

Lipofuscin and Macular Degeneration

The accumulation of the autofluorescent pigment lipofuscin in the retina that occurs with aging has been explained as a side effect of the visual cycle. It occurs when two molecules of all-trans-retinal condense with one molecule of phosphatidylethanolamine in the discs of the rod outer segments, and is followed by uptake into retinal pigment epithelium (RPE) and conversion to the stable A2E, a pyridinium bisretinoid that is toxic to RPE cells. The accumulation of A2E, the major component of lipofuscin causes RPE cell apoptosis, thereby explaining age-related macular degeneration and macular degeneration characteristic of Stargardt disease. The drug isotretinoin (13-cis-retinoic acid) prevents accumulation of A2E in mice by slowing down the visual cycle and might therefore be used to prevent macular degeneration.

Key words: age-related macular degeneration, Stargardt disease, lipofuscin, retina, all-trans-retinal

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doi: 10.131/nr.2003.oct.342-346

Age-related accumulation of pigments in tissues such as the nervous system was noted as long ago as 1842 by Hannover.¹ These lipid-soluble pigments display brown to yellow autofluorescence and are known collectively as lipofuscins. Lipofuscins have interested nutritionists since Robison et al.² noted that lipofuscin deposition was greatly increased in the retinas of vitamin E-deficient rats.

Lipofuscin accumulates in the retinal pigment epithelium (RPE), the layer of cells backing the rods and cones (i.e., the photoreceptor cells constituting the outer segments of the retina, Figure 1). Most of the retina is populated by rods, which are sensitive to dim light and function in black-and-white vision. The central part of the retina, known as the macula, is a circular area of approximately 6 mm diameter that is extremely dense in rods and cones. In its center is the so-called fovea, populated by a

dense array of cones, which are photoreceptors that function in color vision. The macula contains 150,000 rods per mm², whereas the peripheral retina has only 30,000 rods/mm².

Cells of the RPE continuously phagocytose and degrade the membranous discs of the rods and cones. The discs are made of lipid and rhodopsin, the latter consisting of the protein opsin and the prosthetic group 11-*cis*-retinal. Upon light activation, 11-*cis*-retinal is converted to all-*trans*-retinal that is split off the rhodopsin. New discs are continuously generated at the base of the rods and cones. Degradation of the discs is performed by the lysosomes of the RPE.

A by-product of this degradation process is lipofuscin; lipofuscin accumulates as granules located in the lysosomes of the RPE, which lie over the macula. The formation of lipofuscin begins in the photoreceptor outer segments, where the all-*trans*-retinal reacts spontaneously (i.e., non-enzymatically) with the membrane lipid phosphatidylethanolamine, to form N-retinylidene-phosphatidylethanolamine (NRPE) (Figure 2).⁴ After tautomerization, this compound reacts with a second molecule of all-*trans*-retinal to form a pyridinium ring. This compound, dihydrogen phosphatidyl pyridinium bisretinoid (A2PE-H₂), autoxidizes to yield the phosphatidylpyridinium bisretinoid, A2PE. A2PE is hydrolyzed enzymatically by phospholipase D to the orange-fluorescent pyridinium bisretinoid named A2E (Figure 2). A2E accumulates in the RPE during phagocytosis of the rods and cones and is taken up by the lysosomes. It comprises the principal component of lipofuscin. A2E is a stable compound that is not degradable enzymatically.

Ben-Shabat et al.⁵ point out that A2E will form if the rate of generation of all-*trans*-retinal by light-activation of rhodopsin surpasses the rate at which all-*trans*-retinal is reduced to all-*trans*-retinol in the outer segments (Figure 3). If that is the case, the accumulated all-*trans*-retinal would provide the substrate for A2E formation. If, on the other hand, light-induced formation of all-*trans*-retinal is slow, it will not accumulate but rather will be reduced to all-*trans*-retinol. All-*trans*-retinol then proceeds through the visual cycle (Figure 3) and will not be available for A2E formation.

Because autoxidation is one step (Figure 2, steps 2 to 3) in the formation of A2E, it is clear why vitamin E-deficient rats accumulate lipofuscin.² More recently,

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Sparrow et al.⁷ determined that in the presence of singlet oxygen, A2E forms an epoxide that damages DNA. The active oxygen is, of course, countered by vitamin E.

