Molecular medicine in ophthalmic care

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KEYWORDS
RPE lipofuscin; Dietary polyphenols; Resveratrol

Abstract
BACKGROUND: Lipofuscin is the most consistent and phylogenically constant morphologic marker of cellular aging. Autofluorescence of the A2E fluorophore within retinal pigment epithelial (RPE) lipofuscin affords the opportunity for noninvasive evaluation of age- and disease-related pathophysiological changes in the human retina. It is being used in National Eye Institute/Age-Related Eye Disease Study II to evaluate age-related macular degeneration (AMD) geographic atrophy expansion. Experiments show lipofuscin can be reversed in cell culture and animal models in heart, brain, spinal cord, and retinal tissues, using an array of antioxidants and iron chelators.

METHODS: An 80-year-old man with a gastric resection presented with complaints of unremitting night driving difficulty despite treatment with lutein and omega III fatty acids. Notable parafoveal depression of retinal lipofuscin by 50% fundus auto-fluorescence (580 nm excitation/660 barrier filters) and concurrent abnormalities in non-Snellen measures of visual function–Contrast Sensitivity Function, 6.5% large field tritan threshold, 10% threshold visual fields, and deficits in the National Institutes of Health/National Eye Institute Visual Function Questionnaire (VFQ) 25 subjective night driving/mental health subscale questionnaire were obtained. The patient was placed on an over-the-counter daily oral polyphenolic mixture containing resveratrol and re-evaluated 5 months later.

RESULTS: The data reveal improvements in all measures of visual function, subjective improvement in vision and mental functioning on the VFQ 25, and visible clearing of RPE lipofuscin.

CONCLUSION: To our knowledge, we believe this to be the first reported human clinical case of lipofuscin reversal in the human eye correlated with measured clinical and subjective improvement in visual and mental function after nutraceutical intervention.

Optometry 2009;80:695-701

Modern medicine is being ushered into molecular medicine by the mapping of the human genome and the discovery of molecules that significantly differentiate gene expression, a frontier of scientific discovery called epigenetics. Small molecules have been identified for their broad genomic effects because of their ability to enter both cell membranes and the cell nucleus to ultimately influence genetic machinery.1,2

Small natural molecules known as polyphenols (i.e., resveratrol, quercetin, ferulic acid, curcumin, and catechin), commonly found in grapes, onions, berries, and rice bran, are notable examples of widely available over-the-counter (OTC) nutriceuticals. The impact of these molecules on gene expression, with the aim of determining how they influence human health, is a field called nutrigenomics.3

Plants that produce these molecules are subjected to environmental stress (heat, cold, solar radiation, fungal attack), genetically up-regulating production of these defensive molecules, which are then passed on to humans when consumed in the diet, through a phenomenon called xenohormesis.4 Horsemess then is a generally favorable biological

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1529-1839/09/$ -see front matter–This is a U.S. government work. There are no restrictions on its use. Published by Elsevier Inc. on behalf of the American Optometric Association.
doi:10.1016/j.optm.2009.03.018
response produced to low exposures to toxins and other stressors, such as heat, cold, and sunlight.

**Ocular aging and its marker: lipofuscin**

Just how genomic medicine will impact the future of ophthalmic care is now being evaluated. In question is when to invoke molecular medicine to reverse existing disease: (1) when the first signs of eye aging begin, such as deposition of retinal lipofuscin or (2) later in the pathophysiological process? It is possible that some aging changes can be reversed to some extent, such as lipofuscin deposits, whereas others cannot, such as lost post-mitotic retinal pigment epithelial (RPE) cells.

Lipofuscin is a universal marker of mammalian aging composed of cellular debris. The progressive accumulation of lipofuscin has been called a *garbage catastrophe*. However, lipofuscin is not a benign marker of aging but rather generates free radicals impairing cellular functioning.\(^5\) The amount of lipofuscin within the cytoplasm of rabbit cells is minuscule, representing only 0.29% of the cytoplasm volume at 12 months of life, whereas it reaches 2% of cytoplasm volume at 79 months (7 times increase).\(^6\)

**Lipofuscin and the aging eye**

Electron microscope analysis of tissue extracts of human RPE, the single-cell wall of phagocytic (digesting cells) that reside between the blood layer (choriocapillaris and choroid) and photoreceptors (cones, rods), shows no lipofuscin granules in young eyes but large numbers in older eyes.\(^7\) Accumulation of cellular debris (lipofuscin) at the back of the human eye (retina) does not begin until age 19.\(^8\) Retinal lipofuscin deposits, induced by iron accumulation and calcification in retinal tissues, are generally not found until the third decade of life, and more advanced cellular lipoproteinaceous deposits in the retina, called *drusen*, are not normally observed during an eye examination until the fifth decade of life.\(^9\)

In other words, about when full childhood growth ceases is when lipofuscin begins to accumulate more rapidly. This is also precisely when the demand for iron to make new RBCs declines.

