Antioxidants for Eye Health

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Abstract

Eyes commonly experience age-related changes, such as cloudy eyes (nuclear sclerosis and/or cataract) and retinal degeneration, and leading age-related eye conditions may result in visual impairments. There are similarities among species regarding age-related changes in eyes. Our studies, summarized in this paper, showed that visual impairment as measured by increasing lens reflective dot sizes and refractive error is significantly associated with age in dogs. Antioxidant supplementation in dogs resulted in improvement in retinal/visual response and a reduction in refractive error changes. Antioxidants can be beneficial for preserving and improving eyesight and visual function in dogs as well as humans.

Glossary of Abbreviations

AAFCO: Association of American Feed Control Officials
AMD: Age-Related Macular Degeneration
AREDS: Age-Related Eye Disease Study
D: Diopter
DHA: Docosahexaenoic Acid
EPA: Eicosapentaenoic Acid
ERG: Electroretinography
La: Anterior Lens Reflection
Lb: Posterior Lens Reflection

Introduction

Traditionally, eye health has been viewed as an issue most important to humans. Most research seeking nutritional intervention for eye benefits has been focused on human applications. Our eyes are our window to the world. Our eyesight influences the way in which we view the world. Good eyesight is an important part of well-being and a significant factor in retaining independence and quality of life as we get older. Contrary to popular belief, loss of vision does not need to be an accepted consequence of aging. Vision can deteriorate for many reasons, and even when associated with the aging process, many interventions are available. The World Health Organization estimates that up to 80 percent of blindness and serious visual loss around the world is avoidable through prevention or treatment.[1]

Eyesight is equally important to dogs and cats. Eyesight is one of the key senses for animals to acquire information from their outside environment and is critical for hunting for food and daily survival. Although most pet dogs and cats do not hunt for their food, eyesight is still important to allow them to get visual cues from their owners for their understanding and social interactions.

Common Eye Problems in Humans

Age-related macular degeneration (AMD) is a disease that gradually destroys the macula, the part of the eye that provides sharp, central vision required to see objects clearly. AMD is a leading cause of vision loss in older humans, generally affecting people age 50 and older. Cataract, a clouding of the lens in the eye that affects vision, is another leading cause of vision loss in older humans. By age 80, more than half of all Americans either have a cataract or have had cataract surgery. Globally, the main cause of blindness is cataract (51%), though uncorrected refractive errors are the major cause of visual impairment (43%), according to the World Health Organization.[1]

Cataracts develop for a variety of reasons, including long-term ultraviolet exposure, exposure to radiation, secondary effects of diseases, such as diabetes, or simply due to advanced age. They are usually a result of denaturation of lens proteins, which then clump together and cause cloudiness, resulting in blurred vision and, potentially, blindness. Free radical damage to the lens and retina is a major factor in cataracts and macular degeneration. Prevention strategies have been centered on using antioxidant supplements to block free radical buildup.[2,3]

Cloudy Eye in Dogs and Cats

Development of cloudy eyes (nuclear sclerosis and/or cataract) is common in aging dogs and cats.[4] The cloudy lens of older dogs or cats is readily visible to the naked eye as an observed hazy or bluish appearance and often is viewed by owners as cataract formation. Cloudy eye is one of the common concerns for owners to bring their pets for a veterinary evaluation.[5]

Differences of opinion exist about cloudy lens in veterinary and human medicine. The cloudy lens as seen in older dogs or cats is commonly diagnosed as nuclear sclerosis by veterinary ophthalmologists and considered as part of normal aging.[6] In contrast, nuclear sclerosis is considered a type of cataract in human medicine (nuclear cataract or senile cataract).[7,8] In veterinary ophthalmology, nuclear sclerosis is believed to result from internal compression and an increased density of the lens nucleus, but it is not generally believed to significantly affect vision in dogs, except in unusually dense or advanced cases. However, the clinical distinction between advanced nuclear sclerosis and early nuclear senile cataract in dogs is often indistinct.[6]

We conducted studies to evaluate age-related changes in the eyes of dogs and cats at one of our pet care centers. For the canine

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study, we evaluated eyes for evidence of nuclear sclerosis and cataract in 222 dogs, aged 1 to 17 years, representing Beagles, Labrador Retrievers and English Setters. Lens cloudiness was scored using a 1-5 cloudiness grading scale (Tobias lens cloudiness grading scale).[9] Lens cloudiness score was significantly associated with the age of the dogs (r=0.88, p<0.05). Among the 182 dogs that were 6 years and older, the prevalence of nuclear sclerosis increased from 60% at age 6-8 years to 100% for dogs 9 years and older; 36% of these dogs also had some form of cataract. Cataracts were most prevalent in dogs aged 12 years and older.