Age-related macular degeneration (AMD) is the leading cause of blindness in the aged. It is the loss of vision resulting from degeneration of the dense array of rods and cones in the macula, the portion of the retina that functions in high-resolution and color vision. Sparrow et al.⁸ established the connection between accumulation of A2E and macular degeneration; when a critical intracellular level of A2E has been reached, cell damage begins to occur. This damage is induced by irradiation with blue light. Blue light (wavelength 435 nm) is known to cause damage to RPE cells in vivo and in cultured RPE cells.⁹ A2E strongly absorbs blue light and initiates apoptosis in cultured human RPE cells loaded with A2E by activating a proteolytic caspase cascade.¹⁰ An intact RPE is indispensable for survival of rods and cones. Thus, AMD can be traced to increased accumulation of A2E in the RPE.

A more detailed explanation of A2E accumulation came from an investigation of an eye disease called Stargardt disease.¹¹ In Stargardt disease, patients accumulate A2E in their RPE at an early age, causing degeneration of the macula and loss of vision very similar to AMD. Stargardt disease results from a mutation in a gene coding for a protein that is a member of a superfamily of transporter proteins called ATP-binding cassette (ABC) transporters. The particular protein, expressed exclusively in the discs of the rod and cone outer segments of the retina, is called ABCR. ABCR is a 220-kDa glycoprotein coded by the *abcr* gene in a molar ratio of ABCR to rhodopsin of 1:120 in the disc membranes.¹¹

In the visual cycle, the all-*trans*-retinal released from rhodopsin into the lumen of the discs (Figure 4) must be transported across the disc membrane in order to be reduced to all-*trans*-retinol in the cytosolic compartment of the rods. The disc membranes consist of lipid,

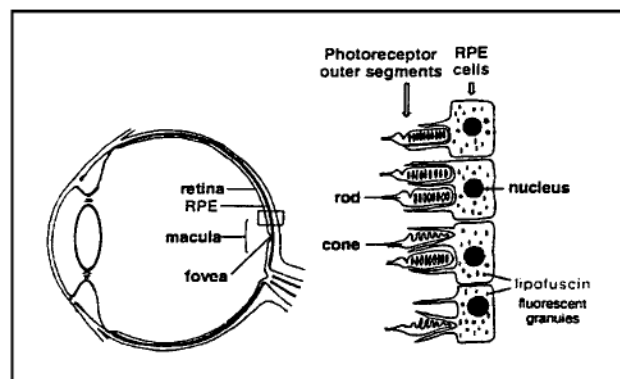


Figure 1. Diagram of the eye. The ratio of outer segment to RPE is simplified for clarity. From reference 3, with permission. RPE = retinal pigment epithelium.

