Emerging Trends in Ocular Nutraceuticals

February 2021

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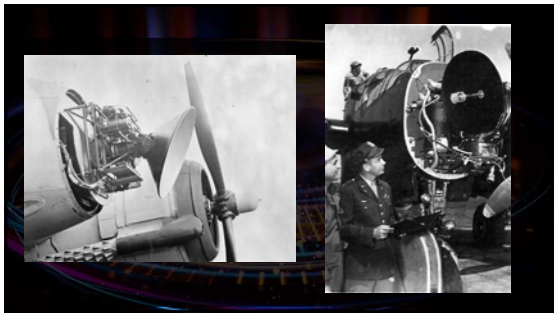


The effect of bilberry nutritional supplementation on night visual acuity and contrast sensitivity

Alternative Medicine Review 5.2 (2000): 164-173.

Purpose:
Investigation on the effects of bilberry on night visual acuity (VA) and night contrast sensitivity (CS).

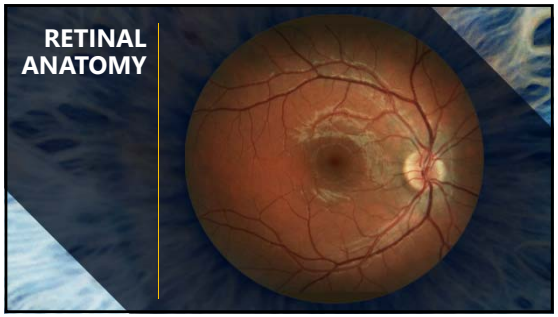
Methods:
- Double-blind, placebo-controlled, crossover design using male subjects (25-47 years) with BCVA ≥ 20/20
- 8 received placebo and 7 received active capsules for 3 weeks.
- Active capsules contained 160 mg of bilberry extract (25% anthocyanosides)
- Subjects ingested one active or placebo capsule three times daily for 21 days.
  - After the three-week treatment period, a 1-month washout period was employed to allow any effect of bilberry on night vision to dissipate. In the second 3-week treatment period, the 8 subjects who first received placebo were given active capsules and the 7 who first received active capsules were given placebo.
- Night VA and night CS was tested throughout the 3-month experiment.

Results:
- ***No difference in mesopic VA during any of the measurement periods***
- ***No difference in mesopic CS during any of the measurement periods***

OVERVIEW

- **Age-Related Macular Degeneration**
  - **Epidemiology**
  - **Age Related Eye Disease Study (AREDS) criterion**
- **Retinal Findings related to Age-Related Macular Degeneration**
  - **Microvascular Changes**
  - **RPE Dysfunction**
  - **Drusen**
  - **Geographic Atrophy**
- **AMD Supplementation Studies**
- **AMD Supplementation: A Walk Down the Rabbit Hole**
  - **Associated Risk Alleles**
  - **Antioxidant and Zinc Limitations**
  - **Polyphenols**
    - **Flavonoids**
      - **Quercetin**
      - **Anthocyanins**
    - **Non-Flavonoids**
      - **Resveratrol**
      - **Curcumin**
  - **Carotenoids**
    - **Lutein, Zeaxanthin and *meso*-zeaxanthin**
- **Clinical Treatments related to Retinal Function and Visual Performance**
  - **Systemic Disease Management**
  - **Oral Supplementation**
- **What's Next?**

RETINAL ANATOMY



RETINAL ANATOMY

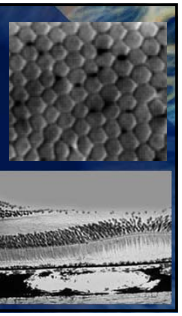
Approximately 100-120 million rods
Peak density of 100,000/mm²

Approximately 4-5 million cones
Peak density of 200,000/mm²

Foveola
0.3mm diameter

Fovea
1.5mm diameter

Macula
5.5mm diameter



Prevalence of undiagnosed AMD in primary eye care

JAMA ophthalmology (2017) 135.6:570-575.

OBJECTIVES
To examine the prevalence of AMD patients seen in primary eye care clinics who purportedly have normal macular health per their medical record

RESULTS
Sample consisted of 1288 eyes from 644 participants (231 [35.9%] male and 413 [64.1%] female; mean [SD] age, 69.4 (6.1) years; 611 white [94.9%]) seen by 31 primary eye care ophthalmologists or optometrists.
- 320 (24.8%) had AMD despite no diagnosis of AMD in the medical record
  - 32 (10.0%) had hyperpigmentation
  - 43 (13.4%) had hypopigmentation
  - 249 (77.8%) had small drusen
  - 250 (78.1%) had intermediate drusen → AREDS II
  - 96 (30.0%) had large drusen → AREDS II

Findings were not different for ophthalmologists and optometrists (age adjusted OR, 0.99; 95% CI,0.71-1.36; P = .94)

CONCLUSIONS AND RELEVANCE
- Approximately 25.0% of eyes deemed to be normal based on DFE had macular characteristics that indicated AMD revealed by fundus photography and trained raters
- ***Total of 30.0% of eyes with undiagnosed AMD had drusen treatable with nutritional supplements had it been diagnosed***
- Improved AMD detection strategies may be needed as more effective treatment strategies become available

Age-Related Macular Degeneration

- Epidemiology
- Age Related Eye Disease Study Criterion

Age Related Macular Degeneration
Epidemiology

20 Million Americans show clinical evidence of AMD*
- 14% of patients over 55 **(Age of enrollment for AREDS)**
- 25% of patients over 65
- 37% of patients over 75
- 66% of patients over 90
- 100% of patients over 100

50 Million Americans have clinical risk factors for AMD development
- Age
- Family History
- Tobacco Use
- Cardiovascular Disease (geographic AMD and exudative AMD)
- Obesity, **Sunlight Exposure**, Ethnicity, Gender

