriocapillaris should encompass more than a hurried discussion on smoking cessation. Besides smoking reduction/cessation and reducing endothelial damage from chronic inflammation, an extended doctor-patient dialogue is needed concerning the importance of cardiovascular physical fitness, reduction of abdominal adiposity (nonalcoholic fatty liver disease [NAFLD]), minimizing glycemic load (with its promotion of insulin resistance), stress reduction (supporting the hypothalamic-pituitary-adrenal axis), microbiome and digestive competence, vascular nitric oxide stimulation (for greater blood flow), and caloric restriction (CR) (fasting or mimicry through the use of metformin and/or resveratrol [RV]).

Individual differences also matter. A dozen physiologic biomarkers, assessed comprehensively in 1000 subjects, can vary by a factor of 3 even in asymptomatic young adults between 28 and 38 years old [2]. Approximately 4% of a European cohort of patients with AMD acquired AMD before age 45 years. Environmental factors, including nutrient intake, are modifiable, affecting an individual’s rate of aging, despite the inherited DNA genetic profile. There are 8 nongenetic factors (age, alcohol use, allergies, education, sunlight exposure, fish consumption, physical exercise, and mineral overload) that provide...
equivalent discrimination between AMD and no AMD comparable with existing DNA single-nucleotide polymorphism (SNP) risk models [3].

**SUPPORTIVE NUTRIENTS BEYOND AGE-RELATED EYE DISEASE STUDY I AND II**

This article examines the limited orthodox toolbox of nutrients applied to AMD by studying the Age-related Eye Disease Study (AREDS) model and experience, and offers additional insight concerning nutrients broadly associated with retinal and choroidal health. These nutrients include glutathione/sele

nuemium (Se) and vitamin D$_3$ status, the family of vitamin E molecules known as tocotrienols/tocopherols, polyphenols, the calcium (Ca)/magnesium (Mg) ratio, omega-3 fatty acids (eg, docohexanoic acid [DHA]), zeaxanthin, and activities that enhance endothelial nitric oxide production. Table 1 lists these nutrients and activities with respect to ocular health (Fig. 1). Ascorbate is the primary “extracellular systemic antioxidant” and most important ocular nutrient. It sets the redox potential of all retinal cells, is included within AREDS science, and is discussed later within the context of meaningful serial dosing given increasing modern environmental stress.

Glutathione master antioxidant and selenium status

Glutathione (gamma-glutamyl cysteinyl glycine) or glutathione-stimulating hormone (GSH) is the key critical intracellular tripeptide associated with both health and life expectancy, with more than 80,000 articles to date. It is abundant in the liver and kidney detoxification organs. GSH detoxifies foreign (xenobiotics) and biological toxins, such as redox reactive heavy metals and man-made compounds found in plastics, pesticides, synthetic hormones, drugs, and carcinogens. Glutathione is the illogical missing AREDS component of the cellular antioxidant network, which includes extracellular ascorbate and cell membrane–bound

<table>
<thead>
<tr>
<th>Nutrient Classification</th>
<th>Classification</th>
</tr>
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<tbody>
<tr>
<td>Vitamin D$_3$ Tocotrienols</td>
<td>Prohormone immune modulator and anticalcification agent</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>Polyphenol bioactives (ie, in tea, coffee, cocoa, RV, curcumin, beets, rosemary, saffron, and quercetin); twenty-first-century multifunctional molecules. Avoid overdosing</td>
</tr>
<tr>
<td>Mg</td>
<td>Essential nutrient; a systemic divalent regulatory metal</td>
</tr>
<tr>
<td>Glutathione (GSH) and Se</td>
<td>GSH: nonprotein thiol tripeptide and primary cellular antioxidant. Both GSH and Se required by RPE cells</td>
</tr>
<tr>
<td>Omega-3 fatty acids including DHA</td>
<td>Antiinflammatory and structural essential dietary fat for retinal photoreceptors and neural tissue</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>Dietary carotenoid for fovea; component of macular pigment (in addition to lutein and its metabolite mesozeaxanthin)</td>
</tr>
</tbody>
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**Table 1**

Nutrients for retinal health

**Abbreviations:** GSH, glutathione-stimulating hormone; Se, selenium.
vitamin E [4]. One clue to GSH deficiency is an increased gamma-glutamyl transferase liver enzyme level, indicating exposure to environmental toxins. Serum GSH level also can be measured directly.

Within the trans-sulfuration pathway of glutathione biosynthesis (ie, liver, lens epithelium), the amino acid cysteine is rate limiting, with methionine a second dietary amino acid precursor. Se is required as a cofactor for the oxidation of GSH with glutathione peroxidase from glutathione disulfide (oxidized state) and is found in metalloamino acids (ie, selenomethionine). These vital nutrients guard against free radicals, eliminate cancer-causing chemicals, and support the immune
The best orally absorbable form of glutathione is 500-mg Setria GSH tablets. GSH is also found within the following dietary sources:

- **GSH**: avocados, spinach, asparagus, watermelon, and walnuts. Proteins such as chicken, chickpeas, and lentils contain all 3 amino acids.
- **Cysteine** (sulfur bearing): turkey breast, eggs, sockeye salmon, yogurt, wheat germ, whey protein, garlic, onions, soy, red bell peppers, and broccoli.
- **Se**: sardines, Brazil nuts, clams, oysters, turkey breast, and garlic.

As damaged proteins accumulate with age against a background of diminished ubiquitin protease levels, endogenous GSH synthesis (ie, cysteine/methionine in whey protein drinks or whole eggs) becomes increasingly desirable.

Other sulfur-based molecules involved as precursors to GSH analogues include taurine, \( \text{N} \)-acetyl cysteine, lipoic acid, methylsulfonylmethane, \text{S}-adenosylmethionine (SAMe), and the herb silymarin (milk thistle extract). SAMe has excellent antiinflammatory and thus antidepressant action in undermethelators via its ability to inhibit homocysteine. In excess, some forms (ie, SAMe) can have druglike side effects in overmethylators by altering neurotransmitter synthesis [5]. The endogenous production of glutathione is limited by, but not activated by, the provision of sulfur-based nutrients. Rather, glutathione synthesis is activated by biological stress. Gene mutations can also compromise an individual’s ability to recycle GSH despite extra being consumed. More efficacious endogenous GSH synthesis is stimulated via mild biological stressors (discussed later), facilitated by activation of the nuclear regulatory factor 2 (Nrf2) gene transcription factor.

Se, abundant within the RPE, efficiently helps prevent the peroxidation of cellular and subcellular lipids and fats (ie, retinal drusen or gold dermal liver spots of ceroid lipofuscin). Se deficiency diseases are ubiquitous, diverse, and underappreciated, with the ability to affect every human stage of growth, from embryo to adult [6–8]. In addition to being a precursor of GSH peroxidase, Se counters environmental methyl mercury toxicity; impedes viral replication; is a required factor for the absorption of vitamin E, thereby aiding in RPE antioxidant activity; as well as preserving RPE genomic stability, and telomere function and length. Humans also maintain endogenous intracellular defenses against reactive oxygen species (ROS) with erythrocyte superoxide dismutase (SOD) (Zn and Mn dependent) and GSH peroxidase (vitamin E and Se dependent). The latter reduces hydrogen peroxide by using GSH as an electron donor. The flavoenzyme GSH reductase which recycles oxidized GSSG, and GSH peroxidase are diminished in both AMD and cataractogenesis [9].

**Vitamin D status**

Beyond its role as a modulator of mineral absorption and parathyroid support, vitamin D plays a pivotal role in both immediate intrinsic and adaptive immune memory, immunologic prevention of neoplastic processes, and neuromodulation and cerebrovascular/cardiovascular diseases. Within ocular systems, low values measured on the inexpensive serum 25-hydroxyvitamin
D liver-reserve status laboratory test, are strongly associated with RPE disease, AMD neovascularization, and the geographic extent of postbleed retinal fibrotic damage. Because sunlight is the biological activator of dermal vitamin D synthesis, there exists a catch-22 between balancing protective absorption and risk of DNA-damaging retinal degeneration, particularly in susceptible young (phakic) and pseudophakic subpopulations. Clinicians should avoid this risk by curtailing recommendations to limit sunlight and overzealous cholesterol reduction while assessing vitamin D status [10]. Vitamin D repletion is particularly imperative for housebound elderly patients with AMD living in northern, sun-deprived latitudes.

Tocotrienols and tocopherols
The full spectrum of 8 vitamin E isomers includes 4 tocopherols and 4 tocotrienols. They protect all cell membranes from free-radical damage; improve endothelial function; modulate clotting; reduce C-reactive protein (CRP) levels; and, in some studies, reduce atrial fibrillation, Alzheimer disease (AD) progression, skeletal fractures, and cerebrocardiovascular diseases. Only higher-dose alpha-tocopherol was used in AREDS I and II studies, whereas the clinical applications of tocotrienols, packed with antioxidant, anti-inflammatory, and antineovascularization properties, remain unexplored. For example, both gamma-tocotrienol and RV synergistically return microRNA (miRNA) expression to basal (preinsult) levels in rat cardiac ischemia-reperfusion experiments [11].

