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## REVIEW

# Oxidation, antioxidants and cataract formation: a literature review

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### Abstract

*Purpose* This review aims to provide a literature survey of the association between photo-oxidation of lens proteins and lipid peroxidation with the genesis of age-related cataract in laboratory studies using rodent models, in epidemiological and interventional studies in humans.

*Materials and methods* A Medline search using initial search terms lens, oxidation, antioxidant, and diet was employed to search for research papers covering the areas noted above from 1995 to 2005. Literature cited in those papers was also reviewed to provide as comprehensive a coverage of research work as possible.

*Results* Lens protein photo-oxidation and lipid peroxidation are widely acknowledged as important steps in age-related cataractogenesis. Dietary antioxidants are central in retarding cataractogenesis, although most evidence for this is gained from laboratory-based work on relatively unphysiologic rodent cataract models, using antioxidant regimes that could not be sustained in clinical practice. Most research in humans is retrospective epidemiology although some interventional research has been undertaken, with mixed results.

*Conclusions* Dietary antioxidants are likely to be important in retarding cataractogenesis in older animals and in humans. Work on companion animals could provide a valuable stepping stone between rodent-based laboratory work and human interventional studies.

**Key Words:** antioxidant, ascorbate, cataract, glutathione, lens, oxidation, tocopherol

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## INTRODUCTION

It is widely accepted that oxidative stress is a significant factor in the genesis of cataract both in experimental animal models<sup>1,2</sup> and in cultured lens systems.<sup>3–5</sup> The concentration of proteins damaged by oxidative processes rises with age in the human lens and is significantly higher in cataractous compared with normal transparent lenses.<sup>6</sup> Light-induced protein oxidation can be prevented by antioxidants<sup>7,8</sup> as can photoperoxidation of lens lipids.<sup>9,10</sup>

For many years and in several different human populations the association between dietary intake and cataract formation has been investigated.<sup>11–13</sup> The role of oxidative stress in cataract development, and thus the importance of antioxidants in prevention of cataract has, for some time, been accepted in human ophthalmology.<sup>14</sup> Here we will first investigate more general questions regarding the mechanisms of cataractogenesis, the role of oxidation in lens opacification, and of dietary antioxidants in preventing this. We will assess the contribution of laboratory-based studies to answering

these questions and then review the results of epidemiological and interventional studies for a number of dietary antioxidants both in isolation and when used in combination. Both prospective and retrospective studies will be reviewed, specifically interventional studies with active vitamin supplementation, and epidemiological research, which correlates tissue and plasma antioxidant levels with cataract prevalence and investigates cataract incidence and prevalence in populations using dietary supplements.

### *Mechanisms of age-related cataractogenesis*

Several factors are postulated to be of importance in the generation of lens opacities in the older individual. These have been helpfully summarized by Taylor<sup>15</sup> as the five 'D's: daylight, diet, diabetes, dehydration and don't know. The latter catch-all category probably predominantly involves genetic influences in nuclear and cortical opacification, if not in posterior subcapsular cataract, in humans.<sup>16,17</sup> But the final common pathway by which these different factors exert their influence is predominantly through oxidation of lens

proteins<sup>18,19</sup> and peroxidation of lipids.<sup>20</sup> In addition, the deleterious effects of glucose metabolism in the lens and associated changes in lens epithelial cell redox potential should not be overlooked, given their exacerbating effect on these oxidative changes.<sup>21</sup> Age-related cataract is thus not the result of one metabolic reaction in the lens but rather a final common pathway of many cataractogenic effects. We should not be looking for a scientific stand-off such as that between those considering light as the key factor, others considering dietary antioxidants as the critical factor and still others focusing on dehydration, as has sometimes seemed to be the case.<sup>22</sup> A complete assessment of factors in age-related cataractogenesis sees photo-oxidation as the key event and thus considers light on the one hand and antioxidants on the other as being central to the problem and its solution with dehydration as a critical third factor.