We performed eye examinations on 80 cats at our center, with at least 16 cats per age group representing age groups of 1-3, 4-6, 7-9, 10-12, and 12+ years of age. Cats started to show nuclear sclerosis at age 7-9 years, and 100% of cats older than 12 years of age showed evidence of nuclear sclerosis. Among the 48 cats aged 7 years and older, 13% had some form of cataract in addition to the presence of nuclear sclerosis.

Lens cloudiness also was evaluated by Williams et al., using a 0-10 score system.[10] The lens cloudiness seemed to increase noticeably around 6 years of age. The age at which prevalence of cataract was 50% among the 2,000 dogs in their study was 9.4±3.3 years.[10] Williams et al. also showed that the onset for nuclear sclerosis and cataract in cats begins at about 7-9 years of age. The age at which prevalence of cataract was 50% among the 2,000 cats in their study was 12.7±3.4 years, but was 5.6±1.7 years for cats with diabetes and 9.9±2.5 years for cats with a history of dehydration crises.[11]

Nuclear sclerosis is an age-related progression of lens cloudiness in both human and veterinary medicine. Change in lens transparency is considered a dynamic phenomenon in aging humans, progressing from nuclear sclerosis to senile cataract.[7] In veterinary medicine, nuclear sclerosis is a consistent finding in dogs greater than 6-7 years of age. Although the clinical appearance and rate of progression of age-related eye clouding in dogs can vary, an increase in nuclear opalescence, and punctuate to striate opacifications of the adult lens nucleus, are usually observed. These changes are generally noted concurrently with, or following the presence of, dense nuclear sclerosis.[6,10]

Humans affected with nuclear sclerosis/nuclear cataract report visual disturbances resulting from a myopic shift (from hardening of the lens nucleus), astigmatism, a shift in contrast sensitivity (especially with low-contrast objects), glare, and visual acuity reduction.[6] A myopic shift in the lens has been reported in older dogs,[12] presumably from a change in refractive status of the sclerotic lens nucleus. This shift likely affects visual acuity.[13] It is possible that other visual alterations occur in the aged dogs as well; however, the ability to detect these subtle visual disturbances, especially in the less-active older dog, has been limited.[6]

Visual Impairment in Dogs

Visual impairment in humans can be studied using different methods involving visual targets and feedbacks from the test subjects. It is challenging to evaluate visual impairment in animals. We have used several new technologies in our recent studies evaluating eye health in dogs.

Visual Impairment: Blurry Vision with Aging

We recently conducted a study to determine if the Tobias cloudiness scale could be used to monitor lens sclerotic changes over time associated with aging. Eye examinations were completed by a veterinary ophthalmologist, and lens cloudiness scores were assessed by two veterinarians independently on each eye of 18 healthy Beagles (age mean 6.48±0.30 year; 7 neutered males and 11 spayed females); and 27 Labrador Retrievers (age mean 8.29±0.82 year; 14 neutered males and 13 spayed females). Lens cloudiness score and reflective dot sizes from the anterior (La) and posterior (Lb) of the lens reflection from a penlight were assessed repeatedly every year for five years. The reflective dot size from the anterior (front) and posterior (back) of the lens measures how scattered an incoming penlight source may become when it shines through the back of lens. The more light-scattering of the incoming light in real life would result in a more blurry vision from a single light source. Statistical evaluations were performed using repeated measures analysis of covariance with the initial age of the dogs as a covariate.

Dogs showed a significant increase in lens cloudiness score as well as reflective dot sizes over time (p<0.05) (Figure 1 and Figure 2a and 2b). There was no breed difference for progression rate of lens cloudiness, with an average increase of 0.33±0.14 units for Labradors and 0.28±0.15 units for Beagles per year, respectively. The reflective dot sizes increased from ~1 mm to ~2 mm for anterior reflective dot size and from ~1.5 mm to 3 mm for posterior reflective dot size over five years for Labradors; and from ~0.5 mm to 1.5 mm for anterior reflective dot size and from ~1.0 mm to ~2.5 mm for posterior reflective dot size for Beagles. The doubling of the reflective size over five years may suggest light scattering due to the lens aging, thus a direct result could be blurry vision.

