Published in final edited form as:

Invest Ophthalmol Vis Sci. 2009 January ; 50(1): 305–310. doi:10.1167/iovs.08-2230.

CHANGES IN FERRITIN H- AND L-CHAINS IN CANINE LENSES WITH AGE-RELATED NUCLEAR CATARACT

Małgorzata Goralska¹, Steven Nagar¹, Carmen M.H. Colitz^{1,2,3}, Lloyd N. Fleisher¹, and M. Christine McGahan¹

¹Department of Molecular Biomedical Sciences, North Carolina State University, Raleigh, North Carolina

²Animal Eye Specialty Clinic, West Palm Beach, Florida

³Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, Ohio

Abstract

PURPOSE—To determine potential differences in the characteristics of the iron storage protein, ferritin and its heavy (H) and light (L) subunits in fiber cells from cataractous and normal lenses of older dogs.

METHODS—Lens fiber cell homogenates were analyzed by SDS-PAGE and ferritin chains were immunodetected with ferritin chain-specific antibodies. Ferritin concentration was measured by ELISA. Immunohistochemistry was used to localize ferritin chains in lens sections.

RESULTS—The concentration of assembled ferritin was comparable in normal and cataractous lenses of similarly aged dogs. The ferritin L-chain detected in both lens types was modified and was about 11 kDa larger (30 kDa) than standard L-chain (19 kDa) purified from canine liver. The H-chain identified in cataractous fiber cells (29 kDa) differed from 21 kDa standard canine H-chain and from 12 kDa modified H-chain present in fiber cells of normal lenses. Histologic analysis revealed that the H-chain was distributed differently throughout cataractous lenses when compared to normal lenses. There was also a difference in subunit makeup of assembled ferritin between the two lens types. Ferritin from cataractous lenses contained more H-chain and bound 11-fold more iron than ferritin from normal lenses.

CONCLUSIONS—There are significant differences in the characteristics of ferritin H-chain and its distribution in canine cataractous lenses as compared to normal lenses. The higher content of H-chain in assembled ferritin allows this molecule to sequester more iron. In addition the accumulation of H-chain in deeper fiber layers of the lens may be part of a defense mechanism by which the cataractous lens limits iron-catalyzed oxidative damage.

INTRODUCTION

Development of age-related cataract is likely determined by both genetic and environmental factors. The crystalline lens has a very high concentration of proteins (up to 35% of its weight), and changes in protein structure could result in cataract formation¹. However, alterations such as various post-translational modifications of lens crystallins and decreased reduced glutathione concentration are present in both cataractous and aging, but still transparent lenses. This makes it difficult to dissociate normal age-related changes from

those correlated with development of age-related cataract². The trigger for the transformation from a clear lens to a cataractous lens has not been determined. It is possible that the accumulation of modified proteins above a critical threshold could initiate cataractogenesis. However, it cannot be excluded that one particular alteration may start this process. It has been demonstrated that a structural change or overexpression of a single protein may cause cataract formation, as it has been for modified β Bp crystallin in Philly cataract³ or ferritin L-chain in hereditary hyperferritinaemia cataract syndrome^{4,5}.

The correlation of cataract progression with a decrease in the level of reduced glutathione⁶ and an increase in the level of modified proteins led to the hypothesis that oxidative changes in protein structure could be responsible for age-related cataract formation^{7,8}. Oxidation of proteins can be exacerbated by the redox-active fraction of iron which can generate free radicals⁹. Iron can be safely stored in ferritin, a ubiquitous protein of high molecular weight (450 kDa), made of 24 subunits of two types, heavy (H; 21 kDa) and light (L; 19 kDa), which controls the level of “free” highly reactive iron¹⁰. The H and L subunit ratio of mammalian ferritins is highly tissue specific. Each ferritin subunit has distinct and complementary role in storing iron. The H subunit which has ferroxidase activity facilitates uptake and oxidation of iron. Ferric ions are subsequently translocated by the L-subunit into the core of ferritin shell for the long term storage. Although ferritin is present throughout the whole lens¹¹, the level of properly assembled ferritin declines with age¹². Additionally, the H- and L-ferritin chains detected in both human and canine fiber cells of different age lenses are significantly altered¹². Substantial changes in ferritin concentration, subunit structure and/or tissue specific H/L ratio could either augment or diminish the capacity of the protein to store iron, thus decreasing or increasing the possibility of oxidative damage¹³.