The lens is designed to focus light onto the retina throughout the individual's life, but a necessary consequence of this is photo-oxidation of lens structures. The lens might appear a relatively inert structure, but has ATP levels as high as those found in muscle, a much more active tissue.<sup>23</sup> Oxidative metabolism is clearly important in maintaining the lens in a transparent state. However, this means that, as well as a continuous bathing in light, the lens is also 'bathed' in oxygen. Even at the time when oxygen was discovered, Joseph Priestley noted that oxygen can become toxic in animals maintained in 100% oxygen.<sup>24</sup> The high reactivity of oxygen was explained at a molecular level by Linus Pauling: oxygen is the most electro-negative element after fluorine and is exceptional in having the two electrons in its  $p^2$  antibonding orbitals in a parallel spin orientation. Pauli's exclusion principle means that, in the reduction of oxygen to water, this reaction must take place through the intermediate of the superoxide radical  $O_2^-$ . So the relatively benign dioxygen molecule  $O_2$  is converted to a highly reactive free radical. Oxidative stress associated with increased reactive oxygen species is known to accelerate cataract formation in laboratory rodent models.<sup>25</sup> Superoxide is converted in most tissues of the body, including the lens, to hydrogen peroxide by superoxide dismutases but even hydrogen peroxide can become highly toxic because it produces the hydroxyl radical OH. This toxicity is prevented by catalase and glutathione peroxidase. These enzymes protect the lens by a system of antioxidant molecules, the lynch-pin of which is glutathione, the role of which is expertly reviewed by Marjorie Lou.<sup>26</sup> The oxidation-reduction cycle of this tripeptide links dietary antioxidants such as ascorbate, riboflavin, carotenoids and tocopherols with the prevention of photo-oxidation.

One key link between photo-oxidation and cataract is that photo-oxidation of thiol groups on lens crystallins produces disulfide bridges between molecules<sup>27</sup> and, given time, the build-up of these will lead to protein aggregation and hence cataract. As Harding notes,<sup>11</sup> these aggregative changes are not confined to the lens – they occur in other age-related tissue degenerations such as central nervous tissue in Alzheimer's disease, but are perhaps particularly evident in a

tissue through which light is continually passing. As Truscott has amply shown, the lens contains UV filters that reduce the effects of the electromagnetic spectrum on lens proteins, but with age these themselves are deleteriously reduced.<sup>28,29</sup> Lipid peroxidation is another key event in cataractogenesis according to some authorities, although the relative importance of protein oxidation and lipid peroxidation is unclear. Again this is unlikely to be an either/or situation but one in which both events may lead to lens opacification. In both cases antioxidants are a key prophylactic agent in preventing oxidation-related cataractogenesis and the dietary route is vital in providing these molecules.

#### *The anticataractogenic effects of dietary antioxidants: laboratory rodent studies*

As we will see later, epidemiological studies on human populations can give powerful indications regarding the influence of dietary antioxidants on cataract formation, but are often flawed because of confounding factors and may yield conflicting results. Studies on laboratory rodents have the advantages of a much shorter timescale, more readily achievable control of dietary intake and fewer practical and ethical dilemmas in their outworking. Yet the problem is that, apart from a very few studies on naturally occurring age-related cataract in rodents, cataracts studied in laboratory rodents are either inherited cataract, or opacities induced by interventions such as selenite or prednisolone treatment or galactose inclusion in the diet, or cataracts occurring in natural and induced diabetes in animals. Studies of lens opacity induced by ultraviolet light also show the importance of exogenous antioxidants in preventing or at least reducing the development of such lenticular pathology, as reviewed by Colitz *et al.*<sup>30</sup> Such models may have little in common with naturally occurring age-related cataract, but at least provide models for the study of effects of antioxidants on cataract prevention. What antioxidants have been studied with regard to their preventative effects on cataract formation?

As a key player in the protection of the lens from oxidation,<sup>31–33</sup> vitamin C or ascorbic acid is considered first. It has been determined that ascorbate plays an important part in lens biology, both as an antioxidant and as a UV filter when present in aqueous. The lenses of diurnal animals contain high levels of ascorbate, as has been recognized for over 40 years.<sup>34</sup> Much research on the association between dietary intake and lens levels of ascorbate has been undertaken in the guinea pig, where production of the vitamin by the animal is not a confounding factor. Dietary deficiency or prolonged marginal sufficiency of the vitamin led to reduction in lens concentrations of ascorbate.<sup>35</sup> Correlation of lenticular concentrations of ascorbate with levels in the diet show that oral intake of ascorbate is crucial in giving adequate levels of the vitamin in the lens, both in guinea pigs<sup>36</sup> and in rats.<sup>37</sup> Ascorbate inhibits galactose cataract in guinea pigs<sup>38</sup> while depressed ascorbate levels were associated with acetone-induced cataract in guinea pigs.<sup>39,40</sup> Ascorbate reduces heat-induced damage to lens proteins<sup>41</sup> and delays