Bioflavonoids
Bioflavonoids are molecularly diverse plant polyphenols found in tea, oranges, grapefruit juice, coffee, chocolate, and red wine (ie, RV, quercetin). They support the gastrointestinal (GI) tract and nervous system, regenerate mitochondria, and serve as chemical messengers and cell cycle inhibitors, and have antiinflammatory, antiallergen, anticlotting, germicidal, and chelating properties. As small molecules, polyphenols act as agents that broadly affect the genome and epigenetic protein expression. Depending on the dose, bioflavonoids show both low-dose and high-dose antioxidant and pro-oxidant properties, respectively. At low doses, polyphenols act as mild biological stressors by triggering internal enzymatic antioxidant defenses (glutathione, catalase, SOD) via the Nrf2 gene transcription factor. The entry of polyphenols into ophthalmology and optometry toolboxes is only hampered by the mandate that these natural molecules must undergo drug trials before being brought into the everyday practice of medicine [12]. Polyphenols, such as RV, quercetin, and catechin, are concentrated 1000-fold in wine via fermentation and thus wine serves as medicine, whereas grape juice does not.

Magnesium shortage with suboptimal magnesium²⁺/calcium²⁺ ratio affecting 50% of the United States population
Mg is an invaluable essential metal with far-reaching biological effects, including vitamin D metabolism and blood coagulation. When Mg is deficient,
toxic metals (lead, mercury, arsenic, cadmium, and nickel) can inhibit enzymatic reactions. Throughout the eye, Ca in excess can precipitate ocular myokymia, blepharospasm, acephalic migraine, and low-tension glaucoma. Mg-depleting stressors are to blame, including modern farming practices; diets high in simple carbohydrates and carbonated beverages; excessive intake of coffee, alcohol, tea, and processed foods; acid blockers; and diuretics. These environmental stressors are particularly damaging to aging individuals. Exacerbating this Mg deficiency further, chronic iatrogenic oversupplementation of Ca for osteoporosis prevention may result in vascular calcification, cardiac arrhythmias, and even death [13]. Ca/Mg imbalance should also be suspected in patients presenting with overzealous Ca supplementation beyond 500 mg/d.

Maintenance of an antiinflammatory omega-6/omega-3 ratio and a high Holman Index with adequate docohexanoic acid intake

Omega-3 fatty acids reduce cardiovascular and AMD risk factors by their anti-inflammatory, antiarrhythmic, antihyperlipidemic, and antithrombotic effects. When used in conjunction with anti-VEGF injections, omega-3 fats seem to have a more robust effect on neovascularization than antioxidants, zinc (Zn), and carotenoids. DHA in particular prevents age-related functional losses, neovascularization, and accumulation of lipofuscin within the retina [14]. Studies have shown highly statistically significant differences between omega-6/omega-3 ratios in patients with neovascular AMD against less affected patients with AMD and controls (P = .002) [15].

The preponderance of evidence suggests the omega-6/omega-3 ratio to be of paramount importance at any stage of AMD, despite it being disregarded in the AREDS II intervention study [16]. Omega-6 proinflammatory vegetable and corn oils are oversupplied in the US diet. Those conclusions with respect to fish oil supplementation were null, in contrast with AREDS I post-hoc data and the preponderance of biological and epidemiologic evidence showing a benefit of fish and fish oil supplementation in AMD. This anomaly likely resulted from higher intake within the well-nourished AREDS II control group, insufficient DHA in the experimental AREDS II formulation, or the half-decade older age of the subject population.

DHA-supplemented patients and those with a higher Holman Index show significant reduction in neovascularization AMD incidence [14]. The Holman Index test is a simple finger-stick blood spot that identifies the complete red blood cell fatty acid profile, specifically focusing on the omega-3 and omega-6 fractions. The total omega-3 score is the percentage of omega-3 fatty acids (eg, eicosapentaenoic acid and DHA) expressed as a percentage of all fatty acids.

In our clinical experience [14], omega-3 fats are synergistic with carotenoids at all AMD stages, desirably enhance macular pigment, and stabilize/improve visual function. DHA sources include sockeye salmon, sardines, herring, arctic char, Pacific blue mussels, and farmed rainbow trout.
Embracing high-dose zeaxanthin
There are 600 carotenoids in nature, 50 commonly found in fruits and vegetables, and 20 circulating within blood. Of the systemic forms, the retina specifically selects 2 as macular pigment: lutein, from leafy greens like spinach, kale, and collard greens; and zeaxanthin, (specifically the RR [trans] stereoisomer) from orange peppers, paprika, and corn. This process begins in the fetus, and continues into infancy when zeaxanthin is preferentially taken up from mother’s milk. High concentrations of foveal zeaxanthin reduce the number of anti-VEGF injection treatment cycles by nearly 25% [17].

Embracing nitric oxide–promoting activities/nutrients for sustained choroidal vasodilation
Nitric oxide (NO) is a simple gaseous molecule of short half-life (seconds), and biological regulator in the fields of neuroscience, physiology, and immunology. The discoverers were awarded the 1998 Nobel Prize for Medicine for its role as a cardiovascular signaling molecule and potent vasodilator with 60,000 articles to date. With age, human blood vessels and the nitric oxide system becomes less efficient because of inactivity, poor diet, smoking, and free-radical damage, causing veins and arteries to deteriorate. Atherosclerosis diminishes endothelial NO production and desirable blood pressure and choroidal perfusion. Nose breathing, exercise, sleep hygiene (correcting sleep apnea), pulsed electromagnetic stimulation (ie, Bemer mattress pad), and far-infrared dry saunas are among the modalities that enhance NO production and improved circulation. The arginine-citrulline cycle is responsible for NO production, with beets, nuts, dark chocolate, watermelon, and spinach particularly good foods for natural NO production. A commercially available dietary supplement, Longevinex, which contains trans-RV, also has been shown at 2 independent medical centers to enhance choroidal thickness via NO synthesis (discussed later).

SUMMARY
Clinicians in collaboration with primary care practitioners should assess and optimize their patients’ GSH and vitamin D statuses, ideally based on baseline serum laboratory testing, and in relation to their age. Adequate GSH and Se are vital in cancer prevention and ocular health. Because of their powerful modulation of both inflammation and angiogenesis, tocotrienols should be considered for prescription. Polyphenols, the so-called medicines of the twenty-first century, should be embraced, in moderation, for their myriad biological attributes. Suboptimal Mg status has been ignored for more than 40 years, against a backdrop of prescriptive overcalcification. Rather than discard fish oil as a therapeutic modality based on the AREDS II study, ophthalmologists and optometrists should consider its proven cardiovascular benefit, and appreciate its beneficial effect on comorbid dry eye, and the recent successful antineovascular AMD clinical data using DHA-rich fish oil. An additional nutrient, high-dose dietary zeaxanthin, seems crucial in protecting the
fovea. Promotion of endothelial health and enhancement of choroidal blood flow is possible with NO-augmenting activities and common nutrients.

MINIMIZING ENVIRONMENTAL STRESS

This article scrutinizes the often-overlooked issue of ascorbate depletion resulting from transporter-competitive simple intake, aspirin, increased blood sugar level, and tobacco use. Other environmental stressors include (1) pharmaceutically induced nutrient depletion, (2) ubiquitous copper (Cu) plumbing, and (3) iatrogenic calcification and oxidation through overzealous Ca and iron (Fe) dietary supplementation respectively (Table 2).

The ability to improve the physiology of the eye through lifestyle/environmental modification should be the core responsibility of progressive optometrists in support of ophthalmologists, achieving superior surgical outcomes. The synergistic relationship of neurologists and neurosurgeons or cardiologists, and cardiac surgeons is logical. In the recent 52-country INTERHEART study concerning acute myocardial infarction, 9 environmental associations were responsible for 90% of population-attributable risk in men, and 94% in women [18]. Thus, if cardiovascular risk can be eliminated by mitigating environmental stress, why not AMD?