Visual Impairment: Refractive Error Change Over Aging

Some more objective evaluation methods that have been used in human studies could be useful to adapt for companion animal studies. One such method uses an auto-refractor for testing refractive errors in infants and toddlers who cannot yet communicate well. The SureSight hand-held auto-refractor has lights and sounds that engage the test subjects' attention, with minimal cooperation required, making it ideal for use on young children, the disabled and when there is a language barrier.[14,15]

We completed a pilot study to evaluate the auto-refractor for use in dogs. Spherical equivalent refractive error was measured using the hand-held auto-refractor (WelchAllyn SureSight) on 9 Beagle dogs (ages 1-14 years) under both indirect and direct lighting conditions with five measurements per condition, per eye

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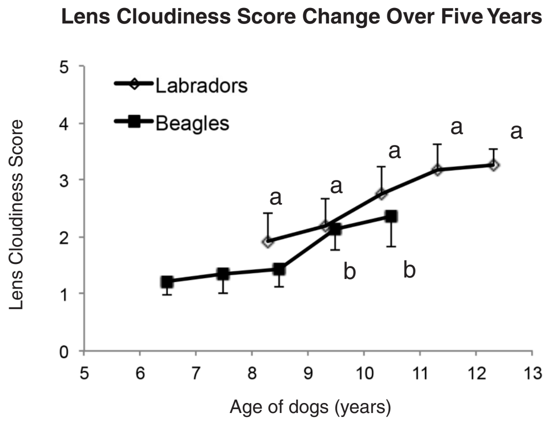

Figure 1. Eighteen healthy Beagles (initial age mean 6.48±0.30 year; 7 neutered males and 11 spayed females) and 27 Labrador Retrievers (initial age mean 8.29±0.82 year; 14 neutered males and 13 spayed females) were followed for five years. Lens cloudiness score was assessed using Tobias grading scale each year. Statistics were done using a repeated measures analysis of covariance with the age of the dogs when they started the study as a covariate.
a and b: Differences between later years to year 1
Statistical significance at $p<0.05$.

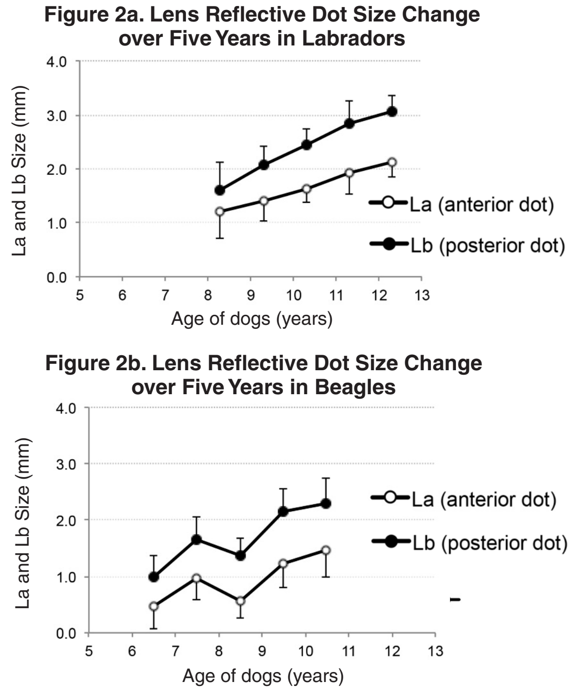

Figure 2a and 2b. Linear correlations between La (anterior lens reflective dot size), Lb (posterior lens reflective dot size), and age of the dogs were analyzed using SAS.

Significant correlation between La and Lb to age in both breeds; $r=0.67$ (La) and $r=0.77$ (Lb) for Labradors; $r=0.58$ (La) and $r=0.72$ (Lb) for Beagles, respectively.

Statistical significance at $p<0.05$.

(Figure 3 a-d). Measures were repeated three different days for each dog within six weeks. Indirect light condition was set with an indoor light from an adjacent room coming into a dark room with dogs facing the incoming light, and direct light condition was set in the same position but with the dark room's light turned on. Eyes were tested under indirect light condition first, followed by direct light at the same setting. Nonparametric statistics were used to detect differences among lighting conditions and test days, and between eyes. Spearmen correlation assessed the visual measurement outcomes' association with age.

