

## Retinal Effects of Resveratrol

Ross A Jarratt, BSc,<sup>1</sup> Hannah Bartlett, PhD, FAAO<sup>2</sup> and Frank Eperjesi, PhD, MBA, FAAO<sup>3</sup>

1. Research Fellow; 2. Senior Lecturer in Optometry; 3. Associate Professor in Optometry, Ophthalmic Research Group, School of Life & Health Sciences, Aston University, Birmingham, UK

### Abstract

Resveratrol is a plant polyphenol that has potent anti-inflammatory and anti-oxidant properties. Age-related macular degeneration is a degenerative condition characterized by elevated levels of oxidation triggered cell damage and a subsequent inflammatory cascade. Resveratrol prevents activation of inflammatory pathways and is also a potent scavenger of reactive oxygen species and free radicals. Experiments using the mouse model have demonstrated that resveratrol reduces angiogenesis. The evidence suggests that resveratrol would be a useful inclusion in ocular nutritional supplements.

### Keywords

Angiogenesis, inflammation, maculopathy, oxidation, resveratrol, retina

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**Correspondence:** Frank Eperjesi, PhD, MBA, FAAO, Ophthalmic Research Group, School of Life & Health Sciences, Aston University, Birmingham, B4 7ET, UK. E: f.eperjesi@aston.ac.uk

Resveratrol is a polyphenol that is found in plants. It is synthesized in response to pathogenic stresses and fungal disease and forms part of a defence mechanism against infection.<sup>1</sup> Red grapes, mulberries, and peanuts are particularly rich sources.<sup>2</sup> A high concentration is found in red wine.

Interest in therapeutic effects of resveratrol began with the French paradox describing a lower incidence of cardiovascular events among French populations despite a high intake of dietary fat.<sup>3</sup> Clinical studies have shown that resveratrol is a potent cardioprotective,<sup>4</sup> chemotherapeutic,<sup>5</sup> and neuroprotective<sup>6</sup> agent and displays anti-aging properties.<sup>7</sup> These effects are most likely attributable to potent anti-inflammatory and anti-oxidant properties.

Age-related macular degeneration (AMD) is the leading cause of visual loss in those over the age of 50 years in the developed world.<sup>8–11</sup> In the US, the number of people affected by this condition is expected to increase from 1.75 million to almost three million by 2020.<sup>11</sup> The condition is characterized in part by an uncontrolled level of oxidation, which damages cell membranes and triggers inflammation. Resveratrol may have the potential to impact on the risk for onset or progression of this condition. Developed world diets contain a very low concentration of resveratrol<sup>12</sup> and increasingly it is being included in nutritional supplementation formulations.

This review explores the scientific evidence documenting the properties of resveratrol relevant to AMD and its potential use in ocular nutritional supplements. The evidence presented here is from published literature located through systematic searches of PubMed and Web of Knowledge.

### Methods

Literature searches of PubMed and Web of Knowledge were undertaken. All searches performed were topic searches and Boolean operators

were used to ensure that the results remain focused. An initial search performed on Web of Knowledge included the search criteria of resveratrol or polyphenol or phytoalexin or stilbenoid and eye or retina or ocular or vision or sight. Other searches used the criteria resveratrol and anti-inflammatory, resveratrol and antioxidant, resveratrol and retina, resveratrol and age-related macul\* and were performed on Web of Knowledge and PubMed. Both databases were set to automatically filter out any search results dated prior to 1950.

The relevancy of the remaining research results was checked by evaluation of the title against the proposed line of enquiry addressed in this review, namely the retinal effects of the resveratrol. If the title proved to be ambiguous, a decision was made by the authors based on the abstract as to whether the result was relevant. By performing the research in this way, it was hoped that the results identified would be specific to the eye. However, many experiments had been performed *in vitro* using cultured retinal or trabecular cells. These studies were included.

To meet inclusion criteria, experimental work had to have a sound method with clear aims, stringent protocols, and a sufficiently substantial analysis together with details of numbers of subjects and appropriate statistical analysis if conducted. Significant variation was evident among the protocol deployed and the analytical techniques used even in literature pertaining to a similar topic.