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Environmental stressors</th>
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<tbody>
<tr>
<td></td>
<td><strong>Physician considerations</strong></td>
</tr>
<tr>
<td>Excess sugar and simple carbohydrate consumption</td>
<td>Counsel high-risk patients with AMD concerning the advantage of low simple-carbohydrate diets and encourage eliminating high-fructose corn syrup and hidden sugars</td>
</tr>
<tr>
<td>Chronic ascorbic acid (vitamin C) deficiency</td>
<td>Encourage consumption of high-bioavailable sources of vitamin C and accompanying phytonutrients, including yellow, red, and green bell peppers, respectively, as well as guava, orange juice, brussels sprouts, strawberries, papaya, broccoli, cantaloupe, and kiwi fruit. Consider supplementation needs beyond AREDS II</td>
</tr>
<tr>
<td>Polypharmacy</td>
<td>Avoid GI acid blockers, β-blockers, and high-dose statins</td>
</tr>
<tr>
<td>Ubiquitous Cu plumbing (United States)</td>
<td>Filters on drinking water faucets</td>
</tr>
<tr>
<td>Fe (ferrous) toxicity</td>
<td>Potential beneficial effects with chelation and blood donation as a long-term preventive treatment of retinal disease involving age-accumulated RPE Fe. Fe chelation has also proved successful in managing cardiovascular disease. Consider diet modifications to replace red meat with leaner meats like chicken</td>
</tr>
<tr>
<td>Oversupplementation of Ca</td>
<td>Calcification in the vascular system and Bruch membrane can be slowed or reversed. Coronary artery Ca(^{2+}) levels directly correlate with hours slept, with fewer than 7 h showing a decline. Supplementation should be considered with natural anticalcifying agents (ie, vitamin D, K [MK4 and 7 form], Mg, and IP6 [the rice bran factor])</td>
</tr>
</tbody>
</table>
Chronic ascorbate depletion/dosing considerations
Ascorbate is the master water-soluble extracellular reducing agent that sets the oxidation/reduction potential, GSH reduction status, and overall energetics of all cells. Ascorbate is actively concentrated in all ocular tissues, including the retina. It downregulates retinal hypoxia-inducible factor antecedent to neovascularization.

Vitamin C status is persuasively linked to reduction of cardiovascular disease, stroke, cancer, and recently mortality, and has a major role in the prevention of atherosclerosis, as delineated in a 17-chapter/650-reference text by cardiologist Thomas Levy [19]. Given a half-life of only 30 minutes, this water-soluble nutrient must be replenished through consistent plant food intake, bioflavonoid potentiation, slowed absorption/time release technologies, or liposomal supplementation. Estimated intake at 110 mg per day for North Americans cannot make up for the postulated universal gene mutation that halts constant ascorbate synthesis by the liver.

Minimal plant food intake (vegetables, fruit), excessive smoking, chronic aspirin/acetaminophen use (see Fig. 3), and high dietary sugar/simple carbohydrate intake all deplete and disrupt the ascorbate redox state. Americans meeting average micronutrient-rich, vitamin C–rich, and plant food (vegetables and fruit) consumption remain inadequate at only 15% to 20% of the population and worse for people of lower socioeconomic status. Cellular damage from ascorbate deficiency is indistinguishable from radiation damage. Vitamin C deficiency is associated with universal disease prevention and health maintenance when average-person, randomized, placebo-controlled, drug-type clinical trials are scaled from lowest to highest micronutrient intake. Vitamin C reduces the upstream stimulus to neovascularization via hypoxia-inducible factor 1 (HIF1). Activation of HIF1 is tied to VEGF. Significantly, blood serum ascorbate is actively transported and concentrated in all ocular tissues. One investigative health journalist reminds readers that it is impractical to believe humans can achieve vitamin C self-sufficiency via diet alone. The issue remains one of ascorbate deficiency and the poor or poorly informed Box 1.

Excess simple sugars against concurrent cellular ascorbate depletion  
(vitamin C deficiency)
Vitamin C is in direct clinical competition with sugar, according to the glucose-ascorbate antagonism hypothesis [20]. Recent data suggest that glucose, ascorbate, and the oxidized form of ascorbate (dehydroascorbic acid) share many of the same cellular membrane transporters [21].

Added sugar, simple carbohydrates, and in particular high-fructose corn syrup have no known nutritional value but are drivers of the pandemic of insulin resistance (IR), preceding a diabetes diagnosis by several years. Counterintuitively, people can be obese without having metabolic syndrome or non-alcoholic fatty liver disease (NAFLD). Fat cells are endocrine cells, secreting hormones (adipokines) regulating insulin sensitivity and satiety, and a plethora of inflammatory cytokines (eg, tumor necrosis factor-alpha [TNF-α], plasminogen activator inhibitor-1, intercellular adhesion molecules, and CRP), as
well as nonesterified free fatty acids. Visceral fat secretes hormones causing IR and increased mitochondrial dysfunction in insulin-independent tissues such as the retina. VAT mobilizes free fatty acids that are oxidized by vascular endothelium to generate ROS. It is this increased oxidative stress that causes inflammation and endothelial dysfunction. Endothelial dysfunction further leads to hypercoagulability, vasoconstriction, and inflammation. NAFLD is a clear and continuous risk factor for hypertension, sleep apnea, and hypoxic stress, all insidiously affecting AMD (Fig. 2).

AMD risk increases with increasing body mass index (BMI) and waist size. In AREDS I, a BMI greater than 30 doubled the risk of neovascularization. Greater waist circumference and prediabetes/diabetes mellitus (DM) are associated with low macular pigment levels, because fat-soluble nutrients like zeaxanthin are stored in adipose tissue before transport to the retina. The 168 forms of environmentally added simple sugar in the American diet is a growing toxic ocular burden, especially in the presence of vitamin C deficiency. Optometrists and ophthalmologists should refer to the USDA (United States Department of Agriculture) 2015 to 2020 Healthy Eating Guidelines to advise patients with AMD to restrict sugar consumption to no more than 10% of total

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**Box 1: Ubiquitous ascorbate deficiency in retinal patients**

*Obstacles for achieving vitamin C sufficiency*

1. Vitamin C–depleting drugs (aspirin, acetaminophen, steroids, diuretics, pain relievers)
2. Vitamin C–depleting habits (ie, smoking, excessive alcohol, refined sugars) and diabetes
3. Excessive emotional or physical stress depletes vitamin C from adrenal glands
4. Chronic or acute viral or bacterial infections enhance need for vitamin C (hepatitis C virus, human papilloma virus, tuberculosis, influenza)
5. Growing children require increased vitamin C to build more connective tissue and bone
6. Pregnant or peak-menstrual-cycle women whose high estrogen levels weaken blood capillaries and induce blood serum leakage and edema
7. Typical older adults in whom there is insufficient gastric acid to properly absorb vitamin C, causing easy bruising and low platelet counts
8. *Helicobacter pylori* impairs vitamin C absorption because of its ability to interfere with gastric parietal cells’ secretion of hydrochloric acid
9. Wound healing requires more vitamin C
10. In atopic patients, increased serum histamine levels quell higher doses of vitamin C
11. *Candida albicans* overgrowth drastically reduces vitamin C levels
12. Anemic individuals require more vitamin C to absorb dietary heme
Fig. 2. (A, B) Biological actions of nutrients in the metabolic syndrome with impact of carotenoids (lutein, zeaxanthin), vitamin D, and sulfur (lipoic acid). B, benfotiamine; C, curcumin; D, vitamin D; L, lutein; LA, lipoic acid; P, pycnogenol; Z, zeaxanthin. Sites of action of micronutrients in DR. Several factors cause the small blood vessel damage that leads to diabetic retinopathy, including high blood sugar levels, high blood pressure, high and abnormal cholesterol levels, production of harmful free-radical molecules, and inflammation. Complex interactions among metabolic abnormalities characteristic of diabetes lead to activation of abnormal biochemical pathways (hexosamine, advanced glycation end product, PKC, polyol), oxidative stress, apoptosis, and inflammation, in addition to breakdown of the blood/retinal barrier, hypoxia, and neovascularization. PKC, protein kinase C. (From Chous AP. Moving the needle: can we influence the course of diabetes? Rev Optom 2013;150(12):40. Figure reprinted with permission of Review of Optometry, Jobson Medical Information.)
calories. Patients with AMD must avoid NAFLD and creation of cytokine-producing and carotenoid-sequestering visceral fat.

The paramount contemporary and scientific public health issue remains antecedent IR, preceding dysglycemia, atypically measured by physicians. All major religions note the benefit of intermittent fasting, which is the single most powerful method for avoiding IR. It should be mentioned that limitation of refined carbohydrates (bread, rice, pasta, cereal) accounts for 70% of the health benefits attributed to calorie-restricted diets. Other approaches beyond total avoidance of refined carbohydrates include ketogenic dieting, high-fiber diets, and the use of nutrients such as vinegar, spices, and herbs such as Ceylon cinnamon, Indian turmeric, or Asian berberine. Movement also matters. Sedentary behavior/sleeping more than 8 hours per day increase the risk of geographic atrophy by a factor of 8. Embracing metformin/RV and other CR mimics, found to reduce cancer/degenerative neovascularization mechanisms, is discussed later.

Polypharmacy and drug-induced nutrient and energy depletion
In Western society, nutrient depletion results from pharmaceutical effects such as those listed in Table 3, rather than simple malnutrition. For example, acid blockers are common pharmaceuticals used to decrease stomach acidity in conditions like gastroesophageal reflux disease, esophagitis, and peptic ulcers. However, these products also carry US Food and Drug Administration (FDA) warnings of blocked absorption of all 8 B vitamins, Zn, Mg, vitamin D, and the carotenoids [22].

