JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 4001–4007 4001
DOI:10.1021/jf903038r

Recent Research on Polyphenolics in Vision and Eye Health[†]

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A long-standing yet controversial bioactivity attributed to polyphenols is their beneficial effects in vision. Although anecdotal case reports and in vitro research studies provide evidence for the visual benefits of anthocyanin-rich berries, rigorous clinical evidence of their benefits is still lacking. Recent in vitro studies demonstrate that anthocyanins and other flavonoids interact directly with rhodopsin and modulate visual pigment function. Additional in vitro studies show flavonoids protect a variety of retinal cell types from oxidative stress-induced cell death, a neuroprotective property of significance because the retina has the highest metabolic rate of any tissue and is particularly vulnerable to oxidative injury. However, more information is needed on the bioactivity of in vivo conjugates and the accumulation of flavonoids in ocular tissues. The direct and indirect costs of age-related vision impairment provide a powerful incentive to explore the potential for improved vision health through the intake of dietary polyphenolics.

KEYWORDS: Flavonoids; retina; antioxidant; rhodopsin

BERRY FLAVONOIDS AND HUMAN HEALTH

Polyphenolics and especially the flavonoids contained in berry crops are being investigated for their potential benefits against cancer, as well as in cardioprotection, neuroprotection, urinary tract health, and antiaging effects (*1*). Concurrent with berry flavonoid research that employs various models of health and disease, investigations are underway to determine the in vivo bioavailability of these compounds (*1*). A somewhat unique yet controversial bioactivity attributed to berry anthocyanins is their benefits in vision, particularly night vision (*2*, *3*). There is a growing need to determine if and how anthocyanins improve human vision because their benefits are promoted commercially and are already widely familiar to consumers, particularly in Japan and Asia. Demonstration of clinical benefits to night vision by anthocyanins could be of great interest because this benefit would be easy for consumers to understand and should be demonstrable in a relatively short time frame.

Recent research suggests that flavonoids may be involved in two major aspects of the diverse processes involved in vision physiology and eye health. Flavonoids may function in visual signal transduction, in ways that are as yet not well understood. Flavonoids may also function in their well-established role as antioxidants, which is particularly important in the eye, where oxidative stress is significant and its damage is implicated in a number of vision pathologies, including macular degeneration (*4*). Studies showing flavonoid effects on visual signal transduction or as antioxidants can be put in context once we understand whether flavonoids are absorbed by the eye and, if so, their relative distribution among ocular tissues.

This paper will focus on the effects of berry polyphenolics on vision physiology, and their bioavailability in ocular tissues will be reviewed. When possible, conclusions will be made and areas for future research will be proposed.

FLAVONOID EFFECTS ON VISUAL SIGNAL TRANSDUCTION IN VITRO

Visual signal transduction involves two major processes: first, the light activation of rhodopsin, leading to a change in its conformation and the creation of a visual signal, and, second, the regeneration of rhodopsin to its original conformation. Rhodopsin has two components, the chromophore retinal and the protein opsin. Rhodopsin is embedded in the membranes of disks in the outer segment of rod photoreceptors (**Figure 1**). Rod photoreceptor cells are responsible for the retinal pathways that give rise to monochrome vision under reduced light (i.e., dark vision). Cone photoreceptors contain either short- (blue), middle- (green), or long − (red) wavelength sensitive light-absorbing molecules called iodopsins. Together the three types of cones are responsible for color vision and visual acuity in bright light. Rhodopsin and iodopsins share the same chromophore, 11-*cis*-retinal, but have different opsin molecules.

[†]Part of the Berry Health Symposium 2009.

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Published on Web 01/26/2010

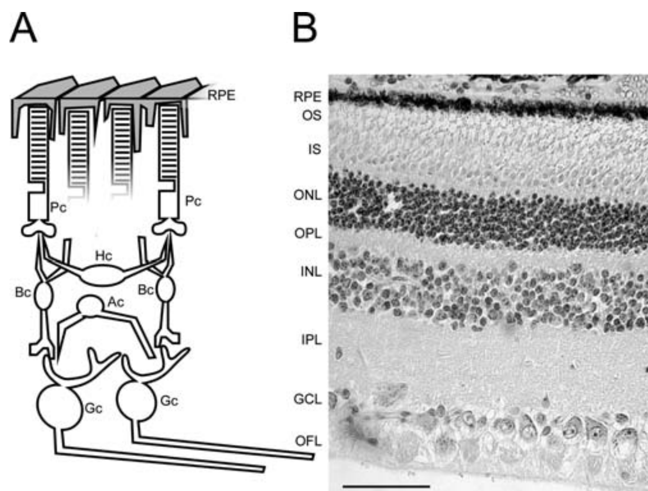

Figure 1. Anatomy of the retina. (**A**) The retina is composed of photoreceptors (Pc, rods and cones, where phototransduction takes place), making synapse to bipolar cells (Bc), the latter making synapse to ganglion cells (Gc), the axon of which constitutes the output of the retina. Horizontal cells (Hc) and amacrine cells (Ac) are responsible for horizontal connections between neighboring cells. (**B**) A cross section of the pig retina shows the retinal pigment epithelium cell (RPE), the outer segments (OS), and inner segments (IS) of the photoreceptors, the cell bodies of which create the outer nuclear layer (ONL). The synapse plexus between photoreceptors and second-order neurons constitutes the outer plexiform layer (OPL). Cell bodies of bipolar cells, along with the ones of horizontal and amacrine cell, make the inner nuclear layer (INL), whereas synapses between bipolar cells, amacrine cells, and ganglion cells occur in the inner plexiform layer (IPL). The optic fiber layer (OFL) is made of the axons of the retinal ganglion cells (GCL). Scale bar = 50 μm.