### Bioavailability

In common with other polyphenols, resveratrol has an extremely limited bioavailability. Although absorption is rapid<sup>13</sup> and the extent of absorption is at least 70 % in humans,<sup>14</sup> studies performed on rats<sup>15</sup> have shown that resveratrol undergoes rapid metabolism in the gut prior

to entering the systemic circulation. The presence of food significantly delays the absorption of resveratrol but does not alter the extent of it.<sup>16</sup> The reduction in the concentration of resveratrol entering the systemic circulation is compounded further by recirculation between the small intestine and the liver. Metabolism occurs so rapidly that a conjugate of resveratrol was detected by high-performance liquid chromatography less than 10 minutes following intravenous infusion to human subjects. Plasma was already devoid of resveratrol in two subjects at just 30 minutes following the onset of infusion.<sup>17</sup> The plasma concentration reached, although high (approximately 25 ng/ml), was far below the amount shown to elicit an effect *in vitro* (>1,000 ng/ml).<sup>7</sup> However, resveratrol is a lipophilic compound that can be bound to the cellular fraction in blood. It is possible that its level in blood and in tissues is underestimated.<sup>18</sup> Urinary excretion appears to be the main mode of disposal in humans.<sup>17</sup>

## Safety

Studies conducted on humans to date have shown that resveratrol is safe and tolerated well. The only reported adverse effect in six human subjects who received 200 mg of resveratrol twice daily was incipient diarrhea but no clinically significant changes occurred in hematology or biochemistry.<sup>19</sup> In 40 human subjects receiving between 25 mg to 150 mg of resveratrol six times daily,<sup>20</sup> five subjects reported headaches. Other reported adverse effects included drowsiness, dizziness, urinary tract infections, epididymitis, dyspepsia, and nasopharyngitis, but these were all reported only in individual subjects. Isolated incidences of nasopharyngitis have been reported in two studies.<sup>16,20</sup> Overall, adverse effects have been minor and resveratrol appears to have a good safety profile.

## Inflammation and Age-related Macular Degeneration

There is an overwhelming body of evidence implicating inflammation in the pathogenesis of AMD. A review<sup>21</sup> described an altered balance of autoimmunity, chronic inflammation, and dysregulated parainflammation as triggers. The Blue Mountains Eye Study<sup>22</sup> found that the white blood cell count correlated positively with pigmentary abnormalities, drusen, and the incidence of early AMD. Another investigation<sup>23</sup> revealed that the retinas of rats with experimentally induced choroidal neovascularization (CNV) contained a greater amount of lymphocyte-producing cells around the retinal vasculature compared with retinas without CNV. It is plausible that increased vascular permeability during AMD permits the influx of macrophages to the retina.

There is unequivocal evidence for involvement of the complement system. Drusen have been found to contain components of the complement cascade and other immunoreactive material.<sup>24</sup> Hard and small drusen from eyes of postmortem AMD patients were found to contain complements known to promote vascular endothelial growth factor (VEGF) secretion in human retinal pigment epithelial (RPE) cells.<sup>25</sup> VEGF is pro-angiogenic. A dysregulated complement system that results in angiogenesis could be a contributor to the macrophage influx. Activated complement has also been found at the outer edge of human basal laminar deposits and VEGF has been detected at the basal surface of the RPE.<sup>26</sup> Further evidence for involvement of the complement cascade comes from studies that have shown that polymorphisms in complement factor H, an anti-inflammatory substance, predispose to AMD.<sup>27,28</sup>

## Anti-inflammatory Properties of Resveratrol

There is good evidence substantiating the use of resveratrol as an anti-inflammatory agent. Of particular relevance is the effect resveratrol has on the adhesion of inflammatory molecules to the retinal vasculature. Administration of resveratrol to male mice with induced uveitis reduced the total number of leukocytes adhering to the retinal vasculature.<sup>29</sup> The same researchers found that resveratrol reduced the expression of two chemotactic adhesion molecules: in the retina and the RPE/choroid again in the mouse model. Under conditions of hyperglycemia vascular permeability increases as does the secretion of VEGF. This has been shown to be inhibited by resveratrol (1.25  $\mu$ M).<sup>30</sup> Resveratrol may be useful in the treatment of hyperglycemia-induced inflammation.