****Refer to 2017 JAMA ophthalmology article mentioned at outset***

Age Related Macular Degeneration
AREDS Criterion

FIRST EYE (Must have VA >20/32, no advanced AMD and no disqualifying lesions)				SECOND EYE
AMD Category	Drusen Size*	Drusen Area*	Pigment Abnormalities**	
1	None or <63um	<125um diameter	None	Same as 1st
2	<63um **Or** >63um, <125um None if pigment abnormalities	>125um diameter **Or** >1 druse	Absent or Present **WITHOUT** GA	Same as 1st or Category 1
3a	>63um, <125um **Or** >125um None if GA present	>360um diameter (if soft drusen present) >656um diameter (if soft drusen absent) **Or** At least 1 druse	Absent or Present **WITHOUT central** GA	Same as 1st or Category 1,2
4a	Category 1,2 or 3			Advanced AMD***

* Drusen and GA within 2DD of fovea

** Pigment abnormalities within 1DD of fovea

*** Advanced AMD is:
1) GA involving fovea
2) CNVM development

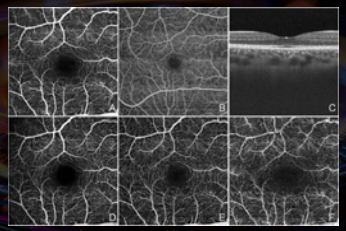
Retinal Findings Related to Age-Related Macular Degeneration
- Microvascular Changes
- RPE Dysfunction
- Drusen Formation
- Geographic Atrophy

Retinal Findings Related to Age-Related Macular Degeneration
Microvascular Changes

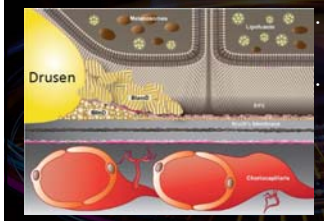
Natural History of Subclinical Neovascularization in Nonexudative Age-Related Macular Degeneration Using Swept-Source OCT Angiography.
***Ophthalmology.* (2018) 125(2):255-266**
- CONCLUSIONS: By 12 months, the ***risk of exudation was greater for eyes with documented subclinical macular neovascularization*** (MNV) compared with eyes without detectable MNV

Association between outer retinal alterations and microvascular changes in intermediate stage age-related macular degeneration: optical coherence tomography angiography study.
***Br J Ophthalmol* (2016):1–6.**
- CONCLUSIONS: Demonstrated association between SD-OCT signs and retinal blood supply in patients with intermediate AMD and showed that ***reduced flow in superficial vascular plexus and damage of the inner and the outer retina is predictive of GA development***

Retinal Findings Related to Age-Related Macular Degeneration
Microvascular Changes



Retinal Findings Related to Age-Related Macular Degeneration
RPE Dysfunction



- Cholesterol accumulation leads to macular deposits (BlinD and BlamD)
  - Peaks in these deposits become clinically-visible drusen
- Extracellular cholesterol deposits:
  - Impact photoreceptor health
  - Initiate inflammation
  - Create CNV predisposition
  - Limit Vitamin A transport across Bruch's membrane

***** Leads to localized Vit A deficiency and impaired dark adaptation ****

Retinal Findings Related to Age-Related Macular Degeneration
RPE Dysfunction

Design:
Prospective, multicenter clinical pilot study
- 26 patients with diagnosis of AMD and the presence of large, soft drusenoid deposits
- Patients received atorvastatin 80mg QD and monitored at baseline and every 3 months with complete ophthalmologic exam to include:
  - BCVA
  - Fundus photographs
  - SD-OCT
  - Blood panel (AST, ALT, CPK, total cholesterol, TSH, creatinine)

Results:
- 23 subjects completed 12 months of follow-up
- High does atorvastatin resulted in regression of drusen deposits associated with increase of 3.3 letters (p = 0.06)
- No patients progressed to exudative AMD

Retinal Findings Related to Age-Related Macular Degeneration
Drusen Formation

7-Ketocholesterol increases retinal microglial migration, activation, and angiogenicity: a potential pathogenic mechanism underlying age-related macular degeneration.
***Scientific reports* (2015) 5:9144**
- CONCLUSIONS: 7-Ketocholesterol is an oxidation product localized to the outer retina with prominent pro-inflammatory effects resulting expression of angiogenic factors transitioning to a neurotoxic and pro-angiogenic phenotype. ***Outer retinal lipid accumulation in intermediate AMD results in neuroinflammation that leads to advanced AMD***

Dry age-related macular degeneration: mechanisms, therapeutic targets, and imaging.
***Invest Ophthal Vis Sci* (2013) 54(14)**
- CONCLUSIONS: Much of the genetic risk for AMD is associated with complement genes. Several ***complement-based therapeutic treatment strategies target protein and/or lipid deposition including anti-amyloid therapies, autophagy and modulation of oxidative stress***

Retinal Findings Related to Age-Related Macular Degeneration
Geographic Atrophy

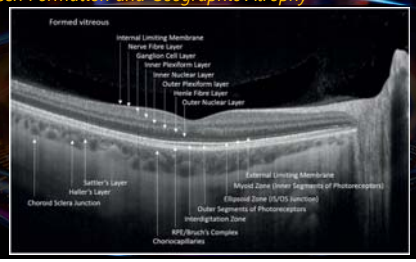
Natural history of geographic atrophy progression secondary to age-related macular degeneration (Geographic Atrophy Progression Study).
***Ophthalmology* (2016) 123(2), 361-368.**
- CONCLUSIONS: Mean change in lesion size from baseline to month 12 was ***significantly greater*** in:
  - ***multifocal atrophic spots compared with unifocal spots (P < 0.001)***
  - ***extrafoveal lesions compared with foveal lesions (P = 0.001)***

  Although differences were observed in mean lesion size, FAF and CFP were highly correlated

Choriocapillaris Degeneration in Geographic Atrophy.
***The American Journal of Pathology* (2019)**
- CONCLUSIONS: Choriocapillaris loss was observed in early AMD with greater loss in GA even in areas of intact RPE. Changes in lumen/stroma ratio in the outer choroid were not found to differ between controls and AMD or GA eyes suggesting ***choriocapillaris changes are more prevalent in AMD than those in the outer choroid***. Additionally, VEGF-A levels were negatively correlated with choriocapillaris vascular density suggesting that ***choroidal microvascular degeneration contributes to atrophic AMD progression.***