The purpose of the current investigation was to characterize ferritin present in fiber cells of aged lenses with nuclear cataract. The results could help to identify possible changes in ferritin concentration, distribution, structure and/or subunit makeup associated with age-related cataractogenesis. These studies could lead to a better understanding of the importance of ferritin in lens antioxidative defense mechanisms and may help to determine its possible involvement in the prevention of age-related nuclear cataract formation.

METHODS

Lens Collection

Eyes were obtained from mixed breed dogs with estimated ages of 8 to 10 years following euthanasia at the Johnston County, North Carolina Animal Shelter. Lenses were divided into two categories: with and without visible opacities in the nucleus of the lens. Additionally, lenses from two dogs with diffuse nuclear cataract with wispy cortical cataracts and subcapsular plaques were obtained from dogs undergoing enucleation for chronic secondary glaucoma. If only one lens from a dog was available, half of the lens was used to prepare the homogenates of lens fiber cells and the other half for immunolocalization of ferritin chains.

Preparation of Homogenates

The anterior capsule of each lens with adherent epithelial cells was removed and the remaining part, which consisted mainly of lens fibers, was sonicated in 10 mM Tris/HCl buffer pH 7.4 (6.0–10 ml/g tissue), containing 2% SDS and protease inhibitor cocktail for mammalian cells (Sigma, St. Louis, MO). The concentration of protein of lens fiber homogenates was determined by BCA Protein Assay Kit (Pierce).

Immunodetection of Ferritin Chains in Canine Fiber Homogenates

The samples containing 50 µg of protein were separated by 15% SDS-PAGE. Proteins were transferred to nitrocellulose membranes (Hydrobond ECL, Amersham Biosciences) by semidry blotting at 20 V for 20–30 min. The chain-specific peptides, designed based on the amino acid sequences of canine H- and L-chains¹⁴, were conjugated and used to produce antibodies in rabbits immunized with conjugated synthetic peptides (Research Genetics Inc.). These antibodies were used in immunodetection of ferritin chains. TrueBlot HRP-anti-rabbit IgG antibodies (eBioscience) were used in the second step of detection.

Immunoreactivity was determined by the ECL Western blotting Analysis System (Amersham, Biosciences) and the images were digitized and evaluated with UN-SCAN-IT gel software (Silk Scientific, Inc).

In Vitro ⁵⁹Fe Labeling of Assembled Ferritin

Samples of lens fiber homogenates (0.7–1.6 mg protein in 25 µl) were incubated with 1 µl of ⁵⁹FeCl₃ (15.8 mCi/ml; 40.3m Ci/mg; (PerkinElmer, Boston,MA) for 1 h at room temperature. Subsequently lens proteins were separated by 8% SDS-PAGE under non-reducing conditions. Binding of ⁵⁹Fe by assembled ferritin was measured in an Instant Imager (Packard –Canberra, Rockville, MD). The protein content of the labeled samples analyzed on each particular gel was the same.

Characterization of Subunit Makeup of Assembled Ferritin

To localize assembled ferritin, lens fiber homogenates containing 1–1.5 mg of protein were separated by 8% SDS-PAGE under non-reducing conditions. Canine heart or liver holoferritin standards, purified according to the method of Cham *et al.*¹⁵, were separated in parallel lanes. The location of assembled ferritin in homogenate samples was assessed based on the mobility pattern of colored ferritin standards. To dissociate assembled ferritin into subunits, the gel pieces containing ferritin were excised and boiled for 10 min with SDS-PAGE loading buffer (25 mM Tris/HCl pH 6.5, 10% glycerol, 6%SDS) containing 50 mM DTT. The gel pieces were inserted into the wells and ferritin chains were separated by 15% SDS-PAGE under reducing condition. The chains were identified by Western blot as described above.

