Changes in Ferritin H- and L-Chains in Canine Lenses with Age-Related Nuclear Cataract

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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 noncataractous 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 noncataractous and cataractous lenses of similarly aged dogs. The ferritin L-chain detected in both lens types was modified and was approximately 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 the 21-kDa standard canine H-chain and from the 12-kDa modified H-chain present in fiber cells of noncataractous lenses. Histologic analysis revealed that the H-chain was distributed differently throughout cataractous lenses compared with noncataractous 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 noncataractous lenses.

Conclusions. There are significant differences in the characteristics of ferritin H-chain and its distribution in canine cataractous lenses compared with noncataractous 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. (*Invest Ophthalmol Vis Sci.* 2009;50:305–310) DOI:10.1167/iovs.08-2230

Development of age-related cataract is likely determined by 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 formation.[1] However, alterations such as various post-translational modifications of lens crystallins and decreased reduced glutathione concentration are present in cataractous and aging, but still transparent, lenses. This makes it difficult to dissociate normal age-related changes from those correlated with the development of age-related cataract.[2] 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 βBp crystallin in Philly cataract[3] or ferritin L-chain in hereditary hyperferritinaemia cataract syndrome.[4,5]

The correlation of cataract progression with a decrease in the level of reduced glutathione[6] 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 radicals.[9] 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), that controls the level of "free" highly reactive iron.[10] The H and L subunit ratio of mammalian ferritin is highly tissue specific. Each ferritin subunit has a 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 to the core of the ferritin shell for long-term storage. Although ferritin is present throughout the whole lens,[11] the level of properly assembled ferritin declines with age.[12] Additionally, the H- and L-ferritin chains detected in human and canine fiber cells of different age lenses are significantly altered.[12] Substantial changes in ferritin concentration, subunit structure, and/or tissue-specific H/L ratio could augment or diminish the capacity of the protein to store iron, thus decreasing or increasing the possibility of oxidative damage.[13]

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 (estimated age range, 8–10 years) after euthanatization at the Johnston County, North Carolina Animal Shelter. 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

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glaucoma. Lenses were divided into two categories: those with and those without visible opacities in the nucleus of the lens. If only one lens from a dog was available, half the lens was used to prepare the homogenates of lens fiber cells, and the other half was used for immunolocalization of ferritin chains. Lenses that were cut in half were cut fresh with a razor blade parallel to the visual axis. The lenses used for biochemical studies were stored at −20°C for no longer than 1 month. Lenses used for immunolocalization were stored at 4°C in fixative for a minimum of 1 week.

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 (6–10 mL/g tissue), containing 2% SDS and protease inhibitor cocktail for mammalian cells (Sigma, St. Louis, MO). Homogenates were centrifuged at 13,000*g* for 10 minutes. The concentration of protein of lens fiber homogenates was determined (BCA Protein Assay Kit; Pierce, Rockford, IL).

Immunodetection of Ferritin Chains in Canine Fiber Homogenates

Samples containing 50 μg protein were separated by 15% SDS-PAGE. Proteins were transferred to nitrocellulose membranes (Hybond ECL; Amersham Biosciences, Piscataway, NJ) by semidry blotting at 20 V for 20 to 30 minutes. Chain-specific peptides, designed based on the amino acid sequences of canine H- and L-chains,[14] were conjugated and used to produce antibodies in rabbits (Research Genetics Inc., Invitrogen, Carlsbad, CA). These antibodies were used in immunodetection of ferritin chains. HRP-anti–rabbit IgG antibodies (TrueBlot; eBioscience, San Diego, CA) were used in the second step of detection. Immunoreactivity was determined (ECL Western Blotting Analysis System; Amersham, Biosciences) and the images were digitized and evaluated with gel software (UN-SCAN-IT; Silk Scientific, Orem, UT).

In Vitro ^{59}Fe Labeling of Assembled Ferritin

Samples of lens fiber homogenates (0.7–1.6 mg protein in 25 μL) were incubated with 1 μL $^{59}FeCl_3$ (15.8 mCi/mL; 40.3m Ci/mg; PerkinElmer, Boston, MA) for 1 hour at room temperature. Subsequently, lens proteins were separated by 8% SDS-PAGE under nonreducing conditions. Binding of ^{59}Fe by assembled ferritin was measured (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 to 1.5 mg protein were separated by 8% SDS-PAGE under nonreducing 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 minutes with SDS-PAGE loading buffer (25 mM Tris/HCl, pH 6.5, 10% glycerol, 6% SDS) containing 50 mM dithiothreitol. Gel pieces were inserted into the wells, and ferritin chains were separated by 15% SDS-PAGE under reducing conditions. The chains were identified by Western blot, as described.