Retinal Findings Related to Age-Related Macular Degeneration

Drusen Formation and Geographic Atrophy

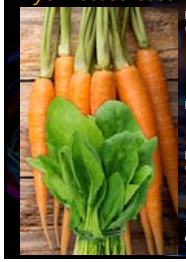


Age-Related Macular Degeneration Studies

- ***Eye Disease Case Control Study (EDCC)***
- Age-Related Eye Disease Study (AREDS)
- Lutein Antioxidant Supplement Trial (LAST)
- Carotenoids in Age-Related Eye Disease Study (CAREDS)
- Lutein Xanthophyll Eye Accumulation (LUXEA)
- Taurine, Omega-3, Zinc, Antioxidant and Lutein (TOZAL)
- Carotenoids in Age-Related Eye Disease Study II (CAREDS2)
- ***Age-Related Eye Disease Study II (AREDS2)***

Age Related Macular Degeneration Studies

Eye Disease Case Control (1994)



Design:
- 5 ophthalmology centers included:
  356 subjects (ages 55-80) with advanced AMD within 1-yr of enrollment
  520 control subjects
  ** Frequency matched to cases of same age and sex **

Methods:
- Multiple Regression analysis controlled for smoking and other risk factors

Results:
- ***Highest quintile carotenoid dietary intake (specifically L and Z) showed 43% lower risk than lowest quintile in the development of advanced AMD***

***Vit A, Vit C and Vit E showed no significant relationship to AMD development*

Age Related Macular Degeneration Studies

Age Related Eye Disease Study II (AREDS2) [2013]

Design:
6 year longitudinal, placebo-controlled study released in May 2013

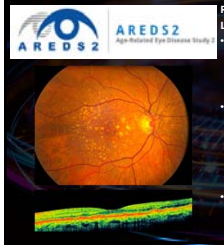
Methods:
4203 men and women ages 50-85 were divided into 4 groups:
1) 10mg L and 2mg Z + AREDS
2) 350mg DHA and 650mg EPA + AREDS
3) 10mg L and 2mg Z and 350mg DHA and 650mg EPA + AREDS
4) control (AREDS formulation only) ***No true placebo group***

All participants were offered:
- Original AREDS formula (Standard of Care)
- Variation of the AREDS formulation (former smokers)

In contrast to AREDS, AREDS II subjects were previously diagnosed with moderate to advanced ARM and/or AMD

Age Related Macular Degeneration Studies

Age Related Eye Disease Study II (AREDS2) [2013]

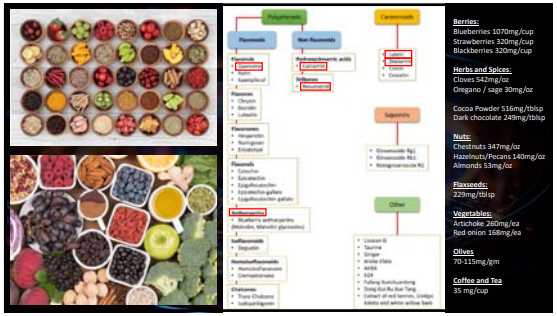


Results:

L/Z plus AREDS formula:
- Reduced progression to advanced AMD 10% over AREDS formulation alone in total cohort
  ✓ ***L/Z substitution for β-carotene resulted in a 18% risk reduction of advanced AMD within 5 years***
- Reduced progression to NV AMD by 11% of AREDS formulation alone in total cohort
  ✓ **Reduced progression to exudative AMD by 26% in subjects with lowest intake of L/Z**
  ✓ ***L/Z substitution for β-carotene resulted in a 22% risk reduction of exudative AMD within 5 years***
- ***NO*** apparent effect of β-carotene elimination or reduction of zinc to 25mg

Clinical Nutraceuticals Related to Retinal Disease

- Oral Supplementation
- Polyphenols
  - Flavonoids
  - Non-Flavonoids
- Carotenoids
  - Xanthophylls



Berries:
Blueberries 1070mg/cup
Strawberries 320mg/cup
Blackberries 320mg/cup

Herbs and Spices:
Cloves 542mg/oz
Oregano / sage 30mg/oz

Cocoa Powder 516mg/tblsp
Dark chocolate 249mg/tblsp

Nuts:
Chestnuts 347mg/oz
Hazelnuts/Pecans 140mg/oz
Almonds 53mg/oz

Flaxseeds:
229mg/tblsp

Vegetables:
Artichoke 260mg/ea
Red onion 168mg/ea

Olives
70-115mg/gm

Coffee and Tea
35 mg/cup

Dietary flavonoids and the prevalence and 15-year incidence of age-related macular degeneration

Am J Clin Nutrition (2018) 108.2:381-387

Background
- Majority of research performed to date has examined the effects of antioxidants such as vitamins C, E, and A and carotenoids on AMD risk and progression. To date, there is limited research on promising phytochemicals with antioxidant and anti-inflammatory properties, including flavonoids

Objective
- Assess the independent associations between dietary intake of total flavonoids and common flavonoid classes with the prevalence and 15-y incidence of AMD

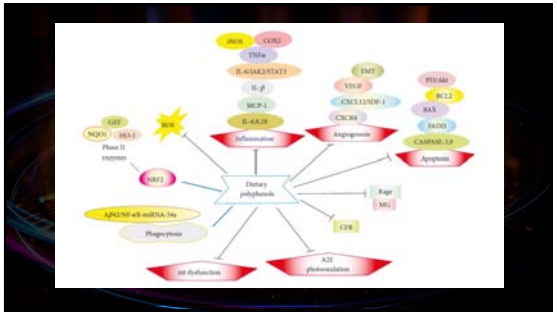
Design
- 2856 adults aged ≥49 y at baseline and 2037 followed up 15 years later were included in prevalence and incidence analyses.
- Dietary intake was assessed by using a semi-quantitative FFQ
- Estimates of the flavonoid contents were assessed by using the USDA Flavonoid, Isoflavone and Proanthocyanidin databases
- AMD was assessed from retinal photographs using AREDS criterion

Dietary flavonoids and the prevalence and 15-year incidence of age-related macular degeneration.