Measurement of Ferritin Content by ELISA in Dog Lens Fiber Homogenates

Ferritin concentration was measured by a sandwich ELISA using goat anti-horse ferritin and HRP-labeled goat anti-horse ferritin antibodies (Bethyl Laboratories Inc.) as described previously¹⁶. ABTS (KPL) was used as a substrate. The optical density of the samples was read at 405 nm.

Immunolocalization of Ferritin Chains

Lenses from cataractous and age-matched normal lenses were collected as described above. In some cases half of the lens was used for Western blotting and the other half was fixed in 4% formaldehyde in 10 mM Sorenson's sodium phosphate buffer at room temperature for several hours and then stored at 4°C in the same fixative until sectioned. Fifty µm sagittal sections were cut using a Vibratome (TPI, St. Louis, MO) and transferred to Netwell (Corning, Inc., Corning, NY) membrane inserts containing 1× phosphate buffered saline (PBS) and stored at 4°C until immunolabeled. At room temperature, sections were permeabilized with 0.2% Triton X-100 in PBS for 20 min, blocked in 5% normal goat serum (Jackson ImmunoResearch Laboratories, Inc, West Grove, PA) in PBS for 1 h, and then incubated with rabbit polyclonal antibodies to canine H- or L- ferritin chains¹⁴ diluted 1:1000 in PBS containing 0.1% IgG-free BSA (PBS/BSA) overnight at 4°C. Control sections were incubated with normal rabbit serum (Jackson ImmunoResearch Laboratories) diluted 1:1000 in PBS/BSA in place of ferritin-chain specific antisera. Following three ten-minute washes in PBS, sections were incubated for 2 hours at room temperature in darkness with Alexa Fluor® 568 goat anti-rabbit IgG antibodies (Invitrogen) diluted 1:1000 in PBS. Following three ten-minute washes in PBS, sections were mounted on slides in ProLong® Gold anti-fade reagent containing DAPI (Invitrogen). Sections were then imaged using a Leica DM5000B microscope with a 5× objective lens and conventional epifluorescence. Images were captured with a Retiga 1300 cooled CCD camera (QImaging) and Simple PCI imaging software (Compix, Inc.), and then arranged using Adobe Photoshop CS2. Multiple images of each section were stitched together to yield single composite images encompassing lens areas from the epithelium to the nuclear regions.

RESULTS

Comparison of Ferritin Chains Immunodetected in Homogenates of Fiber Cells from Cataractous and Non-Cataractous Canine Lenses

Anti-ferritin L-chain antibodies immunodetected a 30 kDa protein in fiber cells of both normal and cataractous lenses. The protein was much larger than the 19 kDa canine liver ferritin L-chain used as a standard (Fig. 1). Fiber cells from cataractous lenses contained three times less modified L-chain in comparison to non-cataractous lens fiber cells from lenses of similar ages. Ferritin L-chain of proper size was not detected in the total homogenates of either tissue.

Ferritin H-chains identified in normal and cataractous lenses differed from that of the canine heart ferritin H-chain standard (21 kDa) used as a control. Anti-ferritin H-chain antibodies detected two different size proteins: a ~29 kDa protein found only in cataractous lenses and a 12 kDa protein in lenses from both groups (Fig. 2).

The content of the 12 kDa protein in cataractous lenses was less than 20% of that in non-cataractous lenses. The combined amount of the 29 and 12 kDa proteins in cataractous lenses was approximately 30% lower than that of the 12 kDa protein in non-cataractous lenses. The 29 kDa protein was not detected in non-cataractous lenses (Fig.2). There was no normal size ferritin H-chain (21 kDa) detected in any lens fiber homogenates.

Detection of Assembled Ferritin in Lens Fiber Cell Homogenates of Normal and Cataractous Lenses

PAGE separation of lens proteins from fiber cell homogenates incubated in vitro with ^{59}Fe revealed the presence of a single ^{59}Fe -labeled protein with the same mobility as that of assembled ferritin standard (canine liver ferritin; Fig. 3). The assembled ferritin from fiber cells of cataractous lenses bound 11 times more iron than ferritin from non-cataractous lenses of similar age.