The generation of a visual signal is initiated by the absorption of light by the rhodopsin chromophore, 11-*cis*-retinal, which results in its isomerization to *all-trans*-retinal. Isomerization to the *all-trans*-retinal causes the molecule to straighten, making its fit in the opsin protein energetically less favorable. This higher energy form, called metarhodopsin II, activates the G-protein, transducin. Transducin in turn activates a cyclic-GMP phosphodiesterase, which hydrolyzes cyclic-GMP, causing the closure of ion channels in the photoreceptor membrane and the hyperpolarization of the photoreceptor cell. The potential difference in the outer segment of the photoreceptor cell is transferred along this cell to its synaptic terminal and then transferred to second-order neurons, including bipolar cells, amacrine and horizontal cells (**Figure 1**), and then to retinal ganglion cells. All of these cells are easily identifiable as a very orderly structure with clearly alternating plexiform (synapses) and nuclear (cell bodies) layers (**Figure 1B**). Lateral interactions maintained by horizontal and amacrine cells ensure that the retina does not process the visual world as a point-to-point detector (such as a camera) but rather analyzes elementary features of the visual scene and transfers that information to the brain via ganglion cell axons that form the optic nerves. In this sense, the retina is considered to be an accessible part of the brain (*5*).

Anthocyanins have been examined with respect to different steps in the visual signal transduction process described above. Cyanidin-3-glucoside (C3g) was recently reported to inhibit the activation of the G-protein transducin by metarhodopsin II (*6*). GDP−GTP exchange leads to the activation of the heterotrimeric G-protein, and the effect of 1 mM C3g on this process was examined using radiolabeled GTP. Transducin activation was reduced by 58% by C3g; however, the high concentration required led the authors to conclude that the effect was only modest. Earlier research reported that anthocyanins could either inhibit (*7*) or activate (*8*) cyclic-GMP phosphodiesterase. However, more recently Matsumoto et al. (*9*) reported insignificant effects of black currant (*Ribes nigrum*) anthocyanins on cyclic-GMP phosphodiesterase activity when tested at various stages of phosphodiesterase activation in the light and dark. The anthocyanins were present in the assay at between 10 and 50 μM, which is well above their in vivo concentration (*10*, *11*).

The second major process involved in visual signal transduction is the regeneration of rhodopsin in a process called the retinoid cycle. Retinoid is a collective term for several intermediates involved in the conversion of photoisomerized *all-trans*-retinal back to the dark form 11-*cis*-retinal. Retinoid translocation and processing contributes to the long time required for human vision to accommodate to low light conditions (i.e., dark adaptation). Lamb and Pugh (*12*) provide an excellent review of these processes, which involve numerous retinoid reactions occurring in different inter- and intracellular locations. In brief, after light activation, rhodopsin (metarhodopsin II) is inherently unstable due to steric hindrance by the more linear *all-trans*-retinal isomer in the opsin protein. As a result, the covalent bond between *all-trans*-retinal and opsin protein is hydrolyzed, and *all-trans*-retinal is released from the opsin protein. *all-trans*-Retinal is then reduced to *all-trans*-retinol, which is transported through the cytoplasm and across the photoreceptor cell outer segment membrane. Once external to the rod photoreceptor cell, *all-trans*-retinol crosses the interphotoreceptor matrix and enters the retinal pigment epithelial (RPE) cells (**Figure 1**). Within the RPE cell *all-trans*-retinol is esterified with lecithin, is de-esterified, then isomerized to 11-*cis*-retinol, and finally oxidized to 11-*cis*-retinal. 11-*cis*-Retinal is then translocated back across the interphotoreceptor matrix and into the outer segment membrane of the rod photoreceptor cell, where opsin is located. Once associated with free opsin, 11-*cis*-retinal enters the opsin-binding pocket and spontaneously forms a covalent Schiff base bond with a lysine residue, to finally regenerate the dark form of rhodopsin (i.e., opsin protein with covalently bound 11-*cis*-retinal).

Matsumoto et al. (*9*) reported that specific black currant anthocyanins stimulate the regeneration of rhodopsin. The kinetics of incorporation of purified 11-*cis*-retinal into an opsin membrane fraction prepared from frog rod outer segments was studied in the presence and absence of 20 μM purified black currant anthocyanins. Of the four anthocyanins contained in black currant, namely, the glucoside and rutinoside of cyanidin and delphinidin, only the two cyanidin glycosides increased the rate of rhodopsin regeneration. Kinetic analysis revealed that the K_m for rhodopsin formation was lowered by the presence of cyanidin glycosides, although it was not possible to determine which specific reaction was affected.

Two recent studies suggest that C3g can interact directly with both the dark and light forms of rhodopsin and opsin protein (*6*, *13*). The direct interaction of purified C3g and rhodopsin purified from bovine retinae was examined in detergent micelles using NMR (*6*). Proton NMR rhodopsin resonances were dampened when 50 μM C3g was added to 50 μM dark state rhodopsin, suggesting that the C3g associated with rhodopsin and restricted its free movement. The effects on proton resonances of dark- and light-state rhodopsin by C3g were different, suggesting that C3g effects were unique to each rhodopsin form (*13*). These studies were carried out at pH 6, at which both the quinoidal and the chalcone forms of C3g would be present.

Computational docking experiments using the various pH-dependent forms of C3g showed docking at the cytoplasmic side of the rhodopsin molecule, with the chalcone having the most favorable binding affinity. The pH of the extracellular environment of the photoreceptor is increased by light exposure (*14*), and vertebrate retinae undergo diurnal pH changes (*15*) such that retinal pH is more alkaline during the day. Differences in photoreceptor pH make the differential effects of pH-dependent forms of anthocyanins particularly interesting.