Stomach acidity naturally decreases with age, but it is essential for enzymatic nutrient metabolism, absorption, and eradication of pathogens, because food is rarely sterile. Intrinsic factor, a glycoprotein protein produced by gastric parietal cells, is necessary to absorb vitamin B\textsubscript{12} and requires stomach acidity for activation. Less intrinsic factor, whether from \textit{Helicobacter pylori} infection or chronic proton pump inhibitor use, depletes vitamin B\textsubscript{12} beyond age-related hypochlorhydria. Depletion of B\textsubscript{12} in turn results in increased levels of homocysteine, a risk factor for AMD. Depletion of B\textsubscript{12} is also seen in 15% of patients with long-term metformin use, used as an off-patent drug by antiaging physicians. Serum total homocysteine, vitamin B\textsubscript{12}, and folate deficiencies predict an increased risk of AMD [23]. B\textsubscript{12} depletion along with decreased DHA is associated with both cognitive decline and AD. It may be useful to use the preferred methylated forms of B vitamins in patients with AMD, because of widespread population gene methylation defects.

Clinicians caring for patients with AMD can take their lead from Indiana University’s Aging Brain Care Medicare center of excellence, which has established that 40% of patients with AD have drug-induced delirium. The analogous drug-induced AMD phenomenon has not been systematically studied to date. The AREDS group should evaluate the habitual use of chronic acid blockers, nonsteroidal antiinflammatory drugs (NSAIDs) (Fig. 3), and high-dose statins in patients genetically susceptible to side effects.
<table>
<thead>
<tr>
<th>Pharmaceutical intervention</th>
<th>Nutrients and cofactors depleted with chronic use</th>
<th>Specific mechanism</th>
<th>Remediation</th>
</tr>
</thead>
</table>
| Gastrointestinal acid blockers | $B_1$, $B_2$, $B_3$, $B_4$, $B_6$, $B_7$, $B_9$, $B_{12}$, Zn, Mg, vitamin D, and carotenoids | - Depletion of $B_{12}$ results in increased homocysteine level, a risk factor for AMD. Intrinsic factor is necessary to absorb vitamin $B_{12}$ and requires a low gastric pH for activation  
- Deficiencies in serum total homocysteine, vitamin $B_{12}$, and folate predict increased AMD risk  
- $B_{12}$ depletion occurs in 15% of patients using long-term metformin [22]  
- All these nutrients are associated with cognitive dysfunction | Address *Helicobacter pylori* infection  
Supplement patients with AMD with a broad-spectrum water-soluble multivitamin/mineral formulation |
| Statins                     | Coenzyme Q10                                      | Depletion of mevalonate pathway metabolite coenzyme Q10, a mitochondrial antioxidant, results in mitochondrial dysfunction, particularly damaging to genetic ARMS2 genotype | Supplementation for patients with statin-induced myopathy and/or extrahepatic peripheral tissue toxicity |
| $\beta$-Blockers and some hypertensive medications | Tissue oxygenation (oxygen) and coenzyme Q10; Zn and multiple water soluble nutrients | Slows heart rate by 12 bpm, reduces BP/blood flow and oxygenation above the heart, triggering VEGF. Associated 70% increase in both early AMD incidence and exudative AMD | Supplementation: ACE inhibitors deplete Zn and loop diuretics deplete multiple water-soluble nutrients |

**Abbreviations:** ACE, angiotensin-converting enzyme; ARMS2, age-related maculopathy susceptibility 2 gene; BP, blood pressure; bpm, beats per minute.
Ubiquitous modern copper plumbing (United States)

Major metallic minerals such as Cu and Fe are essential for life but in its unbound state (unattached from melanin, albumin, ferritin, transferrin, lactoferrin, and other dietary chelators) Cu can generate metal-induced oxidation and resultant tissue destruction. Their toxicity, notwithstanding genetically inherited diseases such as Wilson disease (Cu$^{++}$ overload) and hemochromatosis (Fe$^{++}$ overload), are little appreciated in the pathogenesis of AMD or AD. Dysfunction of ceruloplasmin (Cp), a multifunctional Cu-binding alpha-globulin, which also plays a role in Fe metabolism, can lead to metal overload. Mice deficient in both Cp and its homolog hephaestin, but not each individually, have a striking, age-dependent increase in RPE and retinal Fe levels [24].

With advancing age, Cu and Fe accumulated in tissues are released from binding proteins like melanin and from roaming macrophages that arrive in response to inflammation. Free unbound Cu and Fe may solidify (oxidize) β-amyloid in the brain and cholesterol (ie, drusen) in the retina and liver, impairing efflux (disposal) of fatty biological plaques. Deposition of drusen may be antecedent to wet macular degeneration.

The 80% penetrance of US Cu plumbing is a threat to health according to a major review [25]. As the blood-brain barrier becomes permeable with age, Cu penetrates the brain, increasing production and decreasing clearance of amyloid-β. This process may be the main environmental cause of AD, and a likely contributor to AMD and AD, in which a large percentage of modern populations use Cu piping in home water supplies. For example, Cu mines are prevalent in Colombia, near a remote region where genetic early-onset AD has heavily affected the population. On the other end of the spectrum, India has very low rates of AD (and AMD) and uses plastic piping for the water supply.
Labile iron toxicity

The overmineralization theory of aging postulates that although minerals are necessary for the body to grow and develop, excessive accumulation of divalent metals leads to degeneration. Ca is needed and is difficult to overdose during the growing years, because Ca is needed for bone, Fe for hemoglobin in red blood cells, and Cu for formation of connective tissue. The accumulation of these minerals is largely achieved once full growth is achieved in men and pregnancy and monthly menstruation with accompanying Fe loss cease for women. These minerals then begin to accumulate and their rate of accumulation correlates with the speed of aging [26]. Loss of Fe may be a factor in the longer lifespan of women versus men because women begin to accumulate minerals at a later age. This overmineralization theory of aging also explains the cardiovascular benefit of phlebotomy, blood donation, and chelation.

Life exists at the interface of Fe deficiency and Fe sufficiency, but NAFLD reflects an unnatural Fe burden. It is estimated that one-third of the US population has NAFLD and resultant endoplasmic reticulum stress, leading to end-organ damage. Serum ferritin (stored Fe) is higher in poorly controlled type 2 diabetes than in older nondiabetics. Although hyperferritinemia may exist in the presence or absence of Fe overload, a transferrin saturation cutoff value of greater than 45% has been suggested to discriminate both settings.

The availability of intracellular Fe in its redox active labile form represents the main cause catalyzing extensive oxidative modifications of cellular components such as macromolecular protein, lipids, and DNA by the hydroxyl radical, ultimately leading to accumulation and cellular dysfunction. A 1943 publication notes abundant Fe in retinal and choroidal tissue (Table 4). Universal fortification of US grain products in addition to home-grown NAFLD adds to the retinal Fe burden.

Aging eyes, AMD in particular, represent the overmineralization of tissue not only by divalent Fe$^{2+}$ but also Cu$^{2+}$, and Ca$^{2+}$. Increased total Fe concentrations in both the RPE and Bruch membrane constitute both chelatable and nonchelatable forms. Ultimately, Fe$^{2+}$ impairs RPE phagocytosis and lysosomal function, and is associated with retinal neurodegeneration. Most recently, β-amyloid, a pathologic hallmark of AD, was detected in drusen. β-Amyloid has a

<table>
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<tr>
<th>Ocular tissue</th>
<th>Fe (µg/100 g wet tissue)</th>
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<tr>
<td>Vitreous humor</td>
<td>12</td>
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<tr>
<td>Aqueous humor</td>
<td>16</td>
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<tr>
<td>Lens</td>
<td>32</td>
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<td>Conjunctiva</td>
<td>273</td>
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<tr>
<td>Optic nerve</td>
<td>335</td>
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<tr>
<td>Retina</td>
<td>380</td>
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<tr>
<td>Choroid</td>
<td>1880</td>
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Data from Tauber FW, Krause AC. The role of iron, copper, zinc, and manganese in the metabolism of the ocular tissues with special reference to the lens. Am J Ophthalmol 1943, 26:261.
high affinity for Fe, describing a common pathologic pathway in AD and AMD. Increased transcriptional messenger RNA (mRNA) and levels of Fe-regulating proteins (transferrin, ceruloplasmin, and ferritin) also are present in glaucoma, another probable oculovascular disease [27].