Quantitation of Assembled Ferritin in Canine Fiber Cell Homogenates of Non-Cataractous and Cataractous Lenses

In order to determine if the higher iron binding capacity of cataractous lens homogenates (Fig. 3) was due to an increased concentration of assembled ferritin, ferritin levels were measured by ELISA using the antibodies which predominantly recognize assembled ferritin and have low affinity to individual ferritin H- or L-chains.

There was no difference in the concentration of assembled ferritin in fiber cells from cataractous and non-cataractous lenses (Fig. 4).

Analysis of Subunit Makeup of Assembled Ferritin from Fiber Cells of Normal and Cataractous Lenses

Pieces of gel containing assembled ferritin were excised from 8% SDS-PAGE and subsequently electrophoresed in a 15% gel under reducing conditions as described in the Methods section. Separated ferritin chains were identified by immunoblotting with anti-ferritin chain antibodies. Immunoblotting with ferritin anti-H chain antibodies showed that assembled ferritin of cataractous and non-cataractous lenses contained the 29 kDa modified H-chain (Fig. 5A). Neither ferritin H-chain of the proper size (21 kDa) nor the modified ferritin H-chain 12 kDa, which was detected in total homogenates of normal and cataractous lenses (Fig. 2), was found as a subunit of assembled ferritin. The fiber cells of cataractous lenses contained more of the 29-kDa H-chain than non-cataractous lenses (Fig. 5 C, Table 1). The anti-L ferritin chain antibodies detected the presence of two L-chain types in assembled ferritin from both lens groups: 19 kDa (normal size) and 30 kDa (modified) L-chains (Fig. 5B). The assembled ferritin from cataractous lenses contained less of the proper size, 19 kDa L-chain as compared to that of non-cataractous lenses (Fig. 5D, Table 1). There was no difference in content of modified 30 kDa L-chain in assembled ferritin between normal and cataractous lenses (Fig. 5 D, Table 1). The ratio (H/L) of heavy (29kDa) to light chains (30+19 kDa) in assembled ferritin, based upon quantitated images, was significantly higher for cataractous lenses as compared to non-cataractous lenses. The higher ratio resulted from both higher content of H-chain and lower level of L-chains in assembled ferritin from fiber cells of cataractous lenses (Table 1).

Immunolocalization of Ferritin Chains in Lens Sections

Based on the interesting differences found between normal and cataractous lenses with respect to the ferritin size variants found in fiber cells, we examined the distribution of ferritin H- and L- chains in Vibratome sections of cataractous and similarly aged normal dog lenses using immunofluorescent localization and the same chain-specific antibodies used for

Western blotting. The cataractous lenses examined all exhibited immature (nuclear) opacities with anterior subcapsular or cortical plaques (Fig. 6K). In both normal and cataractous lenses, ferritin H- and L-chains were evenly distributed in outer cortical fiber cells located from just under the epithelium to those located up to approximately 400 microns from the epithelium as measured from the equator (Fig. 6 A, B, D, E, G, H, I, and J). Ferritin L-chain signals in these outermost cortical fiber cells were generally lower in cataractous lenses, compared to normal lenses (Fig 6 B, E, H, and J), consistent with Western results (Fig. 1). Interestingly, additional brighter bands of ferritin H-chain specific signals were detected in fiber layers further in toward the center, but only in cataractous lenses (Fig. 6 D, G, and I). These bands were never observed in normal lenses instead, ferritin H- and L-chain signals were restricted to the outer cortex. (Fig. 6 A, and B). The unique bands of ferritin H-chain labeling extended up to 1.8 millimeters from the epithelium, as measured from the equator and 200 microns in from the epithelium at the apical anterior (data not shown). No inner bands of ferritin L-chain were seen. Interestingly, in some cases, there were gaps devoid of detectable ferritin H-chain labeling between the outer cortical labeling and the inner bands of cataractous lenses. These gaps were also observed between inner ferritin H-chain bands (Fig. 6 G and I). None of the cataractous lenses examined showed bands of ferritin H-chain in the center regions of the lenses. Sections incubated with normal rabbit serum in place of ferritin antibodies showed no ferritin labeling (Fig. 6 C, and F).