How C3g affected the function of bovine rhodopsin was also examined in vitro in detergent micelles (*6*). The regeneration rate of rhodopsin from 11-*cis*-retinal and opsin was increased 1.65-fold in the presence of a 2-fold excess of C3g (1 μM) at pH 6; this observation supports the results of Matsumoto et al. (*9*). However, whereas the rhodopsin regeneration rate was increased by C3g, the total percentage of rhodopsin formed was decreased by 9%. The rate of metarhodospin II decay (which would make opsin available for 11-*cis*-retinal binding) was slightly increased by C3g, but only when present at a 10-fold excess (5 μM). It was concluded that this relatively small effect could not explain the 1.65-fold more rapid rhodopsin regeneration rate observed in the presence of 1 μM C3g. The thermal stability of the dark rhodopsin was monitored spectrally by measuring rhodopsin (A_{max} 500 nm) and free retinal (A_{max} 380 nm). The addition of 0.5 μM C3g had a dramatic destabilizing effect on intact rhodopsin whereby its thermal decay at 50 °C was increased by 3- and 6-fold at pH 6 and 8, respectively.

The flavanone eriodictyol has recently been reported to interact in vitro with opsin and to affect opsin signaling activity (*16*). In computational docking experiments eriodictyol had a very favorable binding energy in the opsin-binding pocket and competed with 11-*cis*-retinal for binding to bovine rod opsin. Eriodictyol also induced the activation of the G-protein transducin in cone cells. It was suggested that eriodictyol behaved as a retinoid surrogate and could potentially modulate physiological changes in photoreceptor function (*16*). Although vision research related to flavonoids has historically been limited to anthocyanins only, these results suggest that research on a broader range of flavonoids is warranted.

OXIDATIVE STRESS IN THE RETINA

The retina is the most metabolically active tissue in the body (*12*), and its high metabolic rate creates a high oxygen demand and vulnerability to oxidative stress (*4*). Other factors that make the retina particularly vulnerable to oxidative stress are the high proportion of PUFA in photoreceptor membranes, chronic exposure to light, and, in the RPE, phagocytosis, which gives rise to reactive intermediates. Evidence of oxidative stress is apparent with increasing age, and oxidative damage may be most prominent in the central portion in the retina, the macula (*17, 18*). Many investigators have proposed that age-related changes within the RPE, including an accumulation of lipofuscin, represent the earliest changes that ultimately lead to age-related macular degeneration (*17, 18*).

The xanthophyll carotenoids (lutein and zeaxanthin) that are embedded in the retinal photoreceptor membrane can interact with oxygen radicals at their polar end groups, which protrude from the lipid bilayer. In addition to direct scavenging of radicals, xanthophyll macular pigments can prevent the oxidation of PUFA-rich photoreceptor cells by filtering light. Because the absorption maxima for lutein and zeaxanthin are between 445 and 472 nm, these macular pigments can reduce the intensity of the most damaging light energy (400−500 nm) impinging on the retinal membranes (*19*).

The results of meta-analyses and systematic reviews of research conclude that there is a lack of adequately designed trials to determine whether or not dietary supplementation with carotenoids can reduce the risk of age-related macular degeneration in healthy populations (*4, 20*). However, the clinical epidemiological Age-Related Eye Disease Study (AREDS) (*21*) reported that certain dietary antioxidants and mineral supplements reduced the progression of visual loss by up to 28% in patients with moderate to advanced disease, and AREDS2 has been initiated to address whether lutein supplementation will be beneficial. Foods that were correlated with ocular benefits in AREDS1 included leafy green vegetables, which are rich in carotenoids and, of relevance to this paper, polyphenolics (*21*).

ANTIOXIDANT EFFECTS OF FLAVONOIDS IN VITRO

The in vitro antioxidant effects of flavonoids and other phenolics have recently been studied using models and ocular cell types that are relevant to vision processes and pathologies. In three studies (*22−24*) cultured RPE cells were employed to investigate how flavonoids affected oxidative damage and metabolic responses to oxidative stress in vitro. The RPE is a relevant tissue for study because oxidative damage to RPE is implicated in age-related macular degeneration (*17, 20*). Functionally, the RPE forms the blood−retina barrier and is the site of active transport of materials such as vitamin A (retinol) from blood to the photoreceptors (**Figure 1**). As mentioned earlier, the RPE is involved in the recycling of retinoid during rhodopsin regeneration and in the phagocytosis of the aged tips of photoreceptor outer segments.

Numerous flavonoid aglycones, having the C-ring structure of flavones, flavonols, flavanols, flavanones, and anthocyanidins, were tested for their effect on the survival of immortalized human RPE cells (ARPE-19) after cells were subjected to oxidative stress by the addition of either *tert*-butyl peroxide (t-BOOH) or hydrogen peroxide (H_2O_2). Selected natural flavones (luteolin and baicalein), flavonols (galangin, fisetin, and quercetin), and the flavanone eriodictyol were effective in improving cell survival with EC_{50} in a range between 6 μM (eriodictyol) and 32 μM (galangin), whereas flavanols and anthocyanidins were not active. Interestingly, in primary RPE cultures the EC_{50} for all flavonoids was lower than in ARPE-19 cells, and epigallocatechin gallate, which was ineffective in ARPE-19 cells, had an EC_{50} of 22 μM (*23*). The authors indicate that clinical treatments are needed that can be effective after oxidative insult has already occurred. By selecting the appropriate time frame after oxidative insult and until cells were committed to cell death, the authors showed that specific flavonoids, namely, luteolin, fisetin, and quercetin, were able to protect cells even when applied 2 h after treatment with t-BOOH or H_2O_2.