There was no difference for day-to-day or intra-eye measurements. Significantly, Beagles showed a myopic shift with aging (average spherical equivalent ranged from plano to -3.00 diopters), suggesting that the older the dog, the more nearsighted ($r=-0.48$ and -0.73 under direct and indirect lights; $p<0.05$ both). Younger dogs were able to make larger accommodation changes from indirect light to direct light conditions, indicating a more flexible lens ($r=-0.50$, $p<0.05$). The results of this pilot study show that the hand-held portable human auto-refractor technique is applicable to dogs, repeatable and sensitive to light conditions.

The myopic refractive shift could be expected to compromise dogs' visual functions with aging. The older dogs in our study had myopic shift close to -2 and -3 diopter (dogs at age 10.2 and age 13.7 years of age that had moderate and advanced nuclear sclerosis). This can be a functionally important difference, as shown by Ofri et al.[16]

Ofri et al.[16] studied seven Labrador Retrievers and one Chesapeake Bay Retriever that were trained for field trial competition. Dogs were commanded to retrieve targets at 150 yards. Each dog participated in three trials while their eyes were fitted with 0- (plano), +1.50- or +3.00-diopter (D) contact lenses, applied in random order. Retrieval times were significantly faster with plano lenses than with +1.50- or +3.00-D lenses, but there were no significant differences in times between +1.50- and +3.00-D lenses. Judges blinded to the specific treatment assigned the best performance scores to dogs with plano lenses and the lowest scores to dogs fitted with +3.00-D lenses. The authors concluded that even mild myopic defocusing, such as -1.5-D, had a significant negative impact on both the subjective and objective assessments of dogs' performances.[16]

Cloudy lens in older dogs may be more similar to human nuclear/senile cataracts than previously acknowledged. It appears that the cloudiness of the lens in dogs, as with humans, may result in similar vision disturbances, such as blurry vision and refractive error, which may have a negative impact on their daily activities. Concerning possible developmental mechanism(s), oxidative damage to the lens has been considered an important factor in the initiation and progression of age-related cataracts.[17]

Antioxidants Benefiting Human Eyes

Balanced nutrition is an integral part of good eye care. Most eye diseases are a result of oxidative damage and/or inflammation, which, in turn, produce free radicals, singlet oxygen and