DISCUSSION

This is the first comparative study to characterize ferritin H- and L-chains in fiber cells of both normal and cataractous aging lenses. Older canine lenses with visible nuclear cataracts were selected for this study because the development of nuclear cataract is often associated with aging. We found similarities but also significant differences in the characteristics of ferritin from fiber cells of both lens types.

The predominant form of L-ferritin chain detected in all lens fiber cell homogenates was a 30 kDa protein, thus larger than the 19 kDa canine liver L-chain used as a standard. The cataractous lens fiber cells however contained significantly less (3-fold) modified L-chain in comparison to non-cataractous lenses of similar age (Fig. 1). The proper size L-chain was only found in assembled ferritin (Fig. 5B) and the amount of the chain was lower (by 40%) in cataractous lenses than in non-cataractous lenses (Table 1). The concentration of assembled ferritin in lens fiber cells from both lens types was low, as compared to lens epithelial cells (60–200 ng/mg protein)¹⁵, and was no higher than 0.08 ng/mg protein (Fig. 4). This may explain why the proper size L-chain could not be immunodetected in the total homogenates of fiber cells without prior separation of assembled ferritin. Interestingly, assembled ferritin also contained small amount of the 30 kDa modified L-chain (Fig. 5D), the content of which was similar in both normal and cataractous lenses (Table 1). We concluded that contrary to what was found for aging but transparent lenses, which maintain steady levels of the 30 kDa L-chain, the development of cataract is associated with a decline of ferritin L-chains in lens fiber cells¹². We previously did not detect the presence of the 30 kDa L-chain in lens epithelial cells, which contained only normal size (19 kDa) L-chain¹². Therefore, based on our previous¹² and current results, we hypothesized that modification of

this chain into a 30 kDa protein is associated with the differentiation of epithelial cells into fibers. Only a small amount of normal size L-chain remained in these differentiated fiber cells, most likely as a subunit of assembled ferritin rather than as a free chain (Fig. 5B,D). There was a further decrease in the content of normal size L-chain associated with the formation of nuclear cataract (Table 1). Since the major role of the L-chain is long-term storage of iron¹⁷, decreased content of the L-chain could limit the long-term iron storage capacity of ferritin in cataractous lenses.

Contrary to what we found for ferritin L-chain we were not able to detect proper size (21 kDa) ferritin H-chain in fiber cells, neither as free chain in total homogenates nor in assembled ferritin. This was true for both normal and cataractous lenses. In our previous studies we identified a 12 kDa modified ferritin H-chain as the predominant form of H-chain present in fiber cells from non-cataractous canine lenses as young as 3 months¹². The predominant form of H-chain immunodetected in cataractous fiber cells was 29 kDa protein, larger than the 21 kDa normal H-chain (Fig. 2). Interestingly, the 29 kDa H-chain was also identified in normal lenses, but only in assembled ferritin and not in total homogenates (Fig. 5). It is difficult to explain the nature of the changes in ferritin H-chain present in normal and cataractous lenses and to speculate about their physiological significance without identifying the structural modifications of this chain. It is possible that H-chain undergoes different posttranslational modifications in cataractous than in normal lens fiber cells but it is also possible that the 29 kDa protein is a dimer of 12 kDa H-chains, since we estimated the molecular weights of both H-chains based on protein standards of a different molecular weight. Alternatively, the modified H-chains could be the products of ferritin H-chain pseudogenes differentially expressed during the processes of differentiation and/or cataractogenesis. In humans, ferritin H-chain-like sequences were mapped to different chromosomes and several H-chain pseudogenes were cloned and characterized^{18,19}. We have cloned and sequenced canine ferritin chains and identified several H-chain pseudogenes by screening their products in an in vitro transcription/translation system (unpublished data). In this scenario 29 kDa H-chain would be synthesized de novo in outer layers of cortical fiber cells and would translocate toward the deeper layers of cortex where it would accumulate as free or assembled H-chain. The bright bands of ferritin H-chain labeling observed in sections of cataractous lenses (Fig. 6 D, G, and I) may reflect such accumulation. It is known that macromolecules can move between lens fiber cells²⁰. Since the antibodies used for immunolabeling sections do not discriminate between the different modified ferritin variants seen in Western immunoblotting, it is unclear whether the high ferritin H-chain labeling observed in the inner fiber cell layers of cataractous lenses represents an accumulation of modified ferritin H-chain, while the outer cortical ferritin H-chain signals are predominantly normal (21 kDa) or modified (12 kDa) ferritin H-chain.