The up-regulation by flavonoids of various phase 2 detoxification proteins, for example, heme-oxygenase and GSH transferase, can contribute to enhanced antioxidant protection and detoxification capacity and therefore may constitute another, and perhaps more important, means for these compounds to protect ocular cells. The cellular expression of phase 2 proteins is under the regulation of the antioxidant response element, which can be activated by the transcription factor Nrf2. Interestingly, quercetin, fisetin, and eriodictyol were found to induce expression of Nrf2 in ARPE-19 cells, as well as hemeoxygenase-1, a phase 2 enzyme under Nrf2 regulation (*23*). Milbury et al. (*22*) examined ARPE-19 cells treated with a purified bilberry (*Vaccinium myrtillus* L.) extract that contained either anthocyanins or non-anthocyanin phenolics before they received an oxidative challenge with H_2O_2. Both the anthocyanin and non-anthocyanin extracts were found to reduce the abundance of reactive oxygen

species arising from H_2O_2. However, neither extract was sufficiently protective to affect cell survival. Both bilberry extracts increased the expression of the phase 2 proteins hemeoxygenase-1 and GSH transferase. It is notable that bilberry phenolics were found to up-regulate hemeoxygenase-1 protein expression even without H_2O_2 treatment. Similarly, hemeoxygenase-1 and GSH transferase mRNA expression in APRE-19 cells was increased after pretreatment with bilberry extracts.

With a relatively high antioxidant efficacy compared to other flavonoids, the flavanone eriodictyol was studied further to investigate its effects on the cell's endogenous antioxidant defense system and phase 2 gene expression (*24*). Eriodictyol was shown to up-regulate the antioxidant response element transcription factor Nrf2 and increase the expression of both hemeoxygenase-1 and NADPH:quinone reductase, another phase 2 enzyme (*25*). Overexpression of these enzymes enhanced long-term antioxidant protection in ARPE-19 cells, demonstrating that their up-regulation by eriodictyol could contribute to enhanced protection. Eriodictyol effects on the expression of Nrf2 and phase 2 proteins as well as GSH concentration were temporally correlated. Approximately 24 h after treatment with 50 μM eriodictyol, the intracellular concentration of GSH had doubled (*24*).

Similar flavonoid antioxidant studies have been conducted in another retinal cell type, the retinal ganglion cells (RGC) (*25*,*26*). Whereas the RPE cells are localized on the outer layer of the retina facing the vitreous humor, RGC are near the inner surface. RGC receive visual stimuli from the photoreceptor cells via the amacrine and bipolar neurons (**Figure 1**). There are many types of RGC, which all have in common a long axon that extends into the brain, thereby connecting the brain with the retina. Oxidative damage to RGC has been associated with glaucoma, diabetic retinopathy, and damage arising from retinal ischemia (*27*−*29*). RGC survival after oxidative stress generation by the addition of H_2O_2 or t-BOOH or by GSH depletion (using glutamate and buthionone sulfoxime) was used as models to test the effects of different classes of flavonoid aglycones. Similar to the results obtained in RPE cells (*23*), the flavanone eriodictyol, as well as specific flavones and the flavonol quercetin, was protective in mitigating cell death after all three types of in vitro oxidative stress. Catechin, epicatechin, epigallocatechin gallate, and cyanidin were ineffective in the GSH depletion assay and were therefore not tested in the other oxidative stress models. Protective flavonoids were also tested after oxidative insult to determine their ability to rescue cells, rather than protect them, from damage by either H_2O_2, t-BOOH, or GSH depletion. In the GSH depletion assay no flavonoids provided protection greater than was achieved by simply removing the oxidative stress (changing media) except for the synthetic flavonoid 3,7-dihydroxyflavone, which improved cell survival when applied up to 18 h after GSH depletion. Among 10 flavonoids tested, up-regulation of Nrf2 and activation of the antioxidant response element in RGC were observed in response to galangin, fisetin, and quercetin treatment (*25*).

Transient ischemia was modeled in RGC-5 cells by the addition, and subsequent removal, of the glycolytic toxin iodoacetic acid (IAA) (*26*). Effects on the concentration of GSH, ATP, reactive oxygen species, and cell survival were monitored in the presence and absence of flavonoids. Resveratrol (25 μM) protected cells against death significantly better than either of the classic antioxidants vitamin C or E at 100 μM. Six naturally occurring flavonoids including two flavones (baicalein, luteolin), three flavonols (quercetin, galangin, fisetin), and the flavonone eriodictyol significantly improved cell survival when applied with IAA or after IAA treatment. The ability of the flavonoids to beneficially affect the concentration of GSH, ATP, and reactive oxygen species was more limited, however. Whereas all of the natural flavonoids reduced the concentration of reactive oxygen species, GSH and ATP levels were maintained only after luteolin and fisetin treatment (*26*).

On the basis of in vitro results obtained in both RPE (*23*) and RGC (*25*, *26*), it is concluded that flavonoids can protect retinal cell types through different mechanisms including direct scavenging of reactive oxygen species, anti-apoptotic activity, and phase-2 induction. The relative potencies of the flavonoids were similar in both RPE (*23*) and RGC (*25*, *26*), suggesting that certain structural features are key to their effects, and the authors indicate that these features are the same as those reported in similar studies with hippocampal cells (*30*). Structural features that increase antioxidant potency of flavonoids and their range of effects include the presence of vicinal hydroxyl groups (i.e., catechol structure), unsaturation of the C ring, and a high degree of hydrophobicity (*30*).