UV-induced damage to lens proteins,<sup>42</sup> although neither of these models can be described as particularly physiologic. In diabetic rats, ascorbate reduces crystallin leakage from lenses undergoing cataractogenesis.<sup>43</sup> The complicating factor with ascorbic acid is that its oxidation products are themselves cataractogenic: lenses subjected to millimolar concentrations of dehydroascorbate, a natural oxidation product, become opacified.<sup>44</sup> Ascorbate regeneration from ascorbate free radical by ascorbate free radical reductase and from dehydroascorbate by thiolreductase is essential to the maintenance of lens transparency, as is a high level of ascorbic acid itself.

Tocopherol has an important part to play in lenticular antioxidant status. The same group that showed the influence of ascorbate on the diabetic rat lens above demonstrated the importance of tocopherol on retardation of similar opacities in galactose-fed animals,<sup>45</sup> while other groups have shown increased cataractogenesis in vitamin E-deficient animals.<sup>46</sup> It is unclear what the relevance of oxidative stress is in steroid-induced cataract but low levels of vitamin E have also been shown to be a risk factor in the generation of these opacities in rats.<sup>47</sup>

Carotenoids may be important, although, of the large variety in the normal diet, only alpha and gamma tocopherol and the xanthophylls xanthin and zeaxanthin are found in the lens.<sup>48</sup> There has been more interest in the role of these xanthophylls in protection against age-related macular degeneration because these pigments have a critical role in macular health but, in their large survey of the protective effects of lutein and zeaxanthin,<sup>49</sup> Krinsky *et al.* do note epidemiological observations in which high dietary xanthophyll intake is associated with reduced cataract prevalence.<sup>50,51</sup> Riboflavin was noted to be important in preventing cataract formation in the days when the compound was still known as vitamin G. In 1928 Salmon reported cataracts among the many defects in animals fed deficient diets,<sup>52</sup> and within a few years the unique status of riboflavin was documented.<sup>53,54</sup>

Taurine should be considered important when discussing antioxidant levels in cats but little has been documented with regard to its role in the lens in any species. Trevithick's group working on cataractogenesis in diabetic rats noted that taurine reduced lens opacification.<sup>55</sup>

A number of studies have evaluated the anticataractogenic potential of various vegetable-derived nutrients and plant extracts. The antioxidant effects of curcumin, the active agent in the Indian spice turmeric have proved anticataractogenic in more than one experimental model system.<sup>56-58</sup> Tomato extract has been shown to have anticataractogenic properties,<sup>59</sup> probably associated with the carotenoid lycopene.<sup>60</sup> Grape-seed extract (GSE), containing a novel and powerful antioxidant class, the proanthocyanidins, has been shown to have anticataractogenic effects in the rat,<sup>61</sup> and Colitz *et al.* have shown that GSE inhibits UV-induced oxidative stress in lens epithelial cell (LEC) (Colitz, personal communication). There are clearly a number of vegetable- and fruit-derived antioxidants that are worthy of further

evaluation as anticataractogenic agents in animals and humans. How do these experimental studies relate to experiences in human and animal patients?

#### *The anticataractogenic effects of dietary antioxidants: human epidemiological evidence*

Laboratory-based studies can, as we have seen, give some relatively clear results, but studies of cataract incidence in humans using antioxidant dietary supplements have, however, had varied results depending on the design of the study. Recent studies illustrate the variation in relative risk of cataract formation depending on the group of individuals affected in the investigation and the dietary elements concerned. We will assess these studies for each dietary antioxidant.