We can hypothesize what triggers the change from 12 kDa to 29 kDa and leads to the accumulation of 29 kDa H-chain in cataractous fiber cells and higher deposition of the chain into assembled ferritin. What is clear is that the repercussion of that change was significant. The higher concentration of H-chain and lower content of proper size L-chain in cataractous lenses increased the H/L ratio of assembled ferritin over 4- fold in comparison to assembled ferritin from normal lenses (Table 1). It is likely that this change in H/L ratio significantly increased the iron affinity of cataractous lens ferritin. Assembled ferritin from cataractous

lenses bound 11- times more iron than assembled ferritin from non-cataractous lenses (Fig. 3). These results are consistent with the finding that the amount of H-chain in assembled ferritin determines the ability of ferritin to sequester iron, since only H-chain has ferroxidase activity¹⁷. The possibility that an increased concentration of assembled ferritin was responsible for the increase in iron binding properties rather than changes in its subunit makeup is unlikely, since there was no difference in the concentration of assembled ferritin between normal and cataractous lenses (Fig. 4).

The results of this current investigation revealed significant differences in ferritin L- and H-chain characteristics, subunit makeup of assembled ferritin, and the distribution of ferritin chains between normal and cataractous canine lenses. There is evidence that human cataractous lenses have higher concentrations of iron and therefore an increased ability to generate hydroxyl radicals¹¹, which has been implicated in nuclear cataractogenesis²¹. The higher content of H-chain in assembled ferritin of cataractous lenses, which led to increased sequestration of iron by ferritin may be part of a defense mechanism by which the lens limits the degree of oxidative damage. The nuclear section of the lens is particularly prone to oxidative stress. Development of a barrier during aging in the center of the lens limits the movement of the lens' major antioxidant, reduced glutathione, from the metabolically active cortex, to the lens nucleus⁶. Therefore, the location of H-chain-rich ferritin closer to the center of the lens may be the part of the same mechanism. Further investigation of structural changes in ferritin H-chain, are necessary to fully understand the possible role of ferritin in the prevention lens opacification caused or exacerbated by iron-catalyzed oxidative damage.

Acknowledgments

Supported by National Institutes of Health

Grant EY-04900-25

REFERENCES

1. Harding JJ. Viewing molecular mechanisms of ageing through a lens. *Ageing Res Rev.* 2002; 1(3): 465–479. [PubMed: 12067598]
2. Bron AJ, Vrensen GF, Koretz J, Maraini G, Harding JJ. The ageing lens. *Ophthalmologica.* 2000; 214(1):86–104. [PubMed: 10657747]
3. Nakamura M, Russell P, Carper DA, Inana G, Kinoshita JH. Alteration of a developmentally regulated, heat-stable polypeptide in the lens of the Philly mouse. Implications for cataract formation. *J Biol Chem.* 1988; 263(35):19218–19221. [PubMed: 3198622]
4. Girelli D, Corrocher R, Bisceglia L, Olivieri O, De Franceschi L, Zelante L, Gasparini P. Molecular basis for the recently described hereditary hyperferritinemia-cataract syndrome: a mutation in the iron-responsive element of ferritin L-subunit gene (the "Verona mutation"). *Blood.* 1995; 86(11): 4050–4053. [PubMed: 7492760]
5. Brooks DG, Manova-Todorova K, Farmer J, Lobmayr L, Wilson RB, Eagle RC Jr, St Pierre TG, Stambolian D. Ferritin crystal cataracts in hereditary hyperferritinemia cataract syndrome. *Invest Ophthalmol Vis Sci.* 2002; 43(4):1121–1126. [PubMed: 11923255]
6. Sweeney MH, Truscott RJ. An impediment to glutathione diffusion in older normal human lenses: a possible precondition for nuclear cataract. *Exp Eye Res.* 1998; 67(5):587–595. [PubMed: 9878221]
7. Spector A. Oxidative stress-induced cataract: mechanism of action. *Faseb J.* 1995; 9(12):1173–1182. [PubMed: 7672510]