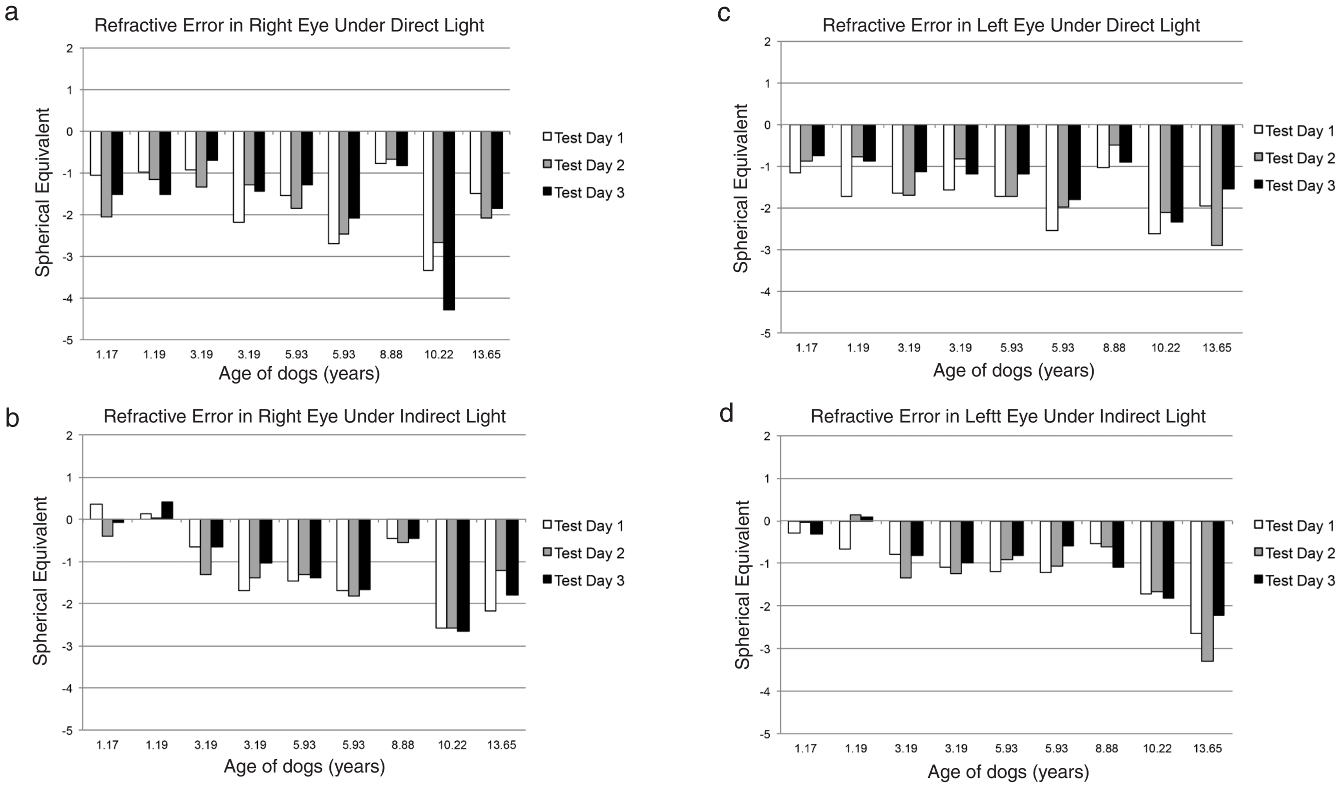

Figure 3a-d. Spherical equivalent refractive error was measured using the hand-held auto-refractor (WelchAllyn SureSight) on 9 Beagle dogs (ages 1-14 years) under both indirect and direct lighting conditions with five measurements per condition, per eye. Measures were repeated on three different days (test day 1-3) for each dog within six weeks. Indirect light condition was set with indoor light from adjacent room coming into a dark room with dogs facing the incoming light, and direct light condition was set in the same position but with the dark room's light turned on. Eyes were tested under indirect light condition first, followed by direct light at the same setting.

Nonparametric statistics were used to detect differences among lighting conditions and test days, and between eyes. Spearmen correlation assessed the visual measurement outcomes' association with age.

Significant correlation between refractive error to age in both light conditions ($r=-0.48$ and -0.73 under direct and indirect lights; $p<0.05$ both); and accommodation changes from indirect light to direct light conditions to age indicating a more flexible lens ($r=-0.50$, $p<0.05$).

There was no difference for day to day or intra-eye measurements.

Statistical significance at $p<0.05$.

reactive oxygen species, which lead to the onset of various eye diseases.[17]

Strong scientific evidence has shown many nutritional factors, such as lutein, zeaxanthin, omega-3 fatty acids (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]), vitamins, and minerals, may be protective for age-related eye problems.[2,3,18-21]

The Age-Related Eye Disease Study (AREDS1), a major clinical trial sponsored by the National Eye Institute and published in 2001, showed that high levels of antioxidants and zinc significantly reduced the risk of progression to advanced AMD by 25% and the risk of moderate vision loss by 19%.[2] Specifically, the AREDS formula contained 500 mg of vitamin C, 400 IU of vitamin E, 15 mg of beta-carotene, 80 mg of zinc, and 2 mg of copper.[2]

Building on these results, the National Eye Institute initiated AREDS2 in 2006, modifying its formula by adding 10 mg lutein, 2 mg zeaxanthin, 1000 mg omega-3 fatty acids (650 mg EPA and 350 mg DHA), and 25 mg zinc and removing beta-carotene. The study results, presented in 2013, showed that the modified supplement had the same overall protective effect on advanced AMD.[3]

Lutein, zeaxanthin and beta-carotene belong to a family of phytonutrients known as carotenoids. Lutein and zeaxanthin are yellow plant pigments that are especially enriched in dark leafy greens such as spinach, kale or collard greens. Beta-carotene is orange plant pigment that is enriched in orange-colored fruits and vegetables, such as carrots, pumpkins and sweet potatoes. In the body, beta-carotene is used to make vitamin A, which is required by the retina to detect light and convert it into electrical signals.

Lutein and zeaxanthin are found in the retina and lens, where they may act as antioxidants and help absorb damaging, high-energy blue and ultraviolet light.