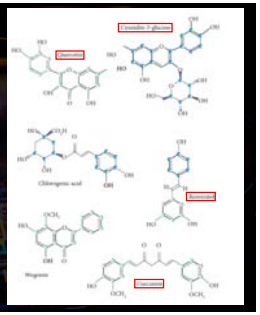
Am J Clin Nutrition (2018) 108.2:381-387

Results
- Cross-sectional and multivariate adjusted
- Each 1-SD increase in total flavonoid intake was associated with a reduced AMD likelihood
  - OR: 0.76 (95% CI: 0.58-0.99)
- Each 1-SD increase in total flavonols intake was associated with reduced prevalence of AMD
  - OR: 0.75 (95% CI: 0.58-0.99)
- Each 1-SD increase in total flavanones was associated with reduced prevalence of any AMD
  - OR: 0.77 (95%: 0.60-0.99)

Conclusions
Findings suggest an ***independent and protective association between dietary intake of flavonoids and the reduced likelihood of developing AMD***. Additional prospective cohort studies are needed to validate these findings.



Retinal nutraceuticals share a common thread....



Clinical Nutraceuticals Related to Retinal Disease

- Oral Supplementation
- Polyphenols
  - Flavonoids
    - ***Quercetin***
    - ***Anthocyanin***
  - Non-Flavonoids
- Carotenoids
  - Xanthophylls

Polyphenols
Flavonoids
Quercetin

Quercetin inhibits choroidal and retinal angiogenesis *in vitro*.
Graefe's Arch Clin Exp Ophthal (2008) 246.3:373-378.

Singlet oxygen quenching-and chain-breaking antioxidant-properties of a quercetin dimer able to prevent AMD.
Biophysical chemistry 243 (2018): 17-23.

Quercetin and cyanidin-3-glucoside protect against photooxidation and photodegradation of A2E in RPE cells.
Experimental eye research 160 (2017): 45-55.

Neuroprotective effects of quercetin in diabetic rat retina.
J Bio Sciences. (2017) 24.6:1186-1194.

Protective effect of quercetin and chlorogenic acid, two polyphenols widely present in edible plant varieties, on visible light-induced retinal degeneration *in vivo*.
J Func Foods (2017) 33, 103-111.

Quercetin protects retina external barrier from oxidative stress injury by promoting autophagy

Cutaneous and Ocular Toxicology (2020) 1-19

Purpose:
Investigate the protective effects of quercetin on the tight junction proteins of human retinal pigment epithelial (ARPE-19) cells during oxidative stress injury and explore the possible mechanism.

Methods:
H_2O_2 was used to establish an oxidative stress model of ARPE-19 cells. ARPE-19 cells were pretreated with different concentrations of quercetin before H_2O_2 exposure. The expression and distribution of tight junction proteins and autophagy-related proteins were detected by Western blot and immunostaining.

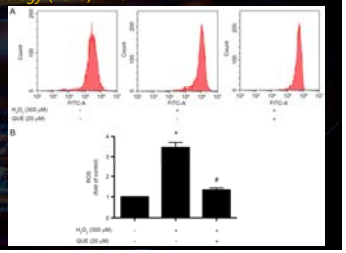
Results:
Cell viability was reduced in the H_2O_2 group compared with the control group. Quercetin treatment significantly enhanced the expression levels of occludin and claudin-1 as compared to the H_2O_2 group. H_2O_2 alone strongly decreased the zonula occludens protein 1 (ZO-1) expression and quercetin supplementation enhanced the accumulation of ZO-1 in ARPE-19 cells. The appearance of LC-3II was enhanced in the quercetin protection group as compared with the control group. The expression levels of beclin-1, LC-3II and P62 increased in the autophagy-inhibited group compared with the quercetin protection group.

Conclusion:
Quercetin prevents the loss of tight junction proteins by upregulating autophagy after oxidative stress in ARPE-19 cells.

Quercetin protects retina external barrier from oxidative stress injury by promoting autophagy

Cutaneous and Ocular Toxicology (2020) 1-19

APRE-19 cells were pretreated with quercetin for 24 hours followed by H_2O_2 administration. Reactive oxygen species (ROS) production was evaluated using flow cytometry (A) and quantification (B)



*****Quercetin reduces generation of reactive oxygen species (ROS) at the RPE level***

Polyphenols
Flavonoids
Anthocyanins

Antioxidant and anti-inflammatory effects of blueberry anthocyanins on high glucose-induced human retinal capillary endothelial cells.
Oxidative medicine and cellular longevity. (2018)

Protective effects of blueberry anthocyanins against H_2O_2-induced oxidative injuries in human retinal pigment epithelial cells.
J Agricultural Food Chem. (2018) 66(7):1638-1648.

Protective effect of anthocyanins and xanthophylls on UVB-induced damage in retinal pigment epithelial cells.
Food and Function (2016) 7(2):1067-1076.

Effects of blueberry anthocyanins on retinal oxidative stress and inflammation in diabetes through Nrf2/HO-1 signaling.
J Neuroimmunology (2016) 301:1-6.

Identification of anthocyanins in the liver, eye and brain of blueberry-fed pigs
J Agric Food Chem (2008) 56.3:705-712

Retinoprotective Effects of Anthocyanins via Anti-Inflammatory and Anti-Apoptotic Mechanisms in a Visible Light-Induced Retinal Degeneration Model in Pigmented Rabbits

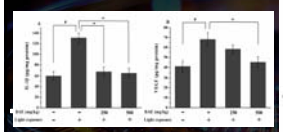
Molecules (2015) 20:22395–22410

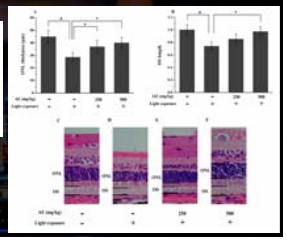
Abstract:
Study created a model of phototoxicity in pigmented rabbits and investigated the protective effect of anthocyanin extract (AE) to explore the possible mechanisms of action in this model. The model of light-induced retinal damage was established by the pigmented rabbits exposed to light at 18,000 lx for 2hrs/d and sacrificed on day 7. Administration of AE at dosages of 250 and 500 mg/kg/day showed retinal dysfunction significantly reduced as measured by ERG and less reduction in the thicknesses of retinal outer nuclear layer and photoreceptor outer segment lengths in treated subjects versus controls. AE attenuated apoptotic proteins (Bax, Bcl-2, and caspase-3) and increased the levels of superoxide dismutase, glutathione peroxidase, and catalase, as well as the total antioxidant capacity, but decreased the malondialdehyde level in the retinal cells. AE inhibited the light-induced elevation of proinflammatory cytokines and angiogenic factors (IL-1β and VEGF).