Conjunctival cells form a protective mucous layer on the white (sclera) portion of the eye, as well as the eyelids. Phenolic acids were reported to protect cultured human conjunctival epithelium cells in vitro in a model designed to simulate damage to the cornea by UV-B exposure (*31*). UV-B-induced damage to DNA, measured by 8-oxo-2-deoxyguanosine, was significantly reduced by 0.5 h of preincubation of cells with 10 μM hydrocaffeic or a mixture or 5 μM hydrocaffeic and 5 μM *p*-coumaric acid. The authors note that topical application of phenolics to the cornea and conjunctival epithelium may overcome the limited bioavailability of orally administered phenolics.

FLAVONOID BIOAVAILABILITY IN THE EYE

Although anthocyanins and other flavonoids may affect visual signal transduction and antioxidant protection, relatively little is known about their in vivo bioavailability to the eye. As presented earlier, selected flavonoids including eriodictyol, luteolin, and quercetin provided antioxidant protection to cultured ocular cells (*23*, *25*, *26*). The bioavailability of eriodictyol, luteolin, and quercetin was studied in a rat in situ perfusion system to determine the extent of their intestinal absorption and intestinal and hepatic metabolism (*32*). The absorption behavior of these flavonoids was influenced more by their lipophilicity than by their three-dimensional structure. Compared to other representative flavonoids, it was determined that quercetin, luteolin, and eriodictyol had relatively high availabilities, with 9, 28, and 20% of the administered dose available, respectively, for deposition in peripheral tissues, such as the eye. Because these flavonoids were present in plasma only as various sulfated, methylated, and glucuronidated phase 2 conjugates, these would likely be the flavonoid forms available for deposition in tissues (*32*). The total plasma levels of the three flavonoid metabolites were similar, ranging between about 0.5 μM (eriodictyol) and 1 μM (luteolin). It would very interesting to determine whether these phase 2 conjugates possess in vitro bioactivity similar to those of the native plant flavonoids; however, they are not commercially available. It would also be interesting to determine whether or not these compounds and/or their metabolites could accumulate in tissues.

Although phase 2 conjugates are also the predominant in vivo form of quercetin in circulation (*32*), unconjugated quercetin has been detected in the retina (*33*). Using gas chromatography of TMS derivatives, quercetin was detected in the pooled extract of 10 bovine retinae at a concentration of 40−70 ng/g of FW of retinal tissue (*33*).

Anthocyanins are not substantially metabolized after ingestion, and native anthocyanidin glycosides have been identified in

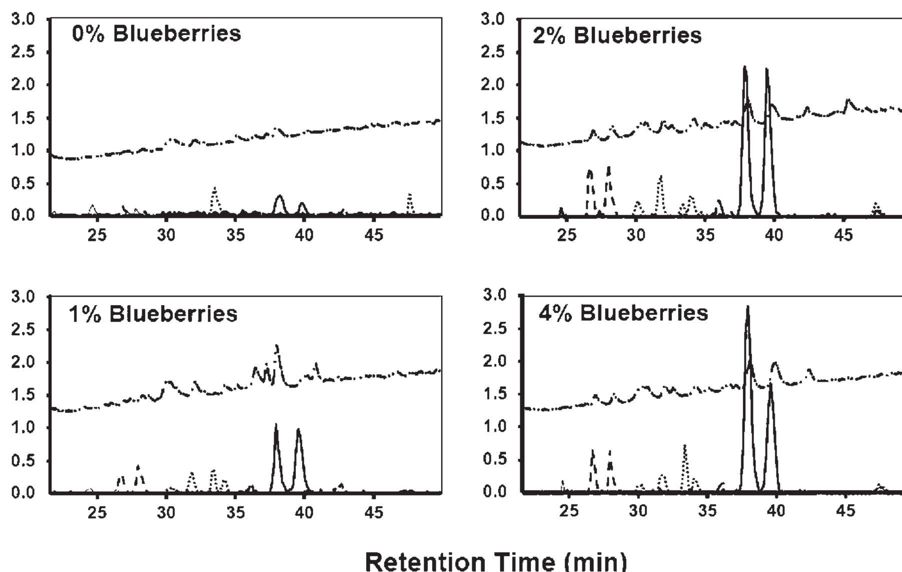

Figure 2. Detection of anthocyanins extracted from tissue of whole eyes of pigs that were fed blueberries (0, 1, 2, or 4%, w/w) for 8 weeks: UV trace (— · · · —); delphinidins (— — —); cyanidins (- - -); peonidins (———); malvidins (—). Details of this study are described ref *34*.

plasma and urine where they occur at nanomolar concentrations (for a review, see ref *10*). However, anthocyanins have also been identified in tissues (*34*), including ocular tissues (*34*, *35*). When 100 mg of anthocyanin/kg of BW was administered to rats by oral gavage, the C_{max} of the whole eye was 115 ± 32 ng/g of FW after 30 min (*35*). When Kalt et al. (*34*) fed blueberries to pigs at 2.4−12 mg of anthocyanin/kg of BW/day for 4 weeks, the anthocyanin concentration measured in the whole eye was 0.709 ng/g of FW. Pigs were fasted for approximately 18 h before tissues were collected, and no anthocyanins were detectable in plasma, suggesting that anthocyanins had accumulated in the tissues. Selected unpublished results obtained from this study also suggest that the accumulation of anthocyanins in the eye was correlated to the dose of blueberries ingested (**Figure 2**).