Ascorbate is, again, an obvious first candidate given its antioxidant properties. One large study showed a relative risk (RR) of 0.23 with a confidence interval (CI) of 0.09–0.60 in women taking approximately 300 mg/day compared with those taking an average of 77 mg/day.<sup>62</sup> This study evaluated individuals taking dietary supplements for over 10 years. Marles-Perlmann<sup>6</sup> found that, while vitamin C supplementation reduced nuclear cataract (RR 0.7; CI 0.5–1.0) it seemed to increase cortical cataract (RR 1.8; CI 1.2–2.9). Robertson used visually impairing cataract as an end point and found a relative risk of 0.30 (CI 0.24–0.77) in consumers of over 300 mg/day compared with those not using any supplementation.<sup>63</sup> Vitale showed no differences between patients taking over 260 mg/day and those taking less than 115 mg/day.<sup>8</sup> Jacques showed, however, that a RR of 0.29 was found when comparing patients with plasma levels of vitamin C over 90  $\mu\text{M}$  with those with levels of less than 40  $\mu\text{M}$ .<sup>11</sup>

What of vitamin E? Again different groups yield different results. Robertson *et al.*<sup>64</sup> showed an inverse relationship between cataract formation and vitamin E use above 400 iu/day. Leske showed an RR of 0.59 in the top 20th percentile of vitamin E users compared with the average of a large group.<sup>65</sup> In comparison, Marles-Perlman found only nonsignificant associations between vitamin E supplementation and nuclear cataract (RR 1.2; CI 0.6–2.3) and a positive, although again nonsignificant association, between the vitamin and cortical cataract (RR 1.2; CI 0.6–2.3).<sup>66</sup> The VECAT study in Australia has recently reported disappointingly inconclusive findings:<sup>67</sup> in 1193 members of the study group 3.25% (39 individuals) had cataract and there was no difference in progression of lens opacity between those taking supplemental vitamin E and controls. However, in a study of 50 individuals with developing cataract the change in lens opacity was significantly less in those taking supplemental vitamin E.<sup>68</sup>

Beta-carotene has also been evaluated with similarly varied results. Hankison *et al.*<sup>69</sup> found a relative risk of 0.73 (CI 0.55–0.97) between those with intakes of over 18,700 iu/day and those with intakes below 5700 iu/day. Jacques *et al.* found the relative risk to be 0.78 (CI 0.03–1.03) when correlating cataract risk at different plasma carotene levels

(> 3.3  $\mu\text{M}$  cf < 1.7  $\mu\text{M}$ ).<sup>70</sup> In recent studies density of nuclear cataract has been inversely correlated with the daily riboflavin intake and in one major study this finding is somewhat complicated by the additional use of vitamin E.<sup>71</sup> There is little likelihood of riboflavin deficiency in western populations, however, while studies in low socioeconomic groups in Mexico<sup>72</sup> and India<sup>73</sup> demonstrate the deleterious effect of vitamin deficiency, including that of riboflavin, on lens transparency.

Given the potential synergistic relationship between different antioxidants what are the results of multivitamin supplementation? While Robertson *et al.* found no benefit from vitamin E and C supplementation,<sup>13</sup> Leske found decreased prevalence of cataracts, with variation in effect for different cataract forms.<sup>74</sup> Most workers, including Seddon's group,<sup>75</sup> Leske *et al.*<sup>13</sup> and Marles-Perlman *et al.*,<sup>76</sup> found significant reductions in both incidence and cataract progression while Hankinson<sup>16</sup> failed to do so.

The vast majority of these studies are evaluations of cataract incidence in groups of patients already on varying dietary supplements. Very few are intervention studies in which eyes are examined prospectively after specific dietary additions. In Sperduto's randomized double-blinded study of vitamin supplementation of almost 4000 individuals in China, those taking riboflavin and niacin were significantly less likely to develop nuclear cataract (RR 0.59; CI 0.45–0.79).<sup>77</sup> The REACT study in populations in the USA and the UK showed a reduction in cataract incidence in humans taking a combination of vitamins consisting of 18 mg/day beta-carotene, 750 mg/day vitamin C, and 600 mg/day vitamin E. After 3 years a positive effect regarding cataract progression was apparent in both the US and the UK groups but, while the effect was statistically significant in the US group, there was no statistically significant benefit of treatment in the UK group.<sup>78</sup> The age-related eye disease study (AREDS) showed a statistically significant benefit for macular degeneration over an average of 6 years of the study but not for cataract.<sup>79</sup> The study had a somewhat less sensitive analysis system for cataract but the reasons for the differences in the REACT and AREDS studies are more likely to be the higher antioxidant doses in the REACT study and possibly, more importantly, that this study evaluated patients at an earlier stage of lens opacification. These two large studies (REACT 297 participants, AREDS 4629 participants) show the difficulties in undertaking such trials and obtaining concrete results.