Results showed that visible light-induced retinal degeneration model in pigmented rabbits was successfully established and ***AE exhibited protective effects by increasing the antioxidant defense mechanisms, suppressing lipid peroxidation and proinflammatory cytokines, and inhibiting retinal cells apoptosis.***

Retinoprotective Effects of Anthocyanins via Anti-Inflammatory and Anti-Apoptotic Mechanisms in a Visible Light-Induced Retinal Degeneration Model in Pigmented Rabbits

Molecules (2015) 20:22395–22410





*****Anthocyanins suppress both lipid peroxidation and proinflamatory cytokine expression and inhibit RPE apoptosis***

Clinical Nutraceuticals Related to Retinal Disease

- Oral Supplementation
  - Polyphenols
    - Flavonoids
    - Non-Flavonoids
      - ***Curcumin***
      - ***Resveratrol***
  - Carotenoids
    - Xanthophylls

Polyphenols
Non-Flavonoids
Curcumin

Therapeutic potential of curcumin in major retinal pathologies.
Int ophth (2019) 39.3:725-734.

Vascular endothelial growth factor: An important molecular target of curcumin.
Crit Review Food Sci Nutrition (2019) 59.2:299-312.

Retinal protection and distribution of curcumin *in vitro* and *in vivo*.
Frontiers in pharmacology 9 (2018) 670.

Curcumin acts to regress macular drusen volume in dry AMD.
Invest Ophth Vis Sci (2020) 61.7:1036-1036.

Curcumin-Based Treatment for Macular Edema from Uncommon Etiologies: Efficacy and Safety Assessment.
Journal of Medicinal Food (2020) 23.8

Protective Effects of Curcumin Ester Prodrug, Curcumin Diethyl Disuccinate against H_2O_2-Induced Oxidative Stress in Human RPE Cells: Potential Therapeutic Avenues for AMD.

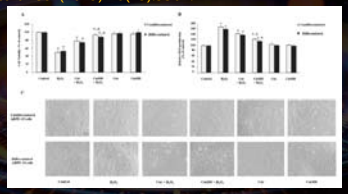
Inter J Molecular Sci (2019) 20(13):3367.

Abstract:
Oxidative stress-induced damage to the RPE, a specialized post-mitotic monolayer that maintains retinal homeostasis, contributes to the development of AMD. Curcumin (Cur) was previously shown to have the ability to protect RPE cells from oxidative stress. However, poor solubility and bioavailability makes Cur a poor therapeutic agent. As prodrug approaches can mitigate these limitations, we compared the protective properties of the Cur prodrug curcumin diethyldisuccinate(Cur DD) against oxidative stress in human ARPE-19 cells.

Both CurDD and Cur significantly decreased H_2O_2-induced ROS production and protected RPE cells from oxidative stress-induced death. Both drugs exerted their protective effects through the modulation of p44/42 and the involvement of downstream molecules Bax and Bcl-2. Additionally, the expression of antioxidant enzymes HO-1 and NQO1 was also enhanced in cells treated with CurDD and Cur. In all cases, CurDD was more effective than its parent drug against oxidative stress-induced damage to ARPE-19 cells. These findings highlight ***CurDD as a more potent drug*** compared to Cur against oxidative stress and indicate that its ***protective effects are exerted through modulation of key apoptotic and antioxidant molecular pathways***.

Protective Effects of Curcumin Ester Prodrug, Curcumin Diethyl Disuccinate against H_2O_2-Induced Oxidative Stress in Human RPE Cells: Potential Therapeutic Avenues for AMD.

Inter J Molecular Sci (2019) 20(13):3367.



Protective effects of curcumin against ROS production and cytotoxicity in ARPE-19 cells
(A) ARPE-19 cells were pre-treated with Cur or CurDD for 24 h, followed by H2O2 treatment for 6 hr
(B) ROS generation was determined by assay. Graphs represent average cell viability (mean ± SD values)

Polyphenols
Non-Flavonoids
Resveratrol

Resveratrol based oral nutritional supplement produces long-term beneficial effects on structure and visual function in human patients.
Nutrients. (2014), 6.10:4404-4420.

Resveratrol suppresses expression of VEGF by human retinal pigment epithelial cells: potential nutraceutical for age-related macular degeneration.
Aging and disease (2014) 5.2:88.

SIRT1 mediated inhibition of VEGF/VEGFR2 signaling by Resveratrol and its relevance to choroidal neovascularization.
Cytokine 76.2 (2015):549-552.

Anti-oxidant, anti-inflammatory and anti-angiogenic properties of resveratrol in ocular diseases.
Molecules 21.3 (2016):304.

Toxic effects of A2E in human ARPE-19 cells were prevented by resveratrol: A potential nutritional bioactive for age-related macular degeneration treatment.
Archives of Toxicology 94.2 (2020): 553-572.

Longevinex® Improves Atrophic AMD Photoreceptor/Retinal Pigment Epithelium Mediated Dark Adaptation.

J Adv Med and Med Res (2017) 1-19.

Aim:
Evaluation of dark adaptation (DA) AS a broad measure of photoreceptor / RPE health with epigenetic modulation using a resveratrol–based caloric-restriction mimic (Longevinex)

Study Design:
Case series, bi-ocular, clinical DA evaluation in deteriorating AMD, before and after supplementation

Methods:
Baseline clinical DA threshold (log dB), time (min) and fixation (%) were taken for patients with established atrophic AMD (n=14 eyes; ages 64 - 89 years) using the AdaptDx and best refraction. Subjects were given Longevinex 1 capsule QD with each eye response considered independent.

Results:
All but 2 eyes improved in one or more DA parameters, with 3 cases showing improvement by retinal macula SD OCT. Expected vs. actual was significant by Chi Square ($p < 0.01$). Additional factors including smoking, alcohol, elevated CRP and statins were retrospectively evaluated.

Conclusion:
Epigenetic-induced ***DA stability and improvement are consistent with previous beneficial effects of Longevinex such as enhanced choriocapillaris circulation***.

Longevinex® Improves Atrophic AMD Photoreceptor/Retinal Pigment Epithelium Mediated Dark Adaptation.