Retinal Fe\(^{2+}\) transport is via endocytosis, transport by transferrin, and storage via ferritin complex. RPE and neuroretinal vasculature act as a blood-retina barrier against Fe\(^{2+}\) and Cu\(^{2+}\). Disruption of these structures in AMD exaggerates Fe-induced ROS stress, creating a cycle of worsening damage. Another cause for Fe overload is alteration of enzyme levels that affect Fe metabolism. Heme oxygenase-1 and oxygenase-2 proteins mediate the release of Fe from heme-containing proteins, and are increased in the RPE of AMD-affected maculas. Ischemia of short duration preconditions the retina by activating an Fe-signaling pathway with marked increase in the Fe sequestering storage protein ferritin.

Cardiovascular disease is intimately associated with AMD, sharing similar risk factors, and “so called ‘mild’ non obstructive coronary artery disease is often anything but” [28]. Higher red meat intake (\(\geq 10\) times/wk vs \(< 5\) times/wk) is associated with early AMD (odds ratio [OR], 1.47; 95% confidence interval [CI], 1.21, 1.79; \(P\)-trend <.001) [29]. Similar trends toward increasing prevalence of early AMD are seen with higher intakes of fresh and processed red meat. In contrast, poultry consumption (chicken \(\geq 3.5\) times/wk vs \(< 1.5\) times/wk) was inversely associated with late AMD (OR, 0.43; 95% CI, 0.20; 0.91; \(P\)-trend = .07), suggesting that different meats differentially affect AMD risk, which provides a target for lifestyle modification [29]. Note that red meat provides many times more highly absorbable heme Fe compared with chicken.

Overzealous Fe supplementation, leading to long-term toxicity, also may have detrimental effects in mitochondria-rich tissues. Labile Fe, via the Fenton reaction, generates activated ROS, mitochondrial genomic mutations, destabilized lysosomes, and ultimately cell death. In neurodegenerative diseases (including AMD and AD) and diabetes, “there are overlapping themes of mitochondrial influence or dysfunction and iron dyshomeostasis is apparent and relatively consistent” [30]. Aceruloplasminemia, an inherited autosomal recessive disease, is characterized by the triad of retinal degeneration, DM, and neurologic disease.

In summary, Fe overload from excess Fe consumption generates ROS-induced inflammation, whether from metabolic syndrome, type 2 DM, genetic susceptibility (ie, haptoglobin genotype), or US universal grain fortification programs. The intake of whole grains containing inositol hexaphosphate (IP6) phytate, a strong Fe chelator, mitigates the effect. Blood donation (aphlebotomy), chelation, and adopting dietary flax seed (ie, 28 g/d [1 ounce/d]) are long-term prevention and treatment strategies for cardiovascular and retinal disease involving age-accumulated RPE Fe.

The retinal lipofuscin connection

Lipofuscin is cellular debris that accumulates with advancing age. Within the retina, the ability of lipofuscin to produce oxidants depends on the amount of transition metals incorporated. Although the amount of oxidants formed
by cellular lipofuscin is moderate, with chronic Fe consumption, lipofuscin autocatalyzes its own formation. There are 3 lines of evidence that suggest absolute CR is not necessary to achieve longevity, reduce morbidity, and achieve a longer health span, and that only mineral control is needed [31].

First, studies show provision of Fe-binding bran to rodents reduced retinal lipofuscin slightly better than a calorie-restricted diet, showing that near starvation is not necessary. When researchers examined whether fiber, germ, or bran influences mortality, only the IP6 bran portion of whole grains significantly reduced mortality [32].

Second, when investigators removed phytoestrogens from soy, they found that soy IP6 was responsible for the cholesterol reduction in soy-based diets. Augmentation of Fe in the culture medium of rat heart myocytes markedly increased the level of lipofuscin accumulation, whereas desferrioxamine, an Fe chelator, had the opposite effect.

Third, it has been suggested that pulse doses of Fe chelators that easily penetrate membranes could be used to diminish lipofuscinogenesis.

Vascular and ocular tissue oxidation by universal overzealous US Fe grain fortification programs, indiscriminate long-term use of Fe$^{2+}$ supplements, and excessive consumption of heme (ie, red meat in full-growth adult men/postmenopausal women), all theoretically lead to increased self-propagating retinal lipofuscin accumulation.

**The cardiac-retinal/choroidal circulation–iron overload connection**

In multiple logistic regression analysis, humans with high ferritin levels are nearly 6 times more likely to have an acute myocardial infarction and 5 times more likely to die if low-density lipoprotein cholesterol level is also increased [33,34]. Does choroidal Fe-induced bleed and heal/unstable plaque also occur? It is clear that tissue Fe homeostasis is maintained by active participation of Fe-regulatory proteins. All 5 genes associated with hereditary hemochromatosis (HFE gene, hemojuvulin, transferrin receptor 2, ferroportin, and hepcidin) are expressed in the retina and RPE. Retinal Fe homeostasis is disrupted in various clinical conditions, such as hemochromatosis, aceruloplasminemia, bacterial and viral infections, and AMD. This finding suggests the benefit of reduced red meat and prescribing natural mineral chelators in ophthalmology and optometry.

**Iatrogenic calcification via overzealous calcium supplementation**

The Bruch membrane regulates the reciprocal exchange of biomolecules, nutrients, oxygen, fluids, and metabolic waste products between the retina and choroid. Bruch membrane calcification is a barometer of systemic calcification. To wit, the diffusional transport of nutrients and removal of toxins across the Bruch membrane in the ninth decade of life is only 6.5% of that in the first decade of life [35].

Ca supplements are generally used as a means to reduce bone fracture risk. However, it is now known that this clinical practice accelerates vascular calcification, increases cardiovascular events in healthy postmenopausal women, and increases mortality in patients with renal failure. In a major recent review, there were few data to support Ca supplementation to prevent hip fractures.
This finding is of concern because prolonged Ca supplementation is associated with increased risk for heart attack [36]. The risk for a mortal heart attack over 5 years is less than 0.5% if the coronary artery Ca or Agatston score, as measured by 64-cut axial cardiac computed tomography scan is zero.

Maintaining a healthy gastrointestinal microbiome

The microbiome is the totality of the biologically active symbiotic microorganisms and their collective genetic material present within the gastrointestinal tract, and understood to exceed the totality of cells within the human body by a factor of 10. Dietary strategies which modulate the gut microbiota or their metabolic activities are emerging as efficacious tools for reducing cardiovascular disease (CVD) risk [37].

—Dr. KM Tuohy

Genetic and acquired gluten intolerance, excessive dietary sugar–induced fungal candida overgrowth, and glyphosate (Round-up) herbicide residuals found on vegetables/fruits and in waterways all result in progressive small intestinal bacterial overgrowth (SIBO). This microbiome disruption leads to less genetic bacterial fauna diversity (typically 30,000 species) and secondary leaky gut syndrome with attendant functional nutrient deficiencies/circulatory pathogenicity. Chronic antibiotic use in animal husbandry and medicine also disturbs a healthy gut microbiota. Pathogens include obligate intracellular bacteria or parasites (Rickettsia typhi, Chlamydia pneumoniae, Treponema pallidum, Toxoplasmosis gondii), obligate spirochetes (Borrelia burgdorferi), and other bacteria such as Streptococcus pyogenes. Imbalances/polarization of pro-inflammatory M1 macrophages versus M2 anti-inflammatory macrophages, with activation of retinal microglia, may be induced by gut bacteria.

Complement factor H (CFH) polymorphisms also have a strong association with AMD, the evolutionary result of human exposure to environmental elements, and disruptive biologics that include pathogens. CFH and the behavior of microglial, as well as neurotrophin levels, all seem to play a role in the phenotypical neurodegeneration of AMD and AD. These polymorphism-associated byproducts are the result of complex inflammatory reactions associated with serologically increased levels of CRP and other inflammatory cytokines, such as matrix metalloproteinase 2 and 9. Treatment of anthropogenic invaders results in disease regression in some individuals. Practitioners should prophylactically prescribe prebiotics, probiotics, and symbiotics to subpopulations with AMD and AD to avoid pathologic GI immune disruption.

It is not a translational leap to conclude that restoration of enterocyte tight junctions (Restore4Life) and microbiome support against SIBO should be part of the foundational treatment protocols against the multifaceted biological malfunctions associated with AMD and AD [38], because such treatments are now emerging in allergy and autoimmune diseases. Macrolides (doxycycline, minocycline) as well as micronutrients (Zn, vitamins C and D, allicin, IP6, carotenoids, quercetin, RV, omega-3 fatty acids, and melatonin) all have antimicrobial and antiinflammatory
properties that have been shown to modulate AMD, DM, or AD disorders. These micronutrients also support other defunct or parainflammatory pathways in AMD pathogenesis, including oxidative stress generated by ROS, advanced glycation end product formation, and mitochondrial dysfunction.

GENETICS, ZINC EPGENETICS, AND HORMETIC ADAPTIVE RESPONSE

Nutrigenomics
Nutrigenomics corresponds with the use of biochemistry, physiology, nutrition, genomics, transcriptomics, proteomics, metabolomics, and epigenomics to seek and explain the reciprocal interactions between genes and nutrients at the molecular level [39] (i.e., nutrients affect every aspect of the genetic pathway).