8. Truscott RJ. Age-related nuclear cataract-oxidation is the key. *Exp Eye Res.* 2005; 80(5):709–725. [PubMed: 15862178]
9. Stadtman ER. Metal ion-catalyzed oxidation of proteins: biochemical mechanism and biological consequences. *Free Radic Biol Med.* 1990; 9(4):315–325. [PubMed: 2283087]
10. Picard V, Epsztejn S, Santambrogio P, Cabantchik ZI, Beaumont C. Role of ferritin in the control of the labile iron pool in murine erythroleukemia cells. *J Biol Chem.* 1998; 273(25):15382–15386. [PubMed: 9624120]
11. Garner B, Roberg K, Qian M, Eaton JW, Truscott RJ. Distribution of ferritin and redox-active transition metals in normal and cataractous human lenses. *Exp Eye Res.* 2000; 71(6):599–607. [PubMed: 11095912]
12. Goralska M, Fleisher LN, McGahan MC. Ferritin H- and L-chains in fiber cell canine and human lenses of different ages. *Invest Ophthalmol Vis Sci.* 2007; 48(9):3968–3975. [PubMed: 17724174]
13. Balla G, Jacob HS, Balla J, Rosenberg M, Nath K, Apple F, Eaton JW, Vercellotti GM. Ferritin: a cytoprotective antioxidant strategem of endothelium. *J Biol Chem.* 1992; 267(25):18148–18153. [PubMed: 1517245]
14. Goralska M, Holley BL, McGahan MC. Identification of a mechanism by which lens epithelial cells limit accumulation of overexpressed ferritin H-chain. *J Biol Chem.* 2003; 278(44):42920–42926. [PubMed: 12920121]
15. Cham BE, Roeser HP, Nikles A, Ridgway K. A procedure for the purification of ferritin from human liver by heating a methanol-treated homogenate. *Anal Biochem.* 1985; 151:561–565. [PubMed: 4096386]
16. McGahan MC, Harned J, Grimes AM, Fleisher LN. Regulation of ferritin levels in cultured lens epithelial cells. *Exp Eye Res.* 1994; 59(5):551–555. [PubMed: 9492756]
17. Harrison PM, Arosio P. The ferritins: molecular properties, iron storage function and cellular regulation. *Biochim Biophys Acta.* 1996; 1275(3):161–203. [PubMed: 8695634]
18. Zheng H, Bhavsar D, Dugast I, Zappone E, Drysdale J. Conserved mutations in human ferritin H pseudogenes: a second functional sequence or an evolutionary quirk? *Biochim Biophys Acta.* 1997; 1351(1–2):150–156. [PubMed: 9116028]
19. Quaresima B, Tiano MT, Porcellini A, D'Agostino P, Faniello MC, Bevilacqua MA, Cimino F, Costanzo F. PCR analysis of the H ferritin multigene family reveals the existence of two classes of processed pseudogenes. *PCR Methods Appl.* 1994; 4(2):85–88. [PubMed: 7580890]
20. Shestopalov VI, Bassnett S. Expression of autofluorescent proteins reveals a novel protein permeable pathway between cells in the lens core. *J Cell Sci.* 2000; 113(Pt 11):1913–1921. [PubMed: 10806102]
21. Fu S, Dean R, Southan M, Truscott R. The hydroxyl radical in lens nuclear cataractogenesis. *J Biol Chem.* 1998; 273(44):28603–28609. [PubMed: 9786852]

FIGURE 1.