J Adv Med and Med Res (2017) 1-19.

Table 1. Demographic post-hoc clinical review

Case (Figure # & initials)	Age	Gender	AMD duration	Current smoker	Current alcohol	Retinal /Corneal dystrophy? (yes / no)	DA improving medication?	Supplements
1 (NS)	86	M	2 years	N	N	N	40 mg simvastatin	1000IU Vit D3 Centrum Silver QD 1000 mg Fish oil BID 420 mg Mg Oxide Centrum®
2 (RS)	64	F	5 years +	N	N	Retinal Pavingstone	20 mg lovastatin	
3 (HJ)	82	M	8 years	N	N	N	40mg atorvastatin	
4 (HJ)	89	M	9 years+	N	N	N	20 mg simvastatin	MaxiVision® AREDS 2, Krill Oil
5 (CJ)	86	F	1 year	N	N	Corneal Pellucid	20 mg atorvastatin	Bright Eyes®
6 (KW)	88	M	1 year	N	N	N/A	N/A	1000 IU Vit D AREDS2 500 mg Vit C, 1000 mg Fish oil
7 (DR)	84	M	New	N	N	N/A	N/A	Vitamin D3

Table 3. Clinical dark adaptation clinical data continued

Case (Figure # & Initials)	Duration prescribed longevinex®	Rod intercept (DS)	Rod intercept (DS)	Baseline log sensitivity (DS)	Baseline log sensitivity (DS)	#Eyes improved OR remained stable in at least 1 DA factor
1 (SN)	5 Weeks	17.38→9.34	2.57→6.10	-1.9→-2.2	2.9→2.1	1 (1 eye parameters worse)
2 (SR)	7 Months	5.73→8.84	2.04→3.90	-1.3→-1.2	-2.1→-2.3	2 (L eye remains normal)
3 (JH)	4.5 Months	>6.5→>6.5	>6.5→N/A (Cannot be calculated)	-1.8→-2.3	-2.2→-2.6	2
4 (JH)	4 Months	>20→>20	>20→>20	-1.1→-1.4	-1.2→-1.6	2
5(JC)	1 Month	>20→>6.5	>20→>6.5	1.2→1.2	1.0→1.1	2
6 (KW)	4 Months	11.9→19.85	>20→>20	-1.5→1.0	-0.9→-1.2	1 (R eye parameters worse)
7 (RD)	~4 Months	>20→>20	>20→>20	-1.1→-0.9	-1.0→-1.1	2

#Eyes improved or remained stable in at least 1 DA factor / 12 (SA) Rod Intercept data (0 = 9/14 same/ improved; < = 4/14 worse, ↓ unavailable. Baseline Log Sensitivity +/- 0.2 (12/14 = same / improved, 9/14 = worsened)

Clinical Nutraceuticals Related to Retinal Disease

- Oral Supplementation
  - Polyphenols
    - Flavonoids
    - Non-Flavonoids
  - Carotenoids
    - Xanthophylls
      - ***Lutein***
      - ***Zeaxanthin***
      - ***Meso-zeaxanthin***

Carotenoids
Xanthophylls
Lutein / Zeaxanthin / Meso-zeaxanthin

Identified an inverse association between AMD findings and retinal lutein and zeaxanthin
Eye (2018) 32.5:992-1004

Mechanisms enhancing the protective functions of macular xanthophylls in the retina during oxidative stress
Experimental eye research (2019) 178:238-246.

Diabetes visual function supplement study (DiVFuSS).
Br J Ophthal (2016) 100.2:227-234.

Supplemental retinal carotenoids enhance memory in healthy individuals with low levels of macular pigment in a randomized, double-blind, placebo-controlled clinical trial.
J Alzheimer's Disease (2018) 61.3:947-961.

Effects of Lutein and Astaxanthin Intake on the Improvement of Cognitive Functions among Healthy Adults: A Systematic Review of Randomized Controlled Trials.
Nutrients (2020) 12.3: 617.

The association between MPOD and visual function outcomes: a systematic review and meta-analysis

Eye (2020): 1–9

METHODS:
MEDLINE®, Cochrane, and Commonwealth of Agriculture Bureau abstracts databases were searched for English-language publications between 1946 and August 2018. Included studies examined correlation of MPOD and visual function in adults with healthy eyes at all timepoints and all designs. Visual function outcomes reviewed included photostress recovery, contrast sensitivity, visual acuity, glare sensitivity/disability and dark adaptation.

RESULTS:
In meta-analysis of 22 publications, MPOD was found to be significantly correlated with contrast sensitivity at 30′ (summary r=0.37) and at 1° eccentricity with a spatial frequency of 7, 11, and 21 cpd (summary r=0.31), with photostress recovery at a 1° eccentricity with a moderate background, 10 cpd, and 16% contrast (summary r= −0.17), and at 30′ (summary r= −0.57), and with glare disability at 30′ eccentricity with a log scale at 460 nm (summary r=0.47). There were insufficient data for meta-analysis for other visual functions.

CONCLUSIONS:
Identified link between MPOD and visual function with ***significant correlations with 1) photostress recovery, 2) glare disability and 3) contrast sensitivity.***

Ocular Nutraceutical Roles

- ***Deposition/Transport Enhancement***
- Supplement Synergy
- Protein Expression Augmentation
- Systemic Disease Associations
- Not Just for AMD.....

Feasibility study of a DHA optimized nutraceutical formulation on the macular levels of lutein in a healthy Mediterranean population

***Ophthalmic Research* (2020)**

Objective:
- Analysis of the influence of DHA supplementation along with lutein on MPOD using the Visucam® retinograph
- Determine variation in the lutein and DHA levels in plasma and red blood cell membranes respectively.