Resveratrol is cytotoxic to macrophages and impairs their ability to proliferate<sup>31</sup> and reduces the expression of inflammatory mediators.<sup>32</sup> The benzene rings appear to play an essential role in the anti-inflammatory properties of resveratrol.<sup>33</sup> Resveratrol has been shown to reduce induced edema by 38.9 % in the mouse model an effect similar to that of ibuprofen (also contains a single benzene ring), which reduced edema by 42.2 %.

## Anti-oxidant Effects of Resveratrol

The retina is exposed to a significant degree of oxidative stress due to the presence of susceptible membranous fatty acids (e.g. docosahexenoic acid [DHA]), the influx of light acts as a catalyst for oxidative reactions and a naturally high oxygen tension.<sup>34</sup> Evidence suggests that oxidative damage to cells reflects an impaired ability of the cells to ameliorate oxidative stress.

Yildirim et al. found higher serum levels of markers for lipid and protein oxidation in subjects with AMD and CNV and compared with unaffected controls.<sup>35</sup> Participants with AMD had a significantly lower level of the anti-oxidant superoxide dismutase. Most of the oxidative damage takes place in the cell membrane and the damaged membrane triggers an inflammatory cascade. Products of the oxidation of DHA were shown to cause lesions in the RPE, activate the complement pathway, and cause a swelling of Bruch's membrane.<sup>36</sup> The likely trigger appears to be disordered phospholipid.<sup>37</sup>

These findings demonstrate the importance of reducing the amount of oxidation that occurs in the retina of AMD patients. Suppression of the complement cascade through the selective targeting of oxidation and inflammation would therefore seem to be important in any therapy for AMD.

Resveratrol has displayed potent antioxidant properties, although many of the investigations have been conducted *in vitro*. However, the limited results from *in vivo* experiments have demonstrated that resveratrol is a potent scavenger of free radical species. Resveratrol is also very effective at scavenging superoxide<sup>38</sup> and hydroxyl radicals.<sup>2</sup>

Resveratrol protects against hydrogen peroxide toxicity and apoptosis of human lens epithelial cells in a dose-dependent manner<sup>39</sup> and was reported to reduce reactive oxygen species (ROS) levels in porcine trabecular meshwork.<sup>40</sup>

## Angiogenesis in Age-related Macular Degeneration

CNV appears to represent changes in Bruch's membrane including thinning in some places, permitting breach by new blood vessels. It is

particularly common after photodynamic therapy,<sup>41</sup> an indication that CNV may be induced by an overexposure to light. The occurrence of CNV denotes the influence of pro-angiogenic mediators dominating anti-angiogenic mediators. In support of this, macular levels of endogenous inhibitors of angiogenesis, such as endostatin, thrombospondin-1, and pigment epithelium-derived factor, are significantly reduced in AMD patients compared with controls.<sup>42</sup> During this pathogenic state, normally observed during the late form of AMD, new blood vessels radiate outward from the choroid, breaching Bruch's membrane, and proliferating into the RPE or subretinal space.<sup>43</sup> Angiogenesis is mediated through the alternative inflammation pathway. Factor H inhibits the alternative inflammatory pathway<sup>44</sup> and a recombinant form of factor H (CR2-fH) reduces the severity of laser-induced CNV in mice.<sup>45</sup> The anti-inflammatory and anti-oxidative properties of resveratrol may help to reduce the incidence of CNV.