Blue light is the highest energy form of visible light and induces photo-oxidative damage by generating reactive oxygen species. Lutein and zeaxanthin together protect the photoreceptor cells and also nervous tissues in the brain from the damage of reactive oxygen species. They are intimately associated with omega-3 fatty acids, which are also abundant in nerve cells, and protect these easily damaged fats from oxidation. Consumption and serum levels of lutein have been shown to be inversely related to the risk for ocular diseases, including AMD and cataracts.[22,23] Long-term supplementation in humans with these carotenoids, including lutein, zeaxanthin and another potent carotenoid astaxanthin, can assist in retaining healthy eyesight, visual performance and acuity, as well as aid glare and contrast sensitivity and support vision in dim light.[2,3,18-21]

Vitamin A is an essential fat-soluble vitamin with antioxidant properties best-known for its role in preventing blindness through the formation of rhodopsin. This photo pigment is responsible for vision under low light. Vitamins C and E both play important roles in protecting the eye through their actions as antioxidants. Zinc is an essential nutrient that acts as a cofactor for more than 100 enzymes. Zinc is also found in high concentrations in the retina, has been shown to help support healthy night vision, and is essential to the activity of dozens of enzymes important for vision health.[17,24]

Antioxidant Supplementation in Dogs

It is reasonable to hypothesize that antioxidants beneficial for human eyes would also be beneficial for dog or cat eyes. To begin to explore this, we completed a study to evaluate the effect of antioxidant supplementation in dogs on eye function measured by full-field flash electroretinography (ERG) and spherical equivalent refractive error.

Twelve Beagles, 6-8 years of age, with normal eyes upon indirect ophthalmoscopy and slit lamp biomicroscopy, were age- and gender-matched and randomly assigned to receive a feeding regimen for six months with or without daily antioxidant supplementation. The control diet was formulated to meet all Association of American Feed Control Officials (AAFCO) nutrition requirements. The antioxidant supplement blend included known antioxidants for human eye health: major carotenoids (20 mg lutein, 5 mg zeaxanthin, 20 mg beta-carotene, 5 mg astaxanthin), 500 IU vitamin E, and 180 mg vitamin C.

Portable mini-Ganzfeld ERG was used with an automated and standardized canine ERG protocol[25,26] in the dogs at baseline and at the end of the supplementation period for ERG measurement. Hand-held auto-refractor was used under indirect light conditions at baseline and at the end of the supplementation period for refractive error measurement.

All ERG a-wave amplitudes were increased in the treatment group compared to those of dogs in the control group, with significant improvements in the scotopic high and photopic single flash cone ($p<0.05$, for both) ERG responses (Figure 4). For the b-wave amplitudes, all responses were increased similarly, with significant improvements in responses for the scotopic high-light intensity stimulation ($p<0.05$) and for photopic single flash cone and 30 Hz flicker ($p<0.01$, for both) recordings (Figure 5).

Scotopic high is for assessing rod photoreceptor function under dark condition. Photopic single-flash cone is for assessing cone photoreceptor function under light condition, and photopic 30 Hz flicker is for cone and rod photoreceptor function under light condition.[25]

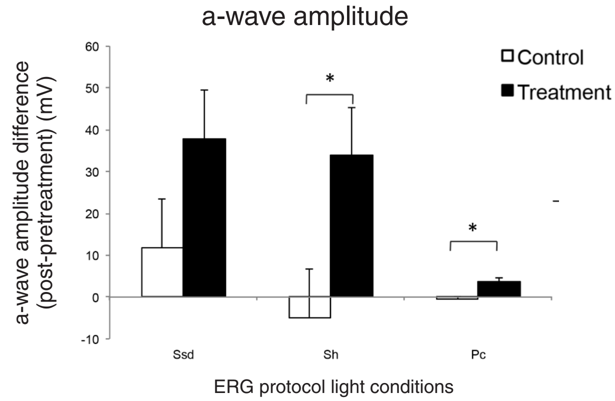

Figure 4. Retinal function a-wave amplitude measured by electroretinography.

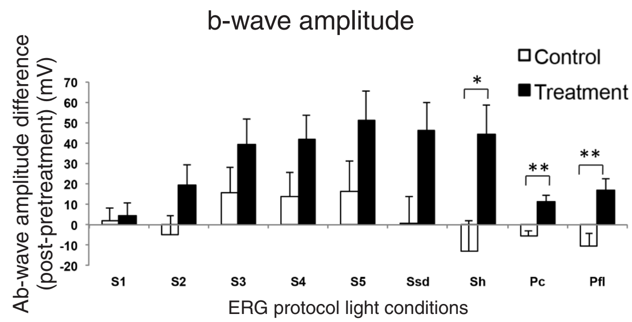

Figure 5. Retinal function b-wave amplitude measured by electroretinography.