Methods:
- One hundred healthy participants with mean age 49.3±13.7 years were randomized in a 1:1 ratio to receive one of following supplements daily for 3 months: 1) Lutein or 2) Lutein + DHA
- MPOD was measured at baseline and end of the follow-up by retinography (Visucam®)
- Lutein in plasma was determined by HPLC
- DHA in red blood cell membranes was analyzed by gas chromatograph/mass spectrometer

Results:
- MPOD was significantly higher in the Lutein/DHA group than in the Lutein group @ 3 months ($p<0.0001$)
- Significantly higher Lutein in plasma ($p<0.0001$) and DHA ($p<0.0001$) levels in the red blood cell membrane were seen in the Lutein/DHA group than in the Lutein group at the 3-month follow-up

Conclusions:
- Lutein supplementation improves MPOD in healthy subjects and is ***significantly increased in the presence of DHA***
- Findings highlight the relevance of the ***adjunctive role of DHA for a better lutein availability***

Ocular Nutraceutical Roles

- Deposition/Transport Enhancement
- ***Supplement Synergy***
- Protein Expression Augmentation
- Systemic Disease Associations
- Not Just for AMD.....

Nutraceuticals for dry age-related macular degeneration: A case report based on novel pathogenic and morphological insights

***Arch. Ital. Biol* 158 (2020): 24-34.**

CASE REPORT
Specific nutraceutical compounds may exert beneficial effects on the progression of atrophic AMD. Antioxidants such as lutein, resveratrol and *Vaccinium myrtillus* have been associated with a reduced risk in the development AMD and when supplemented to diet of an informed patient suffering from dry AMD, showed improvement in visual acuity and lasting reduction in druse volume and number

RESULTS
- OCT indicated a number of drusen beneath the macular region A 6 month diet + intervention decreased drusen volume and thickness in the central area of the macula and was associated with a more regular macular profile
- Subjective point improved in color contrast and a reduction in Amsler grid distortion
- Contrast sensitivity (Pelli-Robson) improved from 1.8 to 2.0 monocularly

CONCLUSIONS AND RELEVANCE
- ***AMD dysfunction occurs on both sides of the RPE*** suggesting that physiological secretion towards the basal membrane is lost leading to ***accumulation of proteins such as unesterified cholesterol, apoE, CFH and vitronectin***
- Possible generalized defects in protein handling by the retinà-choroid junction is ***best targeted by a pharmacological synergism at multiple levels.***

Ocular Nutraceutical Roles

- Deposition/Transport Enhancement
- Supplement Synergy
- ***Protein Expression Augmentation***
- Systemic Disease Associations
- Not Just for AMD.....

Characterizing the effect of supplements on the phenotype of cultured macrophages from patients with age-related macular degeneration.

Molecular vision (2017) *23*, 889.

- CONCLUSIONS: Macrophages may exert oxidative, inflammatory and angiogenic effects in the presence of AMD. Combinations of lutein and carnosic acid with zinc and standardized β-carotene yielded an antioxidative, anti-inflammatory and antiangiogenic effect in M1 and M2 macrophages resulting in ***upregulation of antioxidative genes and downregulation of pro-angiogenic / pro-inflammatory genes***. Combinations of supplements can modify the expression of genes and proteins that may be modulate macrophage phenotype in AMD

Resveratrol based oral nutritional supplement produces long-term beneficial effects on structure and visual function in human patients.

Nutrients (2014) *6*(10):4404-4420.

- CONCLUSIONS: Low dose hormetic OTC oral resveratrol-based matrix of red wine solids, vitamin D_3 and inositol hexaphosphate (IP6) was evaluated against ocular structure measures (FAF and SD-OCT EDI) and ocular function measures (VA, CS and glare recovery) ***Broad bilateral improvements in ocular structure and function were observed suggesting application of epigenetics may have a role in long-term efficacy against AMD***


CFH and LOC387715/ARMS2 Genotypes and Treatment with Antioxidants and Zinc for Age-Related Macular Degeneration

Treatment Response to Antioxidants and Zinc Based on CFH and ARMS2 Genetic Risk Allele Number in the Age-Related Eye Disease Study

Age Related Macular Degeneration Studies

Journal of Vitreo-Retinal Disease (2017)

Methods:
Genetic risk and antioxidant treatment were analyzed as independent and interacting risk factors for the development of intermediate AMD in 554 AREDS individuals

Genetic risk was determined using an allele dosage model based on the total number of CFH and ARMS2 risk alleles.

Conclusion:
Antioxidant treatment has limited to no impact on the development of intermediate AMD in patients without AMD and may reduce the risk of developing intermediate AMD in patients with high genetic risk.

Antioxidant treatment may increase the risk of developing intermediate AMD in patients with low genetic risk.

Genotype-directed antioxidant treatment of patients without AMD may ultimately lead to fewer cases of advanced AMD.


Progression From No AMD to Intermediate AMD as Influenced by Antioxidant Treatment and Genetic Risk: An Analysis of Data From the Age-Related Eye Disease Study Cataract Trial

Ocular Nutraceutical Roles

- Deposition/Transport Enhancement
- Supplement Synergy
- Protein Expression Augmentation
- ***Systemic Disease Associations***
- Not Just for AMD.....

Clinical Treatment
Systemic Disease Management

Serum cytokines as biomarkers for age-related macular degeneration.
Clinical and Experimental Ophthalmology (2015) *253*(5), 699-704.

- CONCLUSIONS: Serum samples from 30 AMD patients and 15 age-matched controls were examined for 16 inflammatory cytokines using multiplex ELISA. Seven (7) ***discrete IL showed serum elevation suggesting that AMD is ultimately an inflammatory disease*** and these ***cytokines may be used as easy-to-obtain risk biomarkers***.

Early Age-related Macular Degeneration with Cardiovascular and Renal Comorbidities: An Analysis of the National Health and Nutrition Examination Survey, 2005–2008.
Ophthalmic epidemiology (2017) *24*(6):413-419.

- CONCLUSIONS: The age-adjusted odds ratio for having early AMD for persons with the selected conditions was 2.6 for any type of heart disease. Having any single condition (AP, CHD, MI, CHF, CKD) was significantly associated with early AMD. The strongest association ***(OR = 6.3)*** was between ***early AMD and the combination of heart disease and stroke***.

Ocular Nutraceutical Roles

- Deposition/Transport Enhancement
- Supplement Synergy
- Protein Expression Augmentation
- Systemic Disease Associations
- ***Not Just for AMD.....***

Clinical Treatment
***Oral Supplementation* for Diabetic Retinopathy**

Application of Lutein and Zeaxanthin in nonproliferative diabetic retinopathy.
Int. J. Ophthalmol (2011) 4, 303–306.