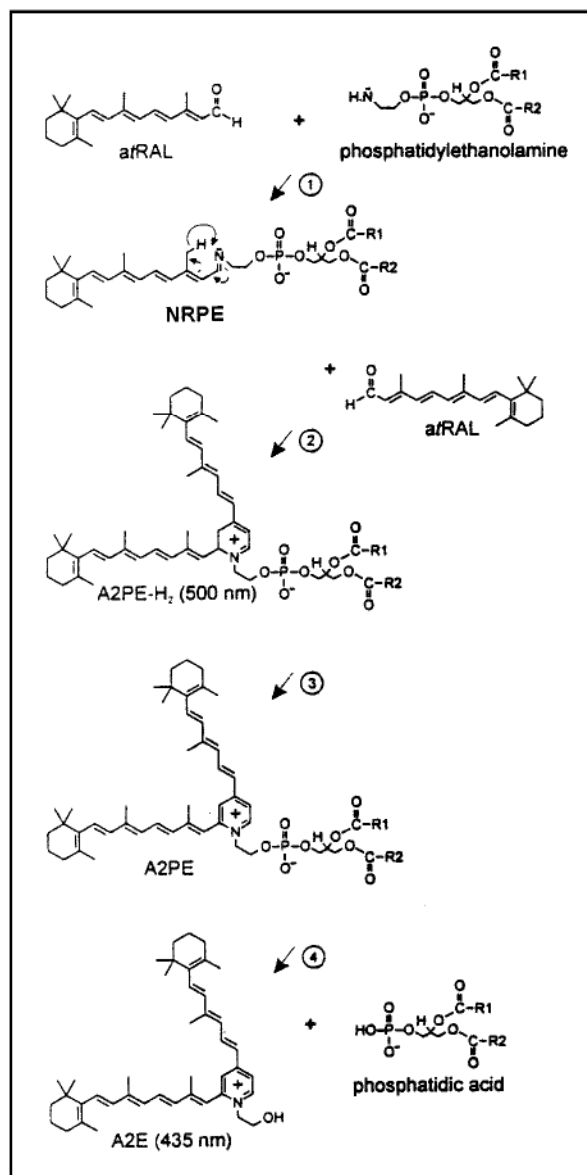


Figure 2. Synthesis of A2E in the retina and RPE. After light exposure, newly released atRAL condenses reversibly with phosphatidylethanolamine to form NRPE (step 1). Rarely, a second molecule of atRAL will condense with NRPE to form A2PE-H₂ (step 2). The wavelength of maximal absorption (λ_{max}) for A2PE-H₂ is 500 nm. Within the acidic and oxidizing environment of the RPE phagolysosome, A2PE-H₂ is oxidized to A2PE (λ_{max} = 435 nm; step 3). Hydrolysis of the phosphate ester yields A2E (λ_{max} = 435 nm) and phosphatidic acid (step 4). From reference 4, with permission. A2E = pyridinium bisretinoid, RPE = retinal pigment epithelium, atRAL = all-*trans*-retinal, NRPE = N-retinylidene phosphatidylethanolamine, A2PE-H₂ = dihydroposphatidylpyridinium bisretinoid, A2PE = phosphatidyl-pyridinium bisretinoid.

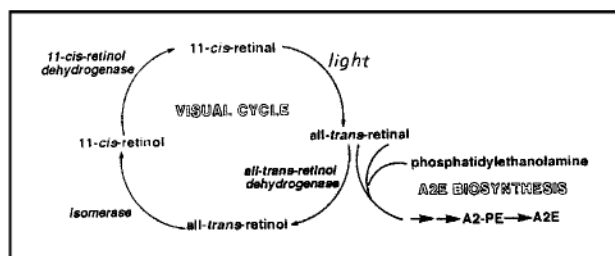


Figure 3. Visual cycle and A2E biosynthesis. atRAL that evades reduction to atROL can react with phosphatidylethanolamine (2:1 ratio) to generate A2E in a multistep process. From reference 6, with permission. A2E = pyridinium bisretinoid, atRAL = all-*trans*-retinal, atROL = all-*trans*-retinol.

specifically 40% phosphatidylethanolamine (PE). The all-*trans*-retinal on the luminal side of the disc membrane interacts spontaneously with PE within the membrane to form an adduct, NRPE (Figure 2). This substance is flipped across the membrane by the ATP-dependent transporter protein ABCR, and releases all-*trans*-retinal on the cytosolic side of the discs.¹² ABCR therefore represents a newly discovered retinoid transporter that occurs in both rods and cones¹³ and that is essential for the functioning of the visual cycle.

Weng et al.¹² proposed that ABCR performs two functions. First, it speeds dark adaptation by flipping the all-*trans*-retinal (in the form of NRPE) from the intraluminal space to the cytosolic space of the rods and cones. Second, it removes the NRPE, which forms spontaneously in the lumen of the discs, to the space outside the discs where the all-*trans*-retinal can be reduced to all-*trans*-retinol. Reduction of all-*trans*-retinal shifts the equilibrium to favor hydrolysis of NRPE to all-*trans*-retinal and PE.

In cases of Stargardt disease, in which there is a loss of ABCR function, NRPE accumulates inside the lumen of the discs, where increasing levels of all-*trans*-retinal, produced by light-activation of rhodopsin, increase A2PE formation (Figure 2). Phagocytosis by the RPE and hydrolysis of the A2PE then lead to accumulation of the stable A2E and degeneration of the macula. This process was shown in mice with null mutations in the *abcr* gene (*abcr*^{-/-}), which were used as models for patients with Stargardt disease.¹⁴ Heterozygote mice (*abcr*^{+/-}) showed a fourfold level of A2E and A2PE in their retinas compared with wild-type mice. The *abcr*^{-/-} mice had approximately 60% more of these products.

In AMD the transporter protein ABCR continues to function. With increasing age, A2E is gradually deposited in the RPE; this occurs as a secondary reaction to the transport of NRPE in the rod and cone discs. Sun et al.¹⁵ calculated that each human RPE cell contacts 50 rod outer segments and degrades 5 outer segments per day, producing a small amount of the stable, nondegradable A2E with each phagocytosis step. Young¹⁶ calculated that a person aged 80 years would have degraded 90 million discs.