Matsumoto et al. (*35*) reported a significantly higher concentration of anthocyanins in ocular tissues when black currant anthocyanins were administered by intravenous and intraperitoneal means. One hour after intraperitoneal administration of 108 mg/kg of BW, total anthocyanin concentration ranged from 0.6 μg/g of lens tissue to 245 μg/g of sclera plus choroid tissue, with a plasma concentration of 2.30 μg/mL. Half an hour after intravenous administration of 4.32 mg/kg of BW, anthocyanins were undetectable in the lens and about 3 μg was detected in both the sclera and choroid, whereas the plasma concentration was 12.42 μg/mL. Most significant, perhaps, was the measurement in the retina of 6.89 and 0.27 μg of anthocyanin/g of tissue after intraperitoneal and intravenous administration, respectively. Taken together, the results suggest that flavonoids, including quercetin and various anthocyanins, can cross the blood−retinal barrier after normal feeding and after acute administration by intraperitoneal and intravenous means.

IN VIVO PROTECTION TO THE EYE BY POLYPHENOLICS

As the most external ocular tissue, the cornea is subject to the greatest irradiation and is most vulnerable to mechanical damage. Corneal damage was studied in rabbits that received topically applied phenolic acids for 3 days prior to, and 5 days after, controlled UV-B exposure (*31*). Compared to saline controls, topical application of phenolic acids resulted in significantly less DNA damage (measured as 8-oxo-2-deoxyguanosine) in the cornea and sclera tissue. The degree of corneal protection was similar after treatment with drops containing either 10 or 100 μM hydrocaffeic acid or a mixture of 5 μM hydrocaffeic and 5 μM *p*-coumaric acid. The hydrocaffeic and *p*-coumaric acid mixture provided slightly better protection to the sclera compared to either the 10 or 100 μM dose of hydrocaffeic acid. Xanthine oxidase, a pro-oxidant enzyme that was elevated in the rabbit eyes after UV-B treatment, was significantly lower in the eyes that were topically pretreated with phenolic acids. Other indices of oxidative damage, namely, malondialdehyde, as a marker of membrane oxidation, and the inflammatory prostaglandin PGE_2, which were both increased after UV-B exposure, were significantly lowered by all combinations of the phenolic acid treatments. Together, the results suggest that these nonflavonoid phenolics may be protective against UV-B in animals, just as they act as UV filters in plants (*36*). As mentioned earlier, topical application to the cornea possibly offers a practical means to overcome the low tissue bioavailability of polyphenolics.

Inflammatory markers were measured in the aqueous humor of rats 24 h after they received the bacterial endotoxin lipopolysaccharide by foot pad injection, in a model of endotoxin-induced uveitis (*37*). To examine the effects of polyphenolics on induced inflammation, black chokeberry (*Aronia melanocarpa* Elliot) extract as well as pure anthocyanins (undefined source) and quercetin were administered intravenously at 1, 10, and 100 mg. The increase in the concentration of tumor necrosis factor-α, prostaglandin E2, and nitric oxide was significantly reduced at all dosage levels of chokeberry extract, whereas monocyte chemoattractant protein-1 was only reduced by the 100 mg dose of black chokeberry extract. Notably, anthocyanin and quercetin alone, which make up 80 and 8% of the total black chokeberry polyphenolics, respectively, were significantly less protective than the whole berry extract against all of the inflammatory markers that were monitored. Whereas the intravenous dosage of the berry extract and the anthocyanin and quercetin doses were massively greater than what could occur in vivo it is noteworthy that the anti-inflammatory effects of these phenolics were apparent in the aqueous humor in this model of endotoxin-induced uveitis.

CLINICAL EVIDENCE FOR ANTHOCYANIN BENEFITS TO VISION

Clinical benefits to vision of anthocyanin-rich fruit such as bilberry were extensively reported in the 1960s−1980s (*2*, *3*).

Indeed, there are widely circulated anecdotal reports that during World War II, Royal Air Force (RAF) pilots consumed bilberry jam before night flying to improve their night vision. Kramer (*38*) provides an alternative explanation, however, suggesting that the improved success of RAF pilots was due to their progress in radar technology and the purported benefits of bilberries was intended as disinformation to mislead the enemy. Canter and Ernst (*3*) systematically reviewed studies that examined the effects of clinical intervention with bilberry extract on vision under reduced light. The authors identified 30 clinical trials, 24 of which were conducted between the 1960s and 1980s. Most of these studies lacked a randomized placebo-controlled design and employed instead a simple prepost design. Unfortunately, psychophysical outcome parameters such as dark adaptation testing are particularly vulnerable to less rigorous study designs. Canter and Ernst (*3*) selected 12 trials for detailed description because they were placebo-controlled. Five of the 12 had a randomized controlled design, and of these 5 trials, 4 reported a negative outcome. The fifth randomized control trial (*39*) reported an increase in the area of the visual field (i.e., angle; $p < 0.005$) of subjects after both acute and long-term feeding. Six trials reviewed by Canter and Ernst employed long-term (between 4 and 28 days) treatment before mesopic vision testing and, where indicated, subjects were relatively young. Subject age is a significant consideration because normal night vision begins to decline in middle age and may be related to the thickening of Brach's membrane (*12*), a tissue that is involved in the translocation of retinoids during the regeneration of rhodopsin. In all of the long-term trials the treatment product was a powdered anthocyanin extract. This may be important because extensive processing and storage can lead to the loss of the more bioavailable monomeric forms of anthocyanins (*40*). Most studies do not indicate whether or not vision was tested at the same time daily, which is potentially important because diurnal changes in vision occur .