In both Figure 4 and 5, ERG protocol light conditions are shown:
S: Scotopic condition
S1-S5: Scotopic condition with increasing light intensity
Ssd: Scotopic condition with standard light intensity
Sh: Scotopic condition with high-light intensity
P: Photopic condition
Pc: Photopic condition for cone
Pfl: Photopic condition flickering light

Statistical analysis was done using t-test comparing changes (after-before) between two treatments (n=6 per treatment group). Statistical significant at *: $p<0.05$ or **: $p<0.01$.

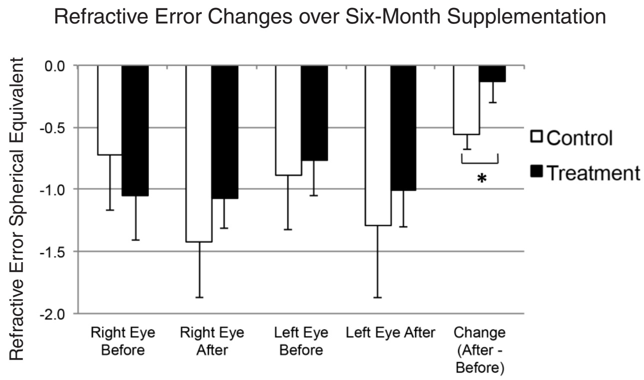

Figure 6. Right and left eyes before or after treatment (n=6 eyes per group).

Change from before to after treatment with all eyes (left and right eyes combined, n=12 eyes per group).

Statistical analysis was done using t-test comparing changes (after-before) between two treatments with all eyes combined (n=12 per group).

* Statistical significance at $p<0.05$.

Retinal function using ERG recordings of scotopic and photopic responses showed increases to varying degrees in the antioxidant supplemented group compared to the control group. This indicates that even in healthy dogs with normal eyes, a better retinal response could be obtained with antioxidant supplementation.

We also observed a marginal but significant difference in the refractive error changes between the two groups. With all eyes combined, the control group had a refractive error change of -0.56 over the six-month study period, while the treatment group had a change of -0.13 ($p=0.0495$) (Figure 6).

Dogs, like humans, experience retinal functional decline and compromised vision with age. Therefore, antioxidant supplementation may be beneficial and effective in the long-term preservation and improvement of eye function in dogs.

Summary

Eyesight is important for the well-being and quality of life of dogs and cats. However, age-related changes in eyes are common in dogs and cats. The age-related lens sclerotic changes were associated with increased lens cloudiness, increased lens reflective dot sizes indicating light scattering and blurry vision, and increased refractive error toward a myopic shift. All these may result in visual impairments that interfere with daily activities of dogs and cats. Antioxidants known for human eye health were able to improve retinal responses as measured by ERG and to slow down the refractive error shifting during a six-month antioxidant supplementation trial in dogs. These results confirmed our hypotheses that these antioxidants benefit dog eyes by improving their retinal and visual functions.

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References

1. World Health Organization. Visual Impairment and Blindness. 2013.

2. Anonymous. A Randomized, Placebo-Controlled Clinical Trial of High-Dose Supplementation with Vitamins C and E, Beta-Carotene and Zinc for Age-Related Macular Degeneration and Vision Loss. AREDS Report No. 8. *Arch Ophthalmol*. 2001; 119(10):1417-1436.

3. Chew EY, Clemons TE, Sangiovanni JP, et al. Secondary Analyses of the Effects of Lutein/Zeaxanthin on Age-Related Macular Degeneration Progression. AREDS2 Report No. 3. *JAMA Ophthal*. 2013.

4. Tobias G, Tobias TA, Abood SK, et al. Determination of Age in Dogs and Cats by Use of Changes in Lens Reflections and Transparency. *Am J Vet Res*. 1998;59(8):945-950.

5. *State of Pet Health: 2012 Report*. Banfield Pet Hospital. 2013.