**Ocular lipofuscin measurement**

Unlike other organs of the body, the accumulation of retinal lipofuscin with advancing age can be observed directly with the use of a scanning laser ophthalmoscope or specialized photographic systems.\(^10\) Thus, theoretically, lipofuscin measurements serve as a way to determine the biological aging of the eye. The human eye, being a transparent organ, is subject to bombardment by solar ultraviolet (UV) and blue light radiation that induces oxygen free radicals accelerating the accumulation of lipofuscin more so than other organs. The human eye may age at a faster rate than the rest of the body. In fact, visual decline is the first sign in lipofuscin disorders (lipofuscinosis).\(^11,12\)

The formation of lipofuscin increases after the removal of a cataractous (cloudy) natural focusing lens of the eye and implantation of a clear plastic intraocular lens. The combined implantation of a yellow intraocular lens (UV blue filter) and use of a vermilion sunglass filter would theoretically reduce lipofuscin formation.\(^13\) The accumulation of...
retinal lipofuscin correlates with declines in contrast sensitivity and visual acuity.14

Prior attempts to reduce lipofuscin
Experiments have found that lipofuscin accumulation can be increased, decreased, or even reversed in cell culture and animal models, in heart, brain, spinal cord and retinal tissues, using an array of oxidants, antioxidants, and iron chelators, particularly vitamin E, lipoic acid, carnitine, grape seed flavonoids, curcumin, coenzyme Q10, and glutathione.15-27

Decades ago, attempts were made to reverse retinal aging and reduce lipofuscin deposits by the use of an acetylcholine precursor (DMAE), which produced mixed results in 2 human studies.28,29 These studies found that a therapeutic intervention in the aging retina is possible.

Iron, retinal lipofuscin and age-related macular degeneration
Lysosomal compartments in mammalian cells produce enzymes that act to scavenge cellular debris, are rich in labile iron, and are subject to oxidative stress through Fenton-type reactions (forming hydroxyl radicals). Lipid peroxidation, in turn, induces the formation of lipofuscin.30 Old cells have 10-fold more iron content than young cells.31 As cells age, they accumulate this iron-generated lipofuscin, which impairs cellular functions. Iron chelators are proposed to retard or erase lipofuscin.32

Experimentally injecting iron into the vitreous gel of the eye induces accumulation of inclusions with lipofuscinline fluorescence in the RPE.33 Age-related macular degeneration (AMD) retinas have more iron within the photoreceptors, RPE, and drusen than do age-matched control retinas. Accelerated AMD-like maculopathy develops in patients with retinal iron overload from the hereditary disease aceruloplasminemia (lack of the major copper transport protein). Mice with retinal iron overload resulting from knockout of ceruloplasmin exhibit retinal degeneration with some features of AMD. Iron chelation can reduce iron overload in mice and protect them from degeneration. The prospect of using iron chelators to reduce oxidative stress in the retina has been proposed.34 The use of iron chelators has also been shown to reverse the accumulation of cellular debris within aged cells, in

Figure 2  A, B, Significantly improved contrast sensitivity.
a similar manner to calorie restriction. Iron chelation also reverses the progressive rupture of lysosomes and prolongs the life of cells.

Calorie restriction

Calorie restriction unequivocally and universally lengthens the average and maximal life span of all organisms including yeast cells, fruit flies, roundworms, fish, rodents, and presumably humans.

While the accumulation of lipofuscin as an aging pigment is a universal feature of aging in animals, particularly in the RPE of the eye, dietary restriction with 40% fewer calories, has been shown to decrease RPE lipofuscin accumulation. Calorie restriction is known to activate autophagy (cell cleansing). Near starvation also increases autophagy and longevity.