In conclusion, phytochemicals play a role in vision physiology and antioxidant protection of the eye, and although the role of carotenoids has been well studied (*19*, *20*), the beneficial effects of polyphenolics in models of vision and eye health, which are reviewed here, constitute a relatively novel bioactivity for this group of compounds, which has only been characterized, for the most part, in vitro.

The renewed interest in the effects of anthocyanins on vision signal tranduction (*6*, *9*, *13*) must be considered in light of the very low concentrations of anthocyanins detectable in ocular tissues (*34*, *35*). Recent research suggests that further investigation is warranted on non-anthocyanin flavonoids (*16*, *23*, *26*). Age-related vision pathologies related to oxidative damage are well documented (*27*−*29*), and possible mitigation of such damage by a variety of non-anthocyanin flavonoids is supported by recent in vitro studies (*23*−*26*). The relative antioxidant potency (*23*−*26*) and in vivo bioavailability (*10*, *32*) of non-anthocyanin flavonoid aglycones provides a rationale for their investigation in other aspects of vision physiology. There is a great opportunity to apply modern experimental and clinical approaches to expand our knowledge of the effects of flavonoids in vision physiology and eye health. Clinical benefit(s) of flavonoids to vision, which would be easily understood by consumers and demonstrable in a relatively short time frame, would be very appealing to public health organizations that wish to increase public awareness of the role of diet in health. Emerging research will likely lead to a renewed and expanded interest in this field that has traditionally been focused on the possible effects of anthocyanins in night vision.

LITERATURE CITED

(1) Stevenson, D.; Hurst, R. Polyphenolic phytochemicals − just antioxidants or much more? *Cell. Mol. Life Sci.* **2007**, *64* (22), 2900–2916.
(2) Kalt, W.; Dufour, D. Health functionality of blueberries. *HortTechnology* **1997**, *7* (3), 216−221.
(3) Canter, P.; Ernst, E. Anthocyanosides of *Vaccinium myrtillus* (bilberry) for night vision − a systematic review of placebo-controlled trials. *Surv. Ophthalmol.* **2004**, *49* (1), 38–50.
(4) Rhone, M.; Basu, A. Phytochemicals and age-related eye diseases. *Nutr. Rev.* **2008**, *66* (8), 465–472.
(5) Dowling, J. *The Retina: An Approachable Part of the Brain*; Belknap Press of Harvard University Press: Cambridge, MA, 1987; pp 286.
(6) Tirupula, K.; Balem, F.; Yanamala, N.; Klein-Seetharaman, J. pH-dependent interaction of rhodopsin with cyanidin-3-glucoside. 2. Functional aspects. *Photochem. Photobiol.* **2009**, *85* (2), 463–470.
(7) Ferretti, C.; Magistretti, M.; Robotti, A.; Ghi, P.; Genazzani, E. *Vaccinium myrtillus* anthocyanosides are inhibitors of cAMP and cGMP phosphodiesterases. *Pharmacol. Res. Commun.* **1988**, *20* (Suppl. 2), 150.
(8) Virmaux, N.; Bizec, J.; Nullans, G.; Ehret, S.; Mandel, P. Modulation of rod cyclic-GMP phosphodiesterase activity by anthocyanidin derivatives. *Biochem. Soc. Trans.* **1990**, *18* (4), 686–687.
(9) Matsumoto, H.; Nakamura, Y.; Tachibanaki, S.; Kawamura, S.; Hirayama, M. Stimulatory effect of cyanidin 3-glycosides on the regeneration of rhodopsin. *J. Agric. Food Chem.* **2003**, *51*, 3560–3563.
(10) Manach, C.; Williamson, G.; Morand, C.; Scalbert, A.; Remesy, C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* **2005**, *81* (1), 230S–242S.
(11) Ichiyanagi, T.; Shida, Y.; Rahman, M.; Sekiya, M.; Hatano, Y.; Matsumoto, H.; Hirayama, M.; Konishi, T.; Ikeshiro, Y. Effect on both aglycone and sugar moiety towards phase II metabolism of anthocyanins. *Food Chem.* **2008**, *110* (2), 493–500.
(12) Lamb, T.; Pugh, E. Dark adaptation and the retinoid cycle of vision. *Prog. Retinal Eye Res.* **2004**, *23* (3), 307–380.
(13) Yanamala, N.; Tirupula, K.; Balem, F.; Klein-Seetharaman, J. pH-Dependent interaction of rhodopsin with cyanidin-3-glucoside. 1. Structural aspects. *Photochem. Photobiol.* **2009**, *85* (2), 454–462.
(14) Yamamoto, F.; Borgula, G.; Steinberg, R. Effects of light and darkness on pH outside rod photoreceptors in the cat retina. *Exp. Eye Res.* **1992**, *54* (5), 685–697.
(15) Dmitriev, A.; Mangel, S. Circadian clock regulation of pH in the rabbit retina. *J. Neurosci.* **2001**, *21* (8), 2897–2902.
(16) Hanneken, A.; Harris, R.; Olson, A.; Crouch, R.; Cornwall, C.; Kono, M. Modulation of opsin signalling by flavonoids. *Invest. Ophthalmol. Vis. Sci.* **2009**, *50* (E-Abstract 2722).
(17) Suzuki, M.; Kamei, M.; Itabe, H.; Yoneda, K.; Bando, H.; Kume, N.; Tano, Y. Oxidized phospholipids in the macula increase with age and in eyes with age-related macular degeneration. *Mol. Vis.* **2007**, *13* (80−85), 772–778.
(18) Lu, L.; Hackett, S.; Mincey, A.; Lai, H.; Campochiaro, P. Effects of different types of oxidative stress in RPE cells. *J. Cell. Physiol.* **2006**, *206* (1), 119–125.
(19) Carpentier, S.; Knaus, M.; Suh, M. Associations between lutein, zeaxanthin, and age-related macular degeneration: an overview. *Crit. Rev. Food Sci. Nutr.* **2009**, *49* (4), 313–326.
(20) Loane, E.; Kelliher, C.; Beatty, S.; Nolan, J. The rationale and evidence base for a protective role of macular pigment in age-related maculopathy. *Br. J. Ophthal.* **2008**, *92* (9), 1163–1168.
(21) AREDS. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, β carotene, and zinc for age-related macular degeneration and vision loss − AREDS report 8. *Arch. Ophthalmol.* **2001**, *119* (10), 1417−1436.
(22) Milbury, P.; Graf, B.; Curran-Celentano, J.; Blumberg, J. Bilberry (*Vaccinium myrtillus*) anthocyanins modulate heme oxygenase-1 and glutathione S-transferase-π expression in ARPE-19 cells. *Invest. Ophthalmol. Vis. Sci.* **2007**, *48* (5), 2343–2349.
(23) Hanneken, A.; Lin, F.; Johnson, J.; Maher, P. Flavonoids protect human retinal pigment epithelial cells from oxidative-stress-induced death. *Invest. Ophthamol. Vis. Sci.* **2006**, *47* (7), 3164–3177.