As mentioned above, vitamin E deficiency in rats leads to increased levels of lipofuscin in the retina,² presumably because an autoxidation step is part of the chain of reactions leading to the formation of A2E.⁴ Vitamin E has been suggested as a possible dietary supplement for the prevention, or at least the retardation, of the development of AMD.¹⁷ However, no evidence has proven its efficacy.

The carotenoids lutein and zeaxanthin are yellow pigments found in high concentrations in the human macula. These pigments presumably filter out the blue light that initiates destruction of the RPE.⁹ Lutein and zeaxanthin have therefore been proposed as dietary supplements; however, Bone et al.¹⁸ state "It remains to be demonstrated whether lutein or zeaxanthin dietary supplements reduce the incidence of AMD."

A recent article entitled "Therapy for macular degeneration: insights from acne"⁶ suggests that a drug therapy has been discovered. This article was based on the research of Radu et al.,⁴ who aimed to find a means of slowing the visual cycle in order to decrease the levels of 11-*cis*-retinal in the rods and cones, and thus its availability for A2E formation.

For some time, scientists have known that the drug used to treat acne, 13-*cis*-retinoic acid, also known as isotretinoin or Accutane, interferes with the visual cycle and causes delayed dark adaptation in humans. This occurs because isotretinoin inhibits a key step in the visual cycle: the oxidation of 11-*cis*-retinol to 11-*cis*-retinal in the RPE by 11-*cis*-retinol-dehydrogenase.¹⁹

As mentioned above, a mouse model for human Stargardt disease and AMD is the *abcr*^{-/-} mouse, which

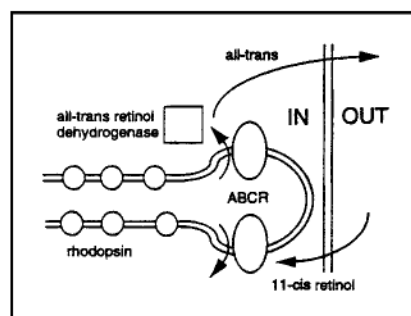


Figure 4. Model for ABCR action in the visual cycle and for pathogenesis of Stargardt disease and AMD, showing the edge of one outer segment disc and the adjacent plasma membrane. A possible path of atRAL and atROL movement following rhodopsin photobleaching is indicated by arrows in the upper half of the figure. atRAL is released into the disc membrane and is transported to atROL dehydrogenase on the cytosolic face of the disc by ABCR. atROL is exported across the plasma membrane to the adjacent RPE. A path of 11cRAL is indicated by arrows in the lower half of the figure. From reference 11, with permission. ABCR = ATP-binding cassette transporter (retinol), AMD = age-related macular degeneration, atRAL = all-*trans*-retinal, atROL = all-*trans*-retinol, RPE = retinal pigment epithelium, 11-c-RAL = 11-*cis*-retinal.

lacks the gene for ABCR, the transporter protein for NRPE. Reaction of NRPE with all-*trans*-retinal results in production of the stable and ultimately toxic A2E. Slowing of the visual cycle reduces the level of rhodopsin, the levels of 11-*cis*-retinal and all-*trans*-retinal formation, and hence NRPE formation.

Radu et al.⁴ injected isotretinoin into dark-adapted mice. One hour after injection, both wild-type and *abcr*^{-/-} mice were exposed to light that bleached 40% of their rhodopsin. Both groups showed a similarly slowed dark-adaptation rate compared with mice that were not injected (although *abcr*^{-/-} mice that were not injected had a slower dark-adaptation rate than wild-type mice that were not injected). Assay by HPLC upon injection of isotretinoin into wild-type mice revealed an approximately 50% dose-dependent decrease in the level of 11-*cis*-retinal in their eyes after light adaptation, evidence of slowed visual cycle. One striking result was the 40% reduction in the level of [³H]NRPE in *abcr*^{-/-} mice treated with isotretinoin and injected with [³H]atROL, compared with *abcr*^{-/-} mice that were not injected. The final proof of isotretinoin's effectiveness was achieved by administering it to 3-month-old *abcr*^{-/-} mice via osmotic pump for 1 month at 40 mg/kg daily. After that treatment, A2E in their RPE was determined by HPLC. The authors⁴ found that during 1 month, the level of A2E had risen 2.3-fold from baseline, and that the isotretinoin completely prevented this increase (Figure 5).