A prevalent misunderstanding in antiaging medicine is the false belief that food-restricted diets exert beneficial effects via limited calories. Researchers at McGill University conclusively showed that calorie restriction limits mineral intake, which then slows aging as evidenced by a rapid increase of aging markers in the brain (when a calorie-restricted diet plus added minerals was compared with a calorie-restricted or ad libitum diet). Thus, it is not calories, but rather mineral intake, that appears to drive the rate of aging. A low-calorie diet limits intake of major minerals such as iron, copper, and calcium, which then retards aging.

Food-deprivation diets as therapy for aging diseases are not likely to be achievable for socioeconomic reasons. This has prompted researchers in the field of aging to search for molecular shortcuts. Once the discovery that calorie restriction activates the SIR2 gene in lower forms of life, homologous with the Sirtuin 1 gene in humans, researchers launched a fervent search for molecules that could mimic calorie restriction via activation of the Sirtuin 1 gene.

Small molecule calorie restriction mimics

It did not take long for investigators in the field of aging to identify candidate molecules for activation of the Sirtuin 1 gene. Small polyphenolic molecules, namely resveratrol, fisetin, and quercetin, have been identified as calorie restriction mimics, activating a key DNA repair gene, Sirtuin 1, that is up-regulated during periods of food deprivation. Resveratrol exerts the strongest Sirtuin 1 activation among the polyphenols tested.

It is not surprising that small molecule therapy is proposed to intervene in the aging process to slow the accumulation of lipofuscin and the eventual onset of the common slow-progressive form of AMD. It is interesting to note that resveratrol activates autophagy (cell cleansing), which can either prolong the life of healthy cells or shorten the life of tumor cells.

As described previously, metal chelation is indicated for preventive and therapeutic intervention in age-related retinal disease, and small polyphenolic molecules both...
activate the Sirtuin 1 gene and are metal chelators. Small polyphenolic molecules are also strong metallic metal chelators, with a higher reducing capacity for copper than iron ions.45

By their ability to chelate minerals, polyphenols alter gene expression at very low concentrations.46 Gene array technology shows that iron excess or depletion affects several hundred genes and that iron chelation is proposed as beneficially influencing the genome.47 Use of small-molecule calorie restriction mimics, which produce their beneficial effects via mineral chelation and subsequently exert measurable genomic effects, is on the therapeutic drawing board.

That human populations who consume a reduced-calorie diet (40% fewer calories by residents of Okinawa, 25% fewer calories by women compared with men) only live another 4 to 5 years48 could be explained by the realization that minerals, and not calories per se, control the rate of aging. The use of iron-chelating polyphenolic molecules has been proposed as an intervention that addresses a wide range of age-related diseases such as Alzheimer’s, Parkinson’s, cardiovascular, and immune-compromised disease.49

The hope is that via molecular medicine, true prevention via inhibition of lipofuscin can be realized before loss of vision, and that chelation of iron and copper from the retina would achieve visual regeneration among those with already existing pathologic visual decline. A case presentation may serve as an early example.

An 80-year-old white man presented to the Department of Veterans Affairs Medical Center Eye Clinic in North Chicago, Illinois, with need for an updated refraction to receive his new driver’s license, with the complaint “I can’t see at night.” Ocular history included early nuclear cataract right eye, pseudophakia (intraocular lens) left eye, anisometropia (unequal refractive error), exophoria, and right pseudoexfolliation with normal intraocular pressures of 15 mmHg in both eyes. Additional medical history includes:
- 65% gastric resection (1956) with impaired vitamin B12 processing (B12 shot twice a month), anemia (iron shot every month)
- Past alcohol abuse (1960s)
- Bilateral hip replacement
- Spinal stenosis of third to fifth vertebrae upper and lower back
- Left carpal tunnel
- Large, efficient slow-pumping heart
- Familial history of AMD (mother in late 80s)

His refraction and Snellen Visual Acuity were:

-1.50 w 1.00 x 10 1BI ————20/20
-1.50 w 0.25 x 150 1BI slab off 20/20
+2.50 add trifocals