Effects of Crocin on Diabetic Maculopathy: A Placebo-Controlled Randomized Clinical Trial.
Am. J. Ophthalmol (2018) 190, 89–98.

Effect of lutein supplementation on visual function in nonproliferative diabetic retinopathy.
Asia Pac. J. Clin. Nutr. (2017) 26, 406–411.

Plasma carotenoids and diabetic retinopathy.
Br. J. Nutr. (2009) 101, 270–277.

Macular Pigment Optical Density Measured by Dual-Wavelength Autofluorescence Imaging in Diabetic and Nondiabetic Patients: A Comparative Study.
Invest Ophthalmol. Vis. Sci. (2010) 51, 5840–5845.

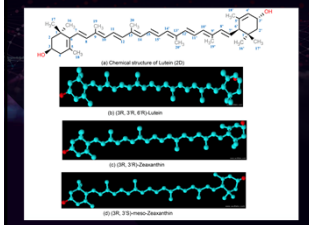
Effect of carotenoids dietary supplementation on macular function in diabetic patients.
Eye Vis. (2017) 4.

Lecithinized curcumin delivery system, in diabetic microangiopathy and retinopathy.
Panminerva Med. (2012) 54, 11–16.

Hypothesized Roles of Macular Pigment

- Optical Hypothesis
- ***Protection Hypothesis***
- Neural Hypothesis

Lutein & Zeaxanthin: Macular Pigment

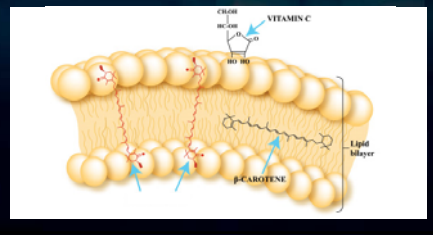


Macular pigment (MP) is the collective name for the isomeric carotenoids **lutein** and **zeaxanthin**
(Bone et al., 1997)

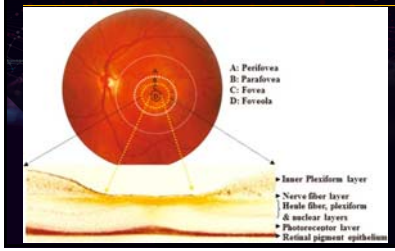
Accumulated within the sensory retina at levels 1000X higher than found in serum to the exclusion of all other carotenoids
(Landrum et al., 1997)

Primary Metabolites:
meso-zeaxanthin
3'-oxolutein
3'-epilutein

Protection Hypothesis
Macular Pigment



Take Home Points: Clinical Macular Pigmentation supplementation

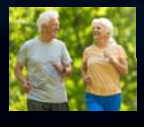


Target a 4:1 L:Z ratio

Generic brands tend to have lower xanthophyll bioequivalencies

Health status matters
- Cardiovascular status
- BMI
- Smoking
- Diet

Take Home Points: Clinical Nutraceutical supplementation – Step 1



Take Home Points: Clinical Nutraceutical supplementation – Step 2

Lutein (20mg) and Zeaxanthin (5mg) [OSL: 40mg/d]
Resveratrol (100mg) [OSL: 2000mg/d]
Curcumin (500mg) [OSL: 3000mg/d]
Quercetin (1000mg) [OSL:
Anthocyanin (500mg) [OSL: N/A]

What's now?

1. Family history, systemic health, genetic predisposition and carotenoid status are all risk factors
2. **NOTHING** will replace:
   i. Healthy diet high in fruits/vegetables (Mediterranean diet)
   ii. Moderate exercise 4 times/week
   iii. Adequate rest targeting 8 hours/night
3. Impaired dark adaptation is an early manifestation of AMD
4. Oral supplementation has been firmly established in treatment and management of non-proliferative AMD
   i. Mild, non-proliferative maculopathy = 20mg L / 5mg Z
   ii. Intermediate, non-proliferative maculopathy = AREDS2 + 20mg L / 5mg Z
   iii. Severe, non-proliferative maculopathy = AREDS2 + 20mg L / 5mg Z

What's next?

1. Serum-based testing for:
   - CFH and ARMS2
   - StARD3 and GSTP1
   - Serum-based cytokines (IL-1α, IL-1β, IL-4, IL-5, IL-10, IL-13, IL-17, IL-18)
2. Patient-tailored health plans for at-risk populations
   - Integrated systemic / supplementation strategies
3. Enhanced bioavailability
4. Risk calculator to incorporate:
   - Clinical biomarkers + Genetic risk
5. Healthy skepticism with reasoned cynicism

What's next?

Prevalence of reticular pseudodrusen in age-related macular degeneration with newly diagnosed choroidal neovascularisation.
Br J Ophth (2007) 91.3: 354-359.

Results:
Patients with newly diagnosed CNV (67 women and 33 men with mean age: 79.5) showed:
- Classic CNV (32 eyes)
- Occult CNVM (41 eyes)
- Vascularized PED (11 eyes)
- Retinal angiomatous proliferation (RAP) with or without PED (13 eyes)
- Hemorrhagic or fibrovascular scarring (3 eyes)

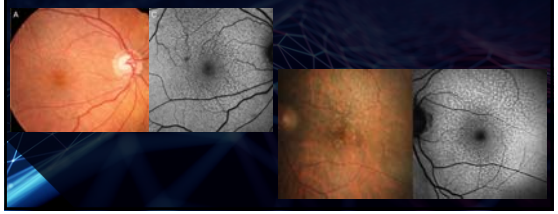
Patients with RPD (155 total eyes) were enrolled in 3 month imaging study. Commonly exhibited signs of ARM:
- soft drusen (101 eyes)
- retinal pigment epithelium abnormalities (70)
- geographical atrophy (27)
- CNV (61)

In both studies, FAF imaging was critical in diagnosing RPD*

Conclusion:
RPD have a high prevalence among patients with AMD with newly diagnosed CNV (24% of cases). ***RPD were commonly associated with ARM or AMD.*** This study suggests that eyes with ***RPD could be classified as a phenotype of ARM.***

What's next?

Prevalence of reticular pseudodrusen in age-related macular degeneration with newly diagnosed choroidal neovascularisation.
Br J Ophth (2007) 91.3: 354-359.



Questions?

Take Home Points: Clinical Macular Pigmentation supplementation