The promise of traditional pharmacogenetics and personalized medicine has been slow to materialize. Single gene–targeted pharmaceuticals, such as Gleevec for chronic myelogenous leukemia or Herceptin for metastatic breast cancer, have proved to have limited success for these specific conditions. Although DNA SNPs in disease susceptibility genes can help foresee a health trajectory, “single gene-targeted therapies developed during the last 2 decades for these diseases have proven to be unsafe, ineffective, and expensive” according to a recent review [40].

Gene mutations (structural changes in nucleotide arrangement) only comprise ~2% of all chronic age-related disease. Expression of gene proteins, or what is called epigenetics, is environmentally and nutritionally controlled and is largely responsible for the rate of biological aging. Rather, it is the absence of nutrients and epigenetic side effects, and not genomic effects, of widely used pharmaceuticals that are involved in the cause of ubiquitous modern maladies such as heart disease, cancer, neurologic cognitive disorders, NAFLD, diabetes, infertility, and sexual dysfunction. This process surely occurs in AMD, but has not been fully evaluated. The effect of inborn genetic defects is useful but overshadowed by stronger epigenetic environmental influences, starting at preconception and within the womb [6].

Epigenetic expression or silencing of genes may differ from one organ to the next. For example, the eyes may age faster than other organs because of the transparent nature of the eyes, which exposes ocular tissues to lifelong unfiltered solar radiation. AMD genetic risk factors are revealed only considering complex relationships among multiple factors. Thus, it is crucial to incorporate environmental exposures such as exogenous estrogen, sugar, Fe, and Cu into tests of genetic association at the SNP, gene, or pathway level. Despite DNA sequencing methodology differences, 2 groups recently showed diametrically opposing predictive results when studying specific high-risk homozygous SNP situations (2 of 2 CFH alleles with 0 of 2 ARMS2 alleles). What is clear from AREDS I, is that approximately one-seventh of all patients with AMD, born homozygous for the complement factor H gene (without the ARMS2 gene), have an overstimulated immune system with high Zn intake [41].
Zn/Cu ratios need to be balanced for optimal health. Zn deficiency is widespread, affecting 70% of Americans, exacerbated by Cu plumbing, and there has been strong randomized controlled trial evidence for Zn efficacy since the late 1970s predating the positive effect found in the 2001 AREDS I RCT. Therefore, while using genetic testing to gauge frequency of retinal examinations in AMD genetic SNP subpopulations is desirable, reducing the level of Zn in formulations is controversial.

Besides evaluating Zn efficacy, the genomic SNP approach remains relevant for determining AMD predisposition. There are now genetic SNP susceptibility versus dietary antioxidant data from 2 large populations: The Blue Mountains Eye Study and Rotterdam Study. In pooled data analyses, significant interaction between AMD genetic risk status and lutein/zeaxanthin intake ($P = .0009$) was found. Among participants with high genetic risk, the highest intake of lutein/zeaxanthin was associated with a greater than 20% reduced risk of early AMD, and weekly consumption of fish was associated with a 40% reduced risk of late AMD. No similar association was evident among participants with low genetic risk, and no interaction was detected between β-carotene or vitamin C and genetic risk status. The investigators concluded that high dietary intake of nutrients with antioxidant properties and fish reduces the risk of early AMD in those at high genetic risk [42]. Therefore, clinicians should provide dietary advice to young susceptible individuals (ie, children and siblings of parents with AMD) to postpone or prevent the vision-disturbing consequences of AMD.

Epigenetics, the epigenome, and hormesis in postdarwinian biology
The primacy of DNA and genetic determinacy (even in identical twins) can no longer be assumed in the postdarwinian scientific world as DNA is altered via epigenetic environmental modification against a backdrop of inherited potentialities of ocular blueprints like AMD SNP defects.

Epigenetics encompasses alterations of genetic material that do not affect the DNA nucleotide sequence; these include DNA methylation patterns, chromatin structure, histone codes, and noncoding small (micro) RNAs. He and colleagues [43] recently completed a review on the role of epigenetics in ocular disease. The complex epigenome is the result of gene expression and gene silencing. Genes are controlled by environmental conditions. Exposure to mild levels of biological stress (hormesis) such as temperature, radiation, high-altitude living, and food or the lack thereof preconditions tissues by activation of endogenous antioxidant enzymes (glutathione, catalase, SOD) via the nuclear response factor 2 transcription factor. Although genetics classically is concerned with mutations and cataloguing invariant SNPs, epigenetics is concerned with variable expression, cellular adaptability, enhancing stem cell survival, and tissue regeneration as manifested through 3 gene-switching mechanisms:

1. DNA methylation: the addition of a methyl group (M) to the DNA base cytosine (C) from folate (B9) and B12, most active during early development in the womb and early childhood, results in activation or deactivation by transfer of methyl
groups. Methylation SNPs are now readily obtained from whole genome analytics (ie, http://www.23andme.com).

2. Histones: proteins that organize DNA strands into nucleosomes by forming molecular complexes around which DNA winds. Gene expression is controlled by winding or unwinding DNA and modulated by histone deacetylase (HDAC) inhibitors such as sulforaphane in cruciferous vegetables, diallyl disulfide in garlic, *Nigella sativa* in black cumin seed oil, and RV in red wine.

3. MicroRNA (MiRNA): short strands of RNA interlacing with mRNA, thus silencing a segment of genes during translation; that is, miRNA blocks the protein-making ability of genes. The primary method for modulation of the epigenome involves miRNA. Specifically, miRNA can exert a profound influence on networks of genes and not just single genes, so often the solitary target of genetic engineers.

There is emerging interest in applying epigenetics to ophthalmology to directly influence the underlying disorders of individual patients. An expanded discussion follows.

**Methylation**

Methylation variation on gene promotor regions leads to AMD ocular damage. GSH S-transferase isoforms, GSTM1 and GSTM2, have antioxidant activity, reducing RPE oxidative damage. However, these gene isoforms are heavily methylated in their promotor regions, decreasing antioxidant expression, resulting in oxidative stress and RPE cell damage. In contradistinction, patients with AMD also show hypomethylation of angiopoietinlike protein 2 and hypomethylation of the interleukin-17 receptor C promoter, each upregulating angiogenesis.

**Histone modification**

Hypoxia-inducible factor (HIF)-1α is the seminal trigger of neovascular angiogenesis under tissue hypoxia. Specifically, excessive HIF senses oxygen deprivation and activates cellular VEGF, stimulating angiogenesis and restoring tissue oxygenation whether in cancerous tumors or AMD. VEGF expression also is upregulated by hyperglycemia, similar to carcinogenesis (discussed earlier). Current AMD treatments focus on reducing VEGF expression. Ascorbate, used in AREDS I and II, significantly downregulates HIF-1, but the half-life is limited unless continuous plant food intake, serial dosing, or time release/liposomal forms are prescribed. Because smoking reduces serum ascorbate levels, supplementation beyond AREDS is especially beneficial when combined with smoking cessation. HIF-1α gene expression is altered by histone modification and reduced by HDAC inhibitors through upregulation of p53 and von Hippel-Lindau proteins.

**MicroRNAs**

MicroRNAs encompass a novel class of small, noncoding endogenous RNAs that regulate gene expression by directing their target mRNAs for degradation or translational suppression. Insidious RPE cell death is the leading cause of atrophic AMD vision loss, and the successful survival of surgically injected retinal RPE stem cells also depends on a healthy RPE. Thus proper modulation of miRNAs is of utmost clinical importance. The downregulation of miRNA 23a...
is associated with the upregulation of the death (apoptosis) receptor FAS (apoptosis stimulating fragment) with additional miRNAs involved. The injection of pre-miRNA-31 or pre-miRNA-150 causes significant downregulation of murine retinal VEGF in mice under ischemic stress. Pre-miRNA-31 also reduces the expression of retinal HIF-1α and platelet-derived growth factor B.

**General principles: actions of sirtuin 3**

Sirtuins are a family of 7 protein deacetylases regulating metabolism, stress responses, and aging. These so-called survival genes are involved in lifespan-extending effects of CR. Mitochondrial sirtuin 3 is essential in enhancing the GSH antioxidant defense system during CR. RV simulates sirtuin 3, mimicking low-caloric diets, underlying the French Paradox. Sirtuin 3 shows a variable-number tandem repeat polymorphism, with allele-specific enhancer activity in this region. This allele lacks enhancer activity in men older than 90 years, suggesting that underexpression of sirtuin 3 is detrimental to longevity. Longevinex, an RV-based matrix, activates sirtuin 3 some 295% better than RV (Fig. 4).