This represented a minimal change in prescription. Slab off was included in the prescription to reduce optically induced anisometric vertical phoria. One degree foveal macular pigment was measured by heterochromic flicker photometry using a QuantifEye® (ZeaVision, St. Louis, Missouri) device and found to be normal albeit slightly reduced in the left eye (0.53 density units [du] right 0.24 du left). Three-dimensional auto-fluorescence macular pigment imaging confirmed the asymmetry (see Figure 1).
A blue-free 3200 Kelvin low-vision reading lamp, traditional OTC ophthalmic supplements, and new eye-glasses were prescribed. However, his night visual symptoms were uncharacteristically not responsive to carotenoid and fish oil supplementation. Better than 75% of male patients in this age group typically respond to these supplements in our clinic.50-56

On closer evaluation, we measured abnormalities on the 100-point National Institute of Health/National Eye Institute Visual Function Questionnaire (VFQ25) including Night Driving and Mental Health Subscales, an abnormal area under the curve (AUC) contrast sensitivity function (Functional Vision Analyzer®; Stereo Optical Company, Inc., Chicago, Illinois) (see Figure 2A), abnormal 6.5° (large field) color vision disturbances (ChromaTest®; CH Electronics, Bromley, United Kingdom), and abnormalities in his central macular 10° kinetic macular visual fields at 5 discrete contrast levels (SimulEyes Kinetic Macula Field Test®; Rush Ophthalmics, Gold Beach, Oregon) (see Figure 3A). There was also notable parafoveal deposition of retinal lipofuscin by 50° fundus autofluorescence (Kowa Medical Instruments, JP modified Fundus Camera, 580 nm excitation/660 barrier filters, high output flash, and high gain Hamamatsu C9440-05G digital black and white camera; Kowa Medical products, Tokyo, Japan) (see Figure 4A).

An OTC mitochondrial-support dietary supplement providing resveratrol (Longevinex®; Reservatol Partners, LLC, Las Vegas, Nevada), quercetin, rice bran IP6, and lecithin was prescribed on September 6, 2007. A 5-month follow-up visit on February 13, 2008, found improvement in all subjective visual function parameters and VFQ 25 night driving and mental health subscales.

- Visual acuity 20/20 + 3 right (better) 20/20 + 1 left (same)
- VFQ25 = 84 versus 81 Overall Score—improved
- 83 versus 67 Driving Subscale Score—improved
- 100 versus 80 Mental Health Subscale Score—dramatic improvement
- StereoOptical Contrast Sensitivity Function:
  - 376 AUC right 533 AUC left—much improved (see Figure 2B)
- 6.5° Tritan Chroma® test, 8.6 SD right; 5.5 SD left—better
- 10° Kinetic Macular Visual Fields—much improved right; normalized left (see Figure 3B)

These dramatic subjective results were supported by objective clearing of retinal lipofuscin (see Figure 4B) and improvement in self-reported night vision and cognitive ability as well as dramatic improvement in contrast sensitivity function (both eyes) by objective testing when an OTC dietary supplement (Longevinex®), providing a blend of proprietary nutriceuticals (resveratrol, quercetin, rice bran phytate) was added to the patient’s dietary supplement regimen. Subjective response: “My night vision and thinking have gotten much better.”

We remain cognizant to the possibility that the original supplements were continued over the 5-month polyphenol intervention and may have had a salutary effect. This additionally argues that a detailed assessment of nutriceuticals should be ascertained before adding another dietary supplement. Nonetheless, this case may serve as an early example of the potential for molecular medicine to make an impact in ophthalmic care. To our knowledge, we believe it to be the first reported human clinical case of lipofuscin reversal in the human eye correlated with measured clinical and subjective improvement in visual and mental function after nutriceutical intervention. Furthermore, it becomes evident that by measuring lipofuscin deposits, not only can the biological age of the human eye be assessed apart from its chronological (calendar) age, but more significantly, such measurement may serve to help determine the biological age of the entire body. Pharmacologic and nutriceutical systemwide age-reversing effects could then be estimated in this manner.

Acknowledgements

This material is based upon work supported by the DVA Medical Center, North Chicago, Illinois and the Department of Veteran’s Affairs. The authors thank Kowa Optimum USA (Torrance, California, and Japan Corporate Headquarters); Rush Instruments (Gold Beach, Oregon); Stereo Optical (Chicago, Illinois), and Zeavision (St. Louis, Missouri) for donation of ophthalmic equipment. The OTC dietary supplement (Logevinex®) was donated by the manufacturer, Resveratrol Partners LLC (Las Vegas, Nevada).

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