(24) Johnson, J.; Maher, P.; Hanneken, A. The flavonoid, eriodictyol, induces long-term protection in ARPE-19 cells through its effects on Nrf2 activation and phase 2 gene expression. *Invest. Ophthalmol. Vis. Sci.* **2009**, *50* (5), 2398–2406.
(25) Maher, P.; Hanneken, A. Flavonoids protect retinal ganglion cells from oxidative stress-induced death. *Invest. Ophthalmol. Vis. Sci.* **2005**, *46* (12), 4796–4803.
(26) Maher, P.; Hanneken, A. Flavonoids protect retinal ganglion cells from ischemia in vitro. *Exp. Eye Res.* **2008**, *86* (2), 366–374.
(27) Roberts, C.; Sindhu, K. Oxidative stress and metabolic syndrome. *Life Sci.* **2009**, *84* (21−22), 705–712.
(28) Izzotti, A.; Bagnis, A.; Sacca, S. The role of oxidative stress in glaucoma. *Mutat. Res. − Rev. Mutat.* **2006**, *612* (2), 105–114.
(29) Dilsiz, N.; Sahaboglu, A.; Yildiz, M.; Reichenbach, A. Protective effects of various antioxidants during ischemia−reperfusion in the rat retina. *Graef. Arch. Clin. Exp.* **2006**, *244* (5), 627–633.
(30) Ishige, K.; Schubert, D.; Sagara, Y. Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. *Free Radical Biol. Med.* **2001**, *30* (4), 433–446.
(31) Larrosa, M.; Lodovici, M.; Morbidelli, L.; Dolara, P. Hydrocaffeic and p-coumaric acids, natural phenolic compounds, inhibit UV-B damage in WKD human conjunctival cells *in vitro* and rabbit eye *in vivo*. *Free Radical Res.* **2008**, *42* (10), 903–910.
(32) Crespy, V.; Morand, C.; Besson, C.; Cotelle, N.; Vezin, H.; Demigne, C.; Remesy, C. The splanchnic metabolism of flavonoids highly differed according to the nature of the compound. *Am. J. Physiol.: Gastrointest. Liver Physiol.* **2003**, *284* (6), G980–G988.
(33) Paulter, E.; Maga, J.; Tengerdy, C. A pharmacologically potent natural product in the bovine retina. *Exp. Eye Res.* **1986**, *42*, 285–288.
(34) Kalt, W.; Blumberg, J.; McDonald, J.; Vinqvist-Tymchuk, M.; Fillmore, S.; Graf, B.; O'Leary, J.; Milbury, P. Identification of anthocyanins in the liver, eye, and brain of blueberry-fed pigs. *J. Agric. Food Chem.* **2008**, *56*, 705–712.
(35) Matsumoto, H.; Nakamura, Y.; Iida, H.; Ito, K.; Ohguro, H. Comparative assessment of distribution of blackcurrant anthocyanins in rabbit and rat ocular tissues. *Exp. Eye Res.* **2006**, *83* (2), 348–356.
(36) Solovchenko, A.; Merzlyak, M. Screening of visible and UV radiation as a photoprotective mechanism in plants. *Russ. J. Plant Phys.* **2008**, *55* (6), 719–737.
(37) Ohgami, K.; Ilieva, I.; Shiratori, K.; Koyama, Y.; Jin, X.; Yoshida, K.; Kase, S.; Kitaichi, N.; Suzuki, Y.; Tanaka, T.; Ohno, S. Anti-inflammatory effects of aronia extract on rat endotoxin-induced uveitis. *Invest. Ophthalmol. Vis. Sci.* **2005**, *46* (1), 275–281.
(38) Kramer, J. Anthocyanosides of *Vaccinium myrtillus* (bilberry) for night vision − a systematic review of placebo-controlled trials. *Surv. Ophthalmol.* **2004**, *49* (6), 618; author reply 618.
(39) Jayle, G.; Aubert, L. Action of anthocyanin glycosides on the scotopic and mesopic vision of the normal subject. *Therapie* **1964**, *19*, 171–185.
(40) Francis, F. Food colorants − anthocyanins. *Crit. Rev. Food Sci. Nutr.* **1989**, *28* (4), 273–314.

Received for review August 28, 2009. Revised manuscript received December 23, 2009. Accepted January 14, 2010.