**General principles: hormesis**

The human organism must be endowed with efficient specialized mechanisms that limit the reaction to stress and prevent stress damage

—Felix Z. Meerson MD, 1991

Stress-inducing treatments such as CR showed positive systemic health effects in 25-year study of 30% calorically restricted rhesus monkeys [44]. CR entails reduced calorie consumption without malnutrition, and is the only natural regimen shown to extend mean and maximum lifespan, as well as health span in a wide range of organisms [45]. The CR mechanism challenges alteration of the epigenetic effects mentioned, as well as telomere shortening, stem cell depletion, cellular senescence, mitochondrial dysfunction, genomic instability, proteostasis imbalance, impaired nutrient sensing, and abnormal intercellular communication. The nicotinamide adenine dinucleotide sirtuin pathways are the specific activated CR routes.

Felix Z. Meerson MD (1926–2010), a Moscow-based biologist, published 578 articles and many books, describing a powerful natural adaptive phenomenon in which humans are deprived of oxygen or food. Meerson discovered that when hypoxia is intermittent rather than constant, the human body switches on defensive mechanisms that produce supernormal resistance to biological, physical, and emotional stress [46]. Through low-oxygen adaptation, Meerson discovered that adaptation to high-altitude environments produces superhuman immunity, circulation, heart pumping power, mental task speed, and improved stress response. Meerson’s work has been largely overlooked by all branches of medicine.

Cardioprotective preconditioning has been studied since the 1970s whereby adapted rodents were first subjected to intermittent hypoxia for 6 weeks, after
Fig. 4. (A, B) The aging cell, sirtuins and criticality of sirtuin 3.
which coronary artery flow was blocked. Preadapted (oxygen-deprived) animals experienced 27% diminished cardiac damage and 2 to 3 times less death \[47\]. Their hearts contracted and pumped oxygenated blood 3 times more efficiently than nonadapted animals. Several decades and more than 500 publications later, Depak Das (1947–2013) and his team at the Cardiovascular Research Center at the University of Connecticut Health Center showed that mortal heart attacks in larger animals (canines) could be turned into non-mortal events when they were preconditioned or by using molecular nutrient mimicry of the phenomenon \[48\]. Stress-inducing treatments likely combat overmineralization. Ischemic preconditioning leads to a transient Fe-signal that increases accumulation of apoferritin and sequestration of reactive Fe released during ischemia. This ischemic stress response alters Fe homeostasis toward cardiac, cerebral, and retinal protection (discussed earlier).

**Resveratrol**

In the Melbourne Collaborative Research Study \[49\], alcohol consumption by itself had modest negative association in both early and late AMD. However, biologists are interested in RV found specifically in red wine because of the French Paradox, and the broad multiple actions supporting healthy aging. Abstention from wine is associated with a mortal myocardial death rate of \(\sim 240\) deaths per 100,000, whereas consumption of 3 to 5 glasses per day is associated with a cardiac mortality \(\sim 90\) deaths per 100,000. The French have similar cholesterol levels to Americans, smoke, and eat fatty foods, but have far more centenarians per 100,000 than any other country \[50\].

RV is a nonflavonoid polyphenol phytoalexin toxin found within a fungus of the skin of plants (eg, grapes) that are stressed by cold or lack of sunlight. It is a small molecule with a molecular weight of 228 Da, similar in size to allicin (in garlic) and therefore able to enter cells. RV is an HDAC inhibitor. There is approximately 1 mg of trans-RV in a 150-mL (5-ounce) glass of the best aged dark-red wine. Oral absorption is approximately 70% through the small intestine with a half-life of 14 minutes if not hepatically metabolized. Bioavailability is dramatically enhanced with half-life extension to 9 hours via hepatic sulfonation or glucuronidation and subsequent release by the glucuronidase enzyme at the sites of infection, inflammation, or malignancy. RV has multiple biological actions of distinct interest to modern medicine and the eye-care professions, which are (1) germicidal (antibacterial, antifungal, antiviral); (2) antiinflammatory (decreasing cyclo-oxygenase [COX]-2, CRP, and TNF-\(\alpha\) levels); (3) vascular (anticholesterol, antihypertensive, antiplatelet, and antiplaque); (4) neurologic (antidepressant [monoamine oxidase inhibitor] and anti–brain plaque [decreased \(\beta\)-amyloid levels]); (5) metabolic (anti-diabetic, rescuing pancreatic beta cells); and (6) anticarcinogenic, against all 3 stages of cancer (initiation, promotion, metastasis).

Nearly 2000 published articles concern RV and cancer. RV is a weak phytoestrogen found to bind estrogen receptor alpha and beta with comparable affinity, but with 7000-fold less affinity than estradiol. RV-induced epigenetic modification alters protective pathways against oxidative stress, DNA damage,
excitotoxicity, apoptosis, and inflammation. RV suppresses VEGF secretion by human RPE cells induced by inflammatory cytokines, TGF-β, and hypoxia.

The 15% failure rate of anti-VEGF therapy against retarding neovascularization has been attributed to a predominance of proangiogenic macrophages in neovascular AMD [51]. RV manifests antiangiogenesis activity through miRNAs, further inhibiting retinal VEGF by modulating Kruppel-like 4 factor and macrophage polarization toward neovascularization. Based on animal data, RV is a prime nutraceutical, controlling pathologic choroidal neovascularization [52].

Mild ischemia (or RV) induces the cardiac preconditioning effect. Short-term mild cardiac ischemia upregulates endogenous protective antioxidants (nitric oxide, heme oxygenase, adenosine, and redox proteins) before a heart attack, thus limiting damage in the event of an abrupt halt in the delivery of oxygenated blood to the heart. RV shows the same anticlotting and pain-reducing antiinflammatory qualities as aspirin without the risk of hemorrhage or bleeding gastric ulcers. RV inhibited blood clotting among high-risk cardiac patients when aspirin did not, while showing superior antiinflammatory action compared with NSAIDs. RV has a much longer half-life than aspirin (30 minutes). RV does not deplete vitamin C as aspirin does. RV also preserves survival of injected stem cells in the heart. The authors found this to be true in the retina (Fig. 5).
Beyond RV, a plethora of red wine constituents affect the epigenome. Small molecular weight (SMW) molecules are preserved in red wine. Although not fully understood, the polyphenolic class of phytonutrients called flavonoids manipulates the human genetic library by suppression of harmful genes and upregulation of genes related to disease prevention. Concentrated unfiltered red wine, an ancient beverage, manifests a biological retinal healing effect, even leading to the enhancement of macular pigment optical density, in the absence of carotenoid supplementation. The authors documented these changes in desperate patients for whom all existing medical options had been unsuccessful. We used molecular medicine observing visible short-time-frame and long-time-frame improvements in retinal architecture, cellular function, and macular pigment enrichment.

**Inositol hexaphosphate, phyate, or phytic acid**

IP6 is nature’s divalent mineral chelator and is derived from rice bran. IP6 exerts beneficial effects against cancer, Parkinson disease, and AD. Bran factor is a normal dietary component with up to 1600 mg/d consumed by vegetarians, but far less by omnivores. Bran diets high in IP6 do as well as CR in reducing RPE lipofuscin [53]. Photoreceptor outer-segment phagocytosis is transduced by an intracellular signal, an increase in intracellular inositol-1,4,5-trisphosphate (IP3), leading to ingestion of the bound photoreceptor outer segment membranes. IP3 is another bran factor.

**Resveratrol–vitamin D$_3$ synergy**

Vitamin D$_3$ assists in maturation and stability of the immune system. As mentioned earlier, there are several emerging clinical studies showing associations of low vitamin D$_3$ level and AMD status, and 2 studies showing that RV increases the sensitivity of the vitamin D receptor.

**Hyaluronic acid**

Hyaluronic acid (HA) contributes to structural organization of extracellular matrix constituting the interphotoreceptor matrix. HA is localized to the chorioretinal complex, disappearing after the fifth decade, leading to retinal fibrosis. HA is used in surgical retinal stem cell repair, functioning as an antioxidant to maintain undifferentiated retinal progenitor cells (RPCs). RPCs that are transplanted in HA-encapsulated form cause little retinal disruption, resulting in an effective method for retinal self-renewal. This finding leads to the hypothesis that activated fibroblast cells induce via supplementation, more HA, providing retinal stem cell protection and mitigating the symptoms common to all retinal diseases.