## Anti-proliferative/Anti-angiogenic Effects of Resveratrol

Resveratrol has demonstrated particularly good anti-proliferative properties. King et al. found that resveratrol reduced the proliferation of RPE cells in a dose-dependent manner.<sup>46</sup> Cao et al. found that resveratrol reduced the migration of human umbilical vein endothelial cells<sup>47</sup> while Hua et al. revealed that resveratrol suppressed the migration of human renal microvascular endothelial cells.<sup>48</sup>

As resveratrol is able to annul cell migration and the proliferation of endothelial cells in particular, it is unsurprising that experimental work has shown that resveratrol reduces caspase angiogenesis. Resveratrol reduced the number of subretinal neovascular lesions in mice and angiogenesis in mice with laser-induced CNV.<sup>49</sup>

Nitric oxide (NOS) is a pro-angiogenic growth factor.<sup>50</sup> Resveratrol has been shown to inhibit production of NOS in mouse macrophages<sup>51</sup> and the secretion of VEGF that occurs in response to the presence of oxysterols. These are products of cholesterol oxidation liberated during degradation of membranes of cells in the RPE. Rats bred in hypoxic conditions have neovascularization, fibrovascular growth, and extravasation of blood<sup>52</sup> partly due to the formation of NOS. This was reduced by resveratrol.

## Conclusion

Resveratrol has poor bioavailability, a good safety profile, and is thought to act on inflammatory, oxidative, and pro-angiogenic mediators that are involved in the pathogenesis of AMD. Resveratrol has the potential to play a role in the treatment of a range of retinal conditions and in particular AMD. Further research using the human model and double-masked placebo-controlled experimental protocols would be of value in order to determine its efficacy and full potential as a component of ocular nutritional supplements. ■