All the research noted above, both laboratory-based and epidemiological, has involved oral dosing of antioxidants. Has topical application anything to offer in prevention of cataracts? Experimental work using topical disulfiram has shown a positive effect in reducing cataractogenesis in the selenite cataract model,<sup>80</sup> while topical vitamin E delivered in liposomes has a beneficial effect in the galactosemic rat model.<sup>81</sup> The most recent work on topical antioxidant delivery has been that on topical n-acetyl carnosine, in which Babizhayev *et al.* report the amelioration of visual effects of

age-related cataracts in elderly people.<sup>82</sup> Babizhayev has used the compound in dogs and the current author has published work on its effects in canine cataracts in the current issue of *Veterinary Ophthalmology*.<sup>83</sup>

#### *The anticataractogenic effects of dietary antioxidants: evidence in companion animals*

There appear, however, to have been no similar studies on cataract formation in aging animals, despite lens opacity being a substantial cause of visual impairment, particularly in older dogs. Veterinary literature on antioxidants and the lens appears limited at present to one laboratory and two clinical studies. Over 20 years ago Gelatt *et al.* showed that cataractous lenses had decreased levels of reduced glutathione than normal lenses.<sup>84</sup> More recently, a Brazilian group defined the systemic antioxidant status of American Cocker Spaniels<sup>85</sup> and Miniature Poodles<sup>86</sup> with and without cataract. Plasma ascorbate levels were lower in cataractous dogs and plasma malondialdehyde was significantly increased. Interesting as these results are, they fail to give a full picture of the lenticular antioxidant status of dogs with and without cataract but, without a doubt, they reflect the paucity of canine data in the field.

The introduction of diets containing antioxidants for the dog population offers an excellent opportunity for a large-scale evaluation of the speed of cataract formation in dogs fed on diets with and without antioxidant supplementation. Such a study would be of value from both a purely veterinary viewpoint and also a comparative perspective, forming, as it does, a stepping stone between laboratory studies and large-scale human population studies. The rodent models described above are flawed by the relatively unphysiologic nature of many of the cataract models and the unrealistically high levels of antioxidant supplementation. On the other hand the human epidemiology studies reviewed above are complicated by the fact that, while close evaluation of cataract formation and progression is possible, strict control on human diet is impossible. This would be considerably easier in the canine population where, given adequate owner compliance, the total dietary input for the animals could come from one commercial dietary source.

#### *The anticataractogenic effects of dietary antioxidants: evidence in fish*

Last, but by no means least, there is much interest in preventing cataract formation in commercially farmed fish. Cataracts in farmed salmon, in particular, are a common and serious problem. Up to over 80% of fish were affected in one study<sup>87</sup> and the condition is widespread in salmon farms in Scotland, Ireland and Norway.<sup>88–90</sup> While there are many potential causes of cataract in farmed fish too numerous to discuss here, lens opacification related to dietary deficiencies are commonly reported and, of these, the most important are those related to levels of dietary pro- and antioxidants. A particularly significant paper is that of Waagbo *et al.* who fed Atlantic salmon in 16 different dietary groups with varying

levels of alpha tocopherol, ascorbate, astaxanthin, lipid (capelin oil), iron, copper and manganese. This substantial study showed reduced cataract formation in groups with high dietary ascorbate, astaxanthin and copper levels while increased incidence of lens opacities were recorded in fish fed diets high in lipid content, iron and manganese.<sup>91</sup> Lenticular ascorbate levels were increased by a diet supplemented with vitamin C while, with regard to vitamin E, increased dietary alpha tocopherol was not reflected by increases in lenticular concentration of the compound. Such data reflect the lack of success in dietary supplementation with vitamin E in humans and reinforce the fact that, as seen with the REACT study, it is a mixture of antioxidants that appear crucial in maintaining lens transparency, rather than over-supplementation with any one antioxidant. Given the network of interactions between different antioxidants and glutathione in the lens, this should surely not come as a surprise.

## CONCLUSION

This review has shown the large amount of evidence, laboratory-based and epidemiological, demonstrating a link between lenticular oxidation processes and cataract and between antioxidant intake and retardation in age-related cataractogenesis. It is thus somewhat depressing to note the equivocal findings with regard to human interventional studies. One hopes that studies in companion animals might, in the future, be able to give more positive results.

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