**Longevinex (2004)**

This is a patented multiple SMW combination of epigenetically active molecules that cross the blood-retinal barrier. Low-dose (100 mg) RV and quercetin (Fe$^{++}$ chelators) are the main bioactives. Because trans-RV is converted to cis-RV when exposed to ultraviolet light, microencapsulation within plant starches and dextrins protects the RV from degradation by light, heat, and oxygen. The second component is IP6, a divalent metal chelator of destructive
labile Cu++, Fe++, and Ca++ (discussed earlier). IP6 dissolves Bruch membrane and arterial Ca++ and aids in double-stranded DNA break repair. The third component is vitamin D₃, a potent antiinflammatory, Bruch membrane/vascular decalcifying agent, antineovascular agent, and gene regulator. The properties of Longevinex extend beyond RV and include:

1. Decreased inflammation (COX-2, CRP)
2. Decreased HIF-1 and VEGF genes (miRNA 21, 20b, 539)
3. Increased levels of Nrf2 mediated endogenous antioxidants (GSH)
4. Decreased blood clotting (platelet adhesiveness)
5. Increased vasodilation (nitric oxide)
6. Increased divalent metal chelation (Fe++, Cu++)
7. Decreased oxidation, peroxidation
8. Decreased cell adhesion (platelets, microbes, tumors)
9. Decreased calcification (ie, arterioles and Bruch membrane)

Longevinex was chosen for experimental human use after considerable scrutiny. The immediate effect of Longevinex is decreased blood clotting. Longevinex-fed rabbits halved their circulating total cholesterol levels, reduced their arterial plaque by more than 50%, and reduced by 35% heart damage area, following intentional heart attack [54]. They also showed improved heart-pumping action and blood flow in both the aorta and the 4 coronary arteries following the intentionally induced heart attack.

In laboratory mice, Longevinex activates more longevity genes over the short-term than long-term CR. That is, short-term use switches 677 of 831 longevity genes in the same direction as long-term calorie restriction, and with beneficial effects on blood sugar levels. Longevinex shows a unique epigenetic profile apart from plain RV. Longevinex exceeded the effect of RV in 15 of the 25 top miRNAs. Longevinex shows a unique genetic pattern (inhibiting miRNA20b controlling hypoxia-inducing and VEGF genes) nearly 2 to 7 times more than RV [55].

In a rodent study of cardiac tissue exposed to ischemia/reperfusion, Longevinex downregulated by 1366 times the miRNA 20b, which controls HIF-1, in turn inhibiting VEGF gene protein induction. Longevinex inhibited HIF-1 6-fold better than plain RV. In addition, unlike high-dose RV, Longevinex does not induce cytotoxicity (cell killing) in megadose concentrations and, therefore, has a desirable margin of safety. The Longevinex matrix has also passed human and animal toxicity testing [56].

In summary, because of the synergy of small active epigenetically acting molecules, Longevinex is profoundly superior compared with RV because it:

1. Does not show cytotoxicity at high dose (no hormesis), whereas RV does
2. Decreases cholesterol, restoring myocardial dysfunction in hypercholesterolemic animals
3. Beneficial against human metabolic syndrome (in Japan) [57]
4. Functions as an antidiabetic drug with insulinlike sugar level-reducing effects
5. Promotes choroidal vasorelaxation and thickening [58]
THERAPEUTIC INTERVENTION
Our clinical publications with respect to stem cells and short-term and long-term treatment of AMD using epigenetically designed SMW nutrients found in Longevinex are discussed here. These nutrients are a stabilized matrix of red wine polyphenols (including trans-RV), vitamin D₃, and IP6. Our cases highlight success in untreatable AMD in octogenarians with both atrophic and neovascular disease (Figs. 6 and 7). Our anatomic and functional clinical observations warrant further large-scale evaluation. This research is a work in progress at other institutions.

Our published clinical experience with Longevinex shows retinal stem cell generation (see Fig. 5); short-term improvements (days, weeks) in retinal anatomy (see Fig. 6); and long-term, multiyear maintenance effects (see Fig. 7).

Fig. 7. Three notable observations of long-time-frame (multiyear) dramatic improvement in retinal anatomy. (Top) Case 1. A 64-year-old white patient suspected to have glaucoma with photophobia, atrophic AMD (worse right eye), and diabetes with declining vision function in the right eye, has been on L/RV for 2.5 years and is maintaining visual function. (Bottom) Case 2. An 89-year-old white patient with chronic kidney disease and cataracts who has been on L/RV for 3 years maintaining his visual function requirements to retain his driver’s license. (Right) Case 3. A 67-year-old white patient with bilateral polypoid choroidal vasculopathy, a treatment-resistant AMD variant, worse in the right eye. He also has a history of central serous retinopathy above the right optic nerve and a left retinal central foveal photoreceptor integrity line defect and impaired color vision. Improved retinal/choroid structure was observed. L/RV, longevinex. (From Richer S, Patel S, Sockanathan S, et al. RV based oral nutritional supplement produces long-term beneficial effects on structure and visual function in human patients. Nutrients 2014;6(10):4413 and 4415.)
SUMMARY
AMD is age dependent with a gradual, insidious onset until the eighth decade when there is an exponential increase in the number of patients. Hence, there is value in the identification of modifying exposures, early in the pathogenesis, that could mitigate risk and slow or halt disease progression. The potential of ocular preventive medicine and functional medicine extends beyond the few AREDS I and II nutrients, important as they are. Although one-third of high-risk patients with AMD delay/avoid catastrophic loss of central vision, two-thirds of the aging population are not helped by AREDS supplements, and one-seventh potentially experience a Zn-induced hyperimmune response.

The “AMD - zinc controversy” has become not only a distraction, but an opportunity for insight. Despite equivalent zinc dosing, the new AREDS II formulation is not strictly equivalent to AREDS I. AREDS II lutein, for example, powerfully dampens inflammatory biomarkers CRP and siCAM as well as the rate - limiting compliment alternative pathway (Factor D) and its’ activation products C5a and C3d [59]. Therefore, post–hoc AREDS 1 genetic modeling predictions cannot strictly adapt to changing eye formulations containing additional nutrients such as lutein, zeaxanthin, omega 3 fats etc.

The seminal issue beyond SNP genomic potentialities, is providing nutrients for maintenance and repair of the photoreceptor/RPE complex and neural retina. A growing number of patients experience suboptimal visual function as they age, or even undergo seemingly successful anti-VEGF intravitreal injections, only to eventually succumb to RPE disease and visual disability.

The future AMD battle encompasses maintenance of optimal cellular ascorbate, GSH/Se, and vitamin D status, all of which are ignored in orthodox AREDS science. Minimizing intake of simple carbohydrates; reestablishment of the microbiome for repletion of vital ocular nutrients such as the B vitamins; minimizing environmental Cu plumbing; minimizing intake of heme Fe and Fe-fortified/IP6-depleted grain products; as well as controlling modern nutrient-depleting polypharmaceutical medical practice is crucial. Zeaxanthin, lutein’s metabolite (mesozeaxanthin) and DHA all merit further clinical evaluation. Methods to promote choroidal blood flow (delivery/removal of waste products) through both activities and nutrients that enhance NO signaling seem beneficial but again appear to be ignored.

Rather than dwell on genetic testing, which addresses ~2% of all chronic disease, and search for genetic causes, clinicians should embrace postdarwinian epigenetics. As shown by Meerson and colleagues [46] and Juhasz and colleagues [48], the human body has an innate ability to heal itself by upregulating its own protective systems under conditions of mild biological stress, such as induced CR or hypoxia, or by using SMW molecules found in unfiltered red wine and crushed garlic.

Fig. 8 postulates synergistic pathways maintaining and restoring retinal health. It emphasizes appropriately controlling divalent metals based on gender and age, promoting mitochondrial renewal, and activating internal endogenous antioxidants through mild biological stress. This approach has been
demonstrated through the prescription of SMW nutrients found in an RV matrix known as Longevinex, to older US veterans, and recently submitted to the FDA for fast-track evaluation [60]. OTC Longevinex remains underused after 12 years.

Epigenetics and the control of aging are at the center of basic science and modern medicine. Epigenetics, the study of non-DNA sequence-related heredity explains the relationship between an individual’s genetic background, the environment, aging, and disease, and why the promise of genetic testing has been unproductive. The epigenetic state varies among tissues during a lifetime, whereas DNA is constant. Predictive SNP genetics is a futile and expensive endeavor unless multiple environmental risk factors are incorporated.

Basic nutritional hygiene incorporating the 90 essential nutrients needed to sustain vibrant cells is the foundation for the health of all end-organ systems and tissues, including the retina [6]. Epigenetic-based therapies controlling minerals while providing a mild biological stress have been documented to reestablishretinal architecture, have anti-VEGF effects, increase macular pigment, and improve visual function, ostensibly through endogenous cellular mechanisms. A so-called AMD diet and epigenetic modulation of genetic expression are the tools of twenty-first-century physicians. Counseling patients involves a team effort (optometrists and ophthalmologists) and staffing commitment. It is worth it, because this process improves overall health status beyond the traditional sphere of eye physicians. Educating patients in foundational nutritional science should also diminish the effects of time, and provide safer and superior ophthalmology treatment outcomes.

Fig. 8. Overlooked pathways beyond AREDS II that maintain and restore retinal health via 1) Mineral Control; 2) assisting ‘Mitochondrial Renewal’ by avoidance of sugars and ‘simple carbohydrates’ and 3) Activation of ‘Biological Stress’ via small molecular weight nutrient molecules such as the RV matrix Longevinex, which activates endogenous antioxidants and cellular repair.
References