- Tosun I, Inkaya AN, Resveratrol as a health and disease benefit agent, *Food Rev Intern*, 2010;26:85–101.
- Leonard SS, Xia C, Jiang BH, et al., Resveratrol scavenges reactive oxygen species and effects radical-induced cellular responses, *Biochem Biophys Res Commun*, 2003;309:1017–26.
- Lippi G, Franchini M, Favalaro EJ, et al., Moderate red wine consumption and cardiovascular disease risk: Beyond the "French paradox", *Semin Thromb Hemost*, 2010;36:59–70.
- Wu JM, Hsieh TC, Resveratrol: A cardioprotective substance, *Ann N Y Acad Sci*, 2011;1215:16–21.
- Gusman J, Malonne H, Atassi G, A reappraisal of the potential chemopreventive and chemotherapeutic properties of resveratrol, *Carcinogenesis*, 2001;22:1111–17.
- Richard T, Pawlus AD, Iglesias ML, et al., Neuroprotective properties of resveratrol and derivatives, *Ann N Y Acad Sci*, 2011;1215:103–8.
- Alarcon de la Lastra C, Villegas I, Resveratrol as an anti-inflammatory and anti-aging agent: Mechanisms and clinical implications, *Mol Nutr Food Res*, 2005;49:405–30.
- Attebo K, Mitchell P, Smith W, Visual acuity and the causes of visual loss in Australia. The Blue Mountains eye study, *Ophthalmol*, 1996;103:357–64.
- Mitchell P, Smith W, Attebo K, et al., Prevalence of age-related maculopathy in Australia – the Blue Mountains eye study, *Ophthalmol*, 1995;102:1450–60.
- Klein R, Wang Q, Klein B, The relationship of age-related maculopathy, cataract, and glaucoma to visual acuity, *Invest Ophthalmol Vis Sci*, 1995;36:182–91.
- Friedman D, O'Colmain B, Tomany S, et al., The Eye Diseases Prevalence Research Group, Prevalence of age-related macular degeneration in the United States, *Arch Ophthalmol*, 2004;122:564–72.
- Espin JC, Garcia-Conesa MT, Tomas-Barberan FA, Nutraceuticals: Facts and fiction, *Phytochem*, 2007;68:2986–3008.
- Azorin-Ortuno M, Yanez-Gascon MJ, Vallejo F, et al., Metabolites and tissue distribution of resveratrol in the pig, *Mol Nutr Food Res*, 2011;55:1154–68.
- Walle T, Bioavailability of resveratrol, Resveratrol and health, *Ann N Y Acad Sci*, 2011;1215:9–15.
- Kuhlne G, Spencer J, Chowrimootoo G, et al., Resveratrol is absorbed in the small intestine as resveratrol glucuronide, *Biochem Biophys Res Commun*, 2000;272:212–17.
- Vaz-Da-Silva M, Loureiro AI, Falcao A, et al., Effect of food on the pharmacokinetic profile of trans-resveratrol, *Int J Clin Pharmacol Ther*, 2008;46:564–70.
- Walle T, Hsieh F, DeLegge MH, et al., High absorption but very low bioavailability of oral resveratrol in humans, *Drug Metab Dispos*, 2004;32:1377–82.
- Soleas G, Yan J, Goldberg D, Measurement of trans-resveratrol, (1)-catechin, and quercetin in rat and human blood and urine by gas chromatography with mass selective detection, *Methods Enzymol*, 2001;335:130–45.
- La Porte C, Voduc N, Zhang GJ, et al., Steady-state pharmacokinetics and tolerability of trans-resveratrol 2000 mg twice daily with food, quercetin and alcohol (ethanol) in healthy human subjects, *Clin Pharmacokinet*, 2010;49:449–54.
- Almeida L, Vaz-da-Silva M, Falcao A, et al., Pharmacokinetic and safety profile of trans-resveratrol in a rising multiple-dose study in healthy volunteers, *Mol Nutr Food Res*, 2009;53:57–S15.
- Buschini E, Piras A, Nuzzi R, et al., Age related macular degeneration and drusen: Neuroinflammation in the retina, *Prog Neurobiol*, 2011;95:14–25.
- Shankar A, Mitchell P, Rochtchina E, et al., Association between circulating white blood cell count and long-term incidence of age-related macular degeneration: The Blue Mountains eye study, *Am J Epidemiol*, 2007;165:375–82.
- Caicedo A, Espinosa-Heidmann DG, Pina Y, et al., Blood-derived macrophages infiltrate the retina and activate muller glial cells under experimental choroidal neovascularization, *Exp Eye Res*, 2005;81:38–47.
- Johnson LV, Leitner WP, Staples MK, et al., Complement activation and inflammatory processes in drusen formation and age related macular degeneration, *Exp Eye Res*, 2001;73:887–96.
- Nozaki M, Raisler BJ, Sakurai E, et al., Drusen complement components c3a and c5a promote choroidal neovascularization, *Proc Natl Acad Sci U S A*, 2006;103:2328–33.
- Lommatzsch A, Hermans P, Muller KD, et al., Are low inflammatory reactions involved in exudative age-related macular degeneration? Morphological and immunohistochemical analysis of amd associated with basal deposits, *Graefes Arch Clin Exp Ophthalmol*, 2008;246:803–10.
- Despriet DD, Klaver CC, Wittman JC, et al., Complement factor h polymorphism, complement activators, and risk of age-related macular degeneration, *JAMA*, 2006;296:301–9.