6. Davidson MG, Nelms SR. *Diseases of the Canine Lens and Cataract Formation*. 2007;Ch18:859-887.

7. Berliner ML. *Nuclear Cataract*. 1966;2:1147-1153.

8. Chylack LT, Khu PM. Subjective Classification and Objective Quantitation of Human Cataract. 2000;3:227-234.

9. Tobias G, Tobias TA, Abood SK, et al. Determination of Age in Dogs and Cats by Use of Changes in Lens Reflections and Transparency. *Am J Vet Res*. 1998;59(8):945-950.

10. Williams DL, Heath MF, Wallis C. Prevalence of Canine Cataract: Preliminary Results of a Cross-Sectional Study. *Vet Ophthal*. 2004;7(1):29-35.

11. Williams DL, Heath MF. Prevalence of Feline Cataract: Results of a Cross-Sectional Study of 2000 Normal Animals, 50 Cats with Diabetes and 100 Cats Following Dehydrational Crises. *Vet Ophthal*. 2006;9(5):341-349.

12. Murphy CJ, Zadnik K, Mannis MJ. Myopia and Refractive Error in Dogs. *Invest Ophthal Vis Sci*. 1992;33(8):2459-2463.

13. Miller PE, Murphy CJ. Vision in Dogs. *J Am Vet Med Assoc*. 1995;207(12):1623-1634.

14. Steele G, Ireland D, Block S. Cycloplegic Autorefraction Results in Preschool Children Using the Nikon Retinomax Plus and the Welch Allyn SureSight. *Optom Vis Sci*. 2003;80(8):573-577.

15. Ying GS, Maguire M, Quinn G, et al. ROC Analysis of the Accuracy of Noncycloplegic Retinoscopy, Retinomax Autorefractor and SureSight Vision Screener for Preschool Vision Screening. *Invest Ophthal Vis Sci*. 2011;52(13):9658-9664.

16. Ofri R, Hollingsworth SR, Groth A, et al. Effect of Optical Defocus on Performance of Dogs Involved in Field Trial Competition. *Am J Vet Res*. 2012;73(4):546-550.

17. Taylor A, Jacques PF, Epstein EM. Relations Among Aging, Antioxidant Status, and Cataract. 1995;62(6 Suppl):1439S-1447S.

18. Parisi V, Tedeschi M, Gallinaro G, et al. Carotenoids and Antioxidants in Age-Related Maculopathy Italian Study: Multifocal Electroretinogram Modifications After One Year. *Ophthal*. 2008;115(2):324-333.

19. Piermarocchi S, Saviano S, Parisi V, et al. Carotenoids in Age-Related Maculopathy Italian Study (CARMIS): Two-Year Results of a Randomized Study. *Eur J Ophthal*. 2012;22(2):216-225.

20. Richer S, Stiles W, Statkute L, et al. Double-Masked, Placebo-Controlled, Randomized Trial of Lutein and Antioxidant Supplementation in the Intervention of Atrophic Age-Related Macular Degeneration. The Veterans LAST Study (Lutein Antioxidant Supplementation Trial). 2004;75(4):216-230.

21. Richer SP, Stiles W, Graham-Hoffman K, et al. Randomized, Double-Blind, Placebo-Controlled Study of Zeaxanthin and Visual Function in Patients with Atrophic Age-Related Macular Degeneration. The Zeaxanthin and Visual Function Study (ZVF). FDA IND #78, 973. 2011;82(11):667-680.

22. Brown L, Rimm EB, Seddon JM, et al. A Prospective Study of Carotenoid Intake and Risk of Cataract Extraction in U.S. Men. 1999;70(4):517-524.

23. Mares-Perlman JA, Fisher AI, Klein R, et al. Lutein and Zeaxanthin in the Diet and Serum and Their Relation to Age-Related Maculopathy in the Third National Health and Nutrition Examination Survey. 2001;153(5):424-432.

24. Chew EY. Nutrition Effects on Ocular Diseases in the Aging Eye. *Invest Ophthal Vis Sci*. 2013;54(14):ORSF42-ORSF47.

25. Katz ML, Coates JR, Cooper JJ, et al. Retinal Pathology in a Canine Model of Late Infantile Neuronal Ceroid Lipofuscinosis. *Invest Ophthal Vis Sci*. 2008;49(6):2686-2695.

26. Narfstrom K, Ekesten B, Rosolen SG, et al. Guidelines for Clinical Electroretinography in the Dog. *Doc Ophthal*. 2002; 105(2):83-92.