Western blot analysis and densitometric quantitation of ferritin L-chain in fiber cells of cataractous (C) and non-cataractous lenses (NC). The blot shown is from a representative experiment. Proteins from lens fiber homogenates (50 µg/sample), were separated by 15% SDS-Page under reducing conditions and blotted onto nitrocellulose membranes. Ferritin L-chain was identified with anti-ferritin L-chain antibodies. Results are expressed as mean $\pm$ SEM for single lens homogenates from 4 different dogs used in each group (* $P < 0.05$; significantly different from value in non-cataractous lenses). Standard: S, canine liver ferritin L-chain.

FIGURE 2.

Western blot analysis and densitometric quantitation of ferritin H-chain in fiber cells of cataractous (C) and non-cataractous lenses (NC). The blot shown is from a representative experiment. Proteins from lens fiber homogenates (50 μ g/sample), were separated by 15% SDS-PAGE under reducing conditions and blotted onto nitrocellulose membranes. Ferritin H-chain was identified with anti-ferritin H-chain antibodies. Results are expressed as mean $\pm$ SEM for single lens homogenates from 4 different dogs used in each group (* P < 0.05; significantly different from value in non-cataractous lenses). Standard: S, canine heart ferritin H-chain.

FIGURE 3.

Comparison of in vitro binding of ^{59}Fe to lens fiber cell ferritin from cataractous (C) and non-cataractous (NC) lenses. Lens fiber cell homogenates containing the same amount of protein were incubated with $^{59}\text{FeCl}_3$. Labeled proteins were separated by 8% SDS-PAGE under nonreducing conditions. The location of assembled ferritin in homogenate samples was compared to the mobility pattern of 450 kDa canine liver holoferritin standards. The autoradiogram shown is from a representative experiment. Iron labeled ferritin was autoradiographed and quantitated using electronic autoradiography. Results are expressed as mean $\pm$ SEM for single lens homogenates from 4 different dogs used in each group (* $P < 0.05$; significantly different from value in non-cataractous lenses).

FIGURE 4.

Comparison of assembled ferritin content in lens fiber cells of cataractous (C) and non-cataractous lenses (NC) as measured by ELISA using goat anti-horse ferritin antibodies. Results are expressed as mean $\pm$ SEM for single lens homogenates from 4 different dogs used in each group.

FIGURE 5.

Comparison of H (A) and L-chain (B) makeup of assembled ferritin from four cataractous (C) and four non-cataractous (NC) lenses. Lens fiber cell proteins were separated by 8% SDS-PAGE under non-reducing conditions. Location of assembled ferritin was identified based on the mobility pattern of colored holo-ferritin standard. Gel pieces containing assembled ferritin were excised, loaded into 15% gel under reducing conditions and ferritin chains were separated by SDS-PAGE. Immunoblotted ferritin chains were detected with anti-ferritin chain antibodies. S; H- and L-chains from canine heart and liver standards respectively. The images were digitalized and were quantitated with gel software (C,D).

FIGURE 6.

Distribution of ferritin H- and L- chains in normal and cataractous canine lenses.

Serial longitudinal sections (cut parallel to the visual axis) from one normal (A–C), and three different cataractous lenses (D–F, G–H, and I–J) were incubated with ferritin H- or L-chain specific antibodies or normal rabbit serum, followed by Alexa Fluor® 568 conjugated secondary antibodies (red fluorescence) and DAPI staining (blue fluorescence). Each panel is a montage of separate low magnification images to provide a view from the equator to the nuclear region of each section. N marks the center most nuclear region of each lens section.

Bars = 1.0 mm.

Table 1

Comparison of subunit makeup of assembled ferritin from non-cataractous (NC) and cataractous lenses (C). Values obtained for non-cataractous lenses were used as 100% reference points.

	L-chain		H-chain		H/total L
	19 kDa	30 kDa	12 kDa	29 kDa	
NC	100	100	–	100	100
C	60.6±8.3	118.7±36.1	–	358.3±157.0	465.8±165.8