So much for the *abcr*^{-/-} mouse as a model for Stargardt disease. Can isotretinoin treatment alleviate AMD?

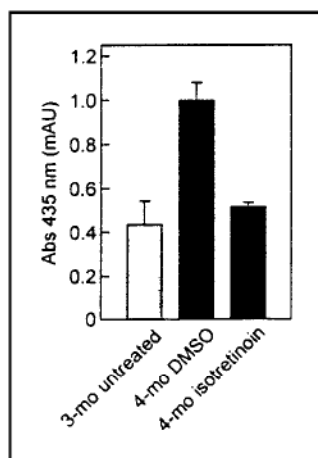


Figure 5. Levels of A2E in RPE after 1 month of treatment with isotretinoin. Levels shown are of A2E in RPE from 3-month-old *abcr*^{-/-} mice before treatment (white bar, *n* = 3), 4-month-old DMSO-treated control mice (left black bar, *n* = 3), and 4-month-old mice treated with 40 mg/kg per day isotretinoin (right black bar, *n* = 3). Values are expressed as peak-height milliabsorption units (mAU) at wavelength λ = 435 nm. Error bars show SD. From reference 4, with permission. A2E = pyridinium bisretinoid, RPE = retinal pigment epithelium, DMSO = dimethylsulfoxide.

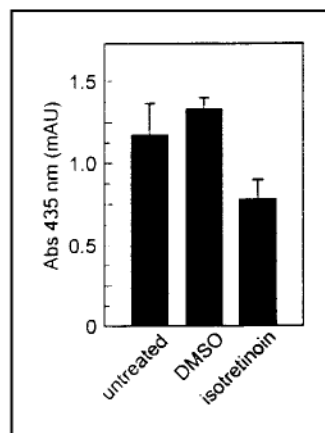


Figure 6. Levels of A2E in 4-month-old wild-type mice after 2 months of treatment with isotretinoin. A2E levels in RPE, as expressed in Figure 5, from untreated mice (*n* = 4), from mice that received daily injections of DMSO vehicle (*n* = 3), and from mice that received daily injections of isotretinoin at 20 mg/kg (*n* = 4). Error bars show SD. From reference 4, with permission. A2E = pyridinium bisretinoid, RPE = retinal pigment epithelium, DMSO = dimethylsulfoxide, SD = standard deviation.

The authors⁴ injected daily doses of 20 mg/kg isotretinoin into 2-month-old wild-type mice for 2 months. After that time, A2E was extracted, isolated by HPLC, and measured spectrophotometrically. The A2E was reduced 40% in the injected mice when compared with the untreated littermates (Figure 6). The isotretinoin therefore drastically reduced the accumulation of the toxic A2E over time.

This work represents the first proven chemopreventive drug for Stargardt disease, at least in an animal model. It cannot be called therapeutic, however, because isotretinoin does not remove the stable A2E once it is formed. Isotretinoin might also be used as a chemopreventive drug for AMD. The authors⁴ point out that they were able to reduce the level of 11-*cis*-retinal in the eyes of wild-type mice after a single injection of as little as 2.0 mg/kg of isotretinoin, evidence that the visual cycle was slowed down and that the side reactions leading to A2E formation were reduced. The amount of isotretinoin in the study is analogous to the amount given daily to humans in the treatment of acne, and may therefore be deemed safe. However, the danger of side effects in humans, such as birth defects in pregnant women²⁰ and depression and suicide reported in many cases of isotretinoin treatment,²¹ would preclude long-term therapy with this drug.

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Genistein Appears to Prevent Early Postmenopausal Bone Loss as Effectively as Hormone Replacement Therapy

Recent data from a randomized, double-blind, placebo-controlled, yearlong clinical trial suggest that supplementation with the dietary phytoestrogen genistein (54 mg/day) may be as effective as hormone replacement therapy in attenuating menopause-related bone loss without causing the associated side effects. These findings may have implications for the development of diet-

based strategies aimed at osteoporosis prevention.

Key words: genistein, phytoestrogens, postmenopausal bone loss, HRT

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doi: 10.131/nr.2003.oct.346–351

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Estrogen deficiency is a major contributing factor to the development of osteoporosis in women. In postmenopausal women, estrogen deficiency is associated with increased bone turnover and acceleration of bone loss, which lead to increased susceptibility to bone fractures.¹