- Narayanan R, Butani V, Boyer DS, et al., Complement factor h polymorphism in age-related macular degeneration, *Am Acad Ophthalmol*, 2006;114:1327–31.
- Kubota S, Kurihara T, Mochimaru H, et al., Prevention of ocular inflammation in endotoxin-induced uveitis with resveratrol by inhibiting oxidative damage and nuclear factor-kappa b activation, *Investigative Ophthalmol Vis Sci*, 2009;50:3512–19.
- Losso JN, Truax RE, Richard G, Trans-resveratrol inhibits hyperglycemia-induced inflammation and connexin downregulation in retinal pigment epithelial cells, *J Agri Food Chem*, 2010;58:8246–52.
- Bilback B, Radkar V, Adiabouh C, *In vitro* evaluation of the cytotoxic and anti-proliferative properties of resveratrol and several of its analogs, *Cell Mol Biol Lett*, 2008;13:553–69.
- Kim MH, Yoo DS, Lee SY, et al., The trif/btk1/rif-3 activation pathway is the primary inhibitory target of resveratrol, contributing to its broad-spectrum anti-inflammatory effects, *Pharmazie*, 2011;66:293–300.
- Chen G, Shan W, Wu YL, et al., Synthesis and anti-inflammatory activity of resveratrol analogs, *Chem Pharm Bull*, 2005;53:1587–90.
- Beatty S, Koh HH, Henson D, et al., The role of oxidative stress in the pathogenesis of age-related macular degeneration, *Surv Ophthalmol*, 2000;45:115–34.
- Yildirim Z, Ucgun NI, Yildirim F, The role of oxidative stress and antioxidants in the pathogenesis of age-related macular degeneration, *Clinics (Sao Paulo)*, 2011;66:743–6.
- Hollyfield JG, Perez VL, Salomon RG, A hapten generated from an oxidation fragment of docosahexaenoic acid is sufficient to initiate age-related macular degeneration, *Mol Neurobiol*, 2010;41:290–98.
- Chen R, Yang L, McIntyre TM, Cytotoxic phospholipid oxidation products. Cell death from mitochondrial damage and the intrinsic caspase cascade, *J Biol Chem*, 2007;282:24842–50.
- Gulcini L, Antioxidant properties of resveratrol: A structure-activity insight, *Innov Food Sci Emerging Technol*, 2010;11:210–18.
- Zheng Y, Liu YH, Ge JY, et al., Resveratrol protects human lens epithelial cells against h(2)o(2)- induced oxidative stress by increasing catalase, sod-1, and ho-1 expression, *Mol Vis*, 2010;16:1467–74.
- Luna C, Li GR, Liton PB, et al., Resveratrol prevents the expression of glaucoma markers induced by chronic oxidative stress in trabecular meshwork cells, *Food Chem Toxicol*, 2009;47:198–204.
- Potter MJ, Szabo SM, Recurrence of choroidal neovascularisation after photodynamic therapy in patients with age-related macular degeneration, *Br J Ophthalmol*, 2007;91:753–6.
- Bhutto IA, Uno K, Merges C, et al., Reduction of endogenous angiogenesis inhibitors in bruch's membrane of the submacular region in eyes with age-related macular degeneration, *Arch Ophthalmol*, 2008;126:670–78.
- Manneros AG, She H, Zambarakji H, et al., Endogenous endostatin inhibits choroidal neovascularization, *FASEB J*, 2007;21:3809–18.
- Rohrer B, Long Q, Coughlin B, et al., A targeted inhibitor of the alternative complement pathway reduces angiogenesis in a mouse model of age-related macular degeneration, *Invest Ophthalmol Vis Sci*, 2009;50:3056–64.
- Rohrer B, Long Q, Coughlin B, et al., A targeted inhibitor of the complement alternative pathway reduces RPE injury and angiogenesis in models of age-related macular degeneration, *Adv Exp Med Biol*, 2010;703:137–49.
- King RE, Kent KD, Bomser JA, Resveratrol reduces oxidation and proliferation of human retinal pigment epithelial cells via extracellular signal-regulated kinase inhibition, *Chemico-Biol Interact*, 2005;151:143–9.
- Cao L, Liu H, Lam DS-C, et al., *In vitro* screening for angiostatic potential of herbal chemicals, *Invest Ophthalmol Vis Sci*, 2010;51:6658–64.
- Hua J, Guerin KI, Chen J, et al., Resveratrol inhibits pathologic retinal neovascularization in vldlr(-/-) mice, *Invest Ophthalmol Vis Sci*, 2011;52:2809–16.
- Khan AA, Dace DS, Ryzanov AG, et al., Resveratrol regulates pathologic angiogenesis by a eukaryotic elongation factor-2 kinase-regulated pathway, *Am J Pathol*, 2010;177:481–92.
- Zhang R, Wang L, Zhang L, et al., Nitric oxide enhances angiogenesis via the synthesis of vascular endothelial growth factor and cgmmp after stroke in the rat, *Circ Res*, 2003;92:308–13.
- Chung EY, Kim BH, Hong J-T, et al., Resveratrol down-regulates interferon-gamma-inducible inflammatory genes in macrophages: Molecular mechanism via decreased stat-1 activation, *J Nutr Biochem*, 2011;22:902–9.
- Kim WT, Suh ES, Retinal protective effects of resveratrol via modulation of nitric oxide synthase on oxygen-induced retinopathy, *Korean J Ophthalmol*, 2010;24:108–18.