Measurement of Ferritin Content by ELISA in Canine 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., Montgomery, TX), as described previously.[16] ABTS (KPL) was used as a substrate. Optical density of the samples was read at 405 nm.

Immunolocalization of Ferritin Chains

Cataractous and age-matched noncataractous lenses were collected as described. In some cases, half the lens was used for Western blotting and the other half was fixed in 4% formaldehyde in 10 mM Sorenson sodium phosphate buffer at room temperature for several hours and then stored at 4°C in the same fixative until sectioned. Fifty-micrometer sagittal sections were cut in a sectioning system (Vibratome; Ted Pella, St. Louis, MO) and transferred to membrane inserts (Netwell; Corning, Corning, NY) 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 minutes, blocked in 5% normal goat serum (Jackson ImmunoResearch Laboratories, West Grove, PA) in PBS for 1 hour, and incubated with rabbit polyclonal antibodies to canine H- or L-ferritin chains[14] 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. After three 10-minute washes in PBS, sections were incubated for 2 hours at room temperature in darkness with goat anti–rabbit IgG antibodies (AlexaFluor 568; Invitrogen) diluted 1:1000 in PBS. After three 10-minute washes in PBS, sections were mounted on slides in antifade reagent containing DAPI (ProLong Gold; Invitrogen). Sections were then imaged using a microscope (DM5000B; Leica, Wetzlar, Germany) with a 5× objective lens and conventional epifluorescence. Images were captured with a cooled CCD camera (Retiga 1300; QImaging, Surrey, BC, Canada) and imaging software (Simple PCI; Compix, Inc.) and then were arranged (Photoshop CS2; Adobe Systems, Inc., San Jose, CA). 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 Noncataractous Canine Lenses

Anti–ferritin L-chain antibodies immunodetected a 30-kDa protein in fiber cells of noncataractous 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 one third of modified L-chain in comparison to noncataractous 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 noncataractous and cataractous lenses differed from those of the canine heart ferritin H-chain standard (21 kDa) used as a control. Anti–ferritin H-chain antibodies detected proteins of two different sizes: an approximately 29-kDa protein found only in cataractous lenses and a 12-kDa protein found in lenses from both groups (Fig. 2).

The content of the 12-kDa protein in cataractous lenses was less than 20% that in noncataractous 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 noncataractous lenses. The 29-kDa protein was not detected in noncataractous lenses (Fig. 2). No normal-sized (21-kDa) ferritin H-chain was detected in any lens fiber homogenates.

Detection of Assembled Ferritin in Lens Fiber Cell Homogenates of Noncataractous 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

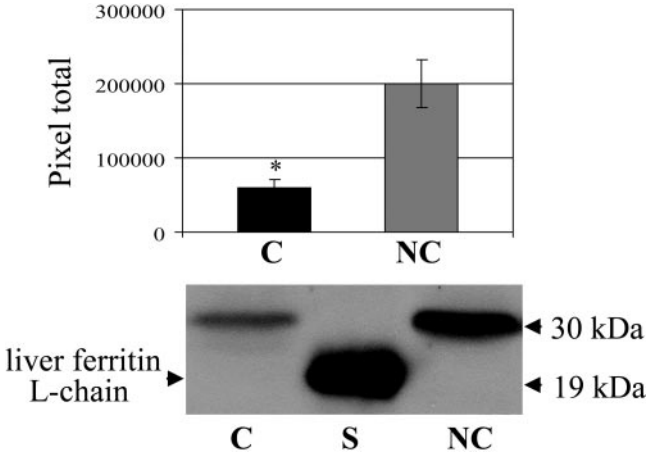

FIGURE 1. Western blot analysis and densitometric quantitation of ferritin L-chain in fiber cells of cataractous (C) and noncataractous (NC) lenses. The blot shown is from a representative experiment. Proteins from lens fiber homogenates (50 μg/sample) were separated by 15% SDS-PAGE under reducing conditions and were blotted onto nitrocellulose membranes. Ferritin L-chain was identified with anti–ferritin L-chain antibodies. Results are expressed as mean ± SEM for single lens homogenates from four different dogs used in each group (*$P < 0.05$; significantly different from value in noncataractous lenses). Standard: S, canine liver ferritin L-chain.

times more iron than ferritin from noncataractous lenses of similar age.

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

To determine whether the higher iron-binding capacity of cataractous lens homogenates (Fig. 3) resulted from an increased concentration of assembled ferritin, ferritin levels were measured by ELISA using the antibodies that predominantly recognized assembled ferritin and had 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 noncataractous lenses (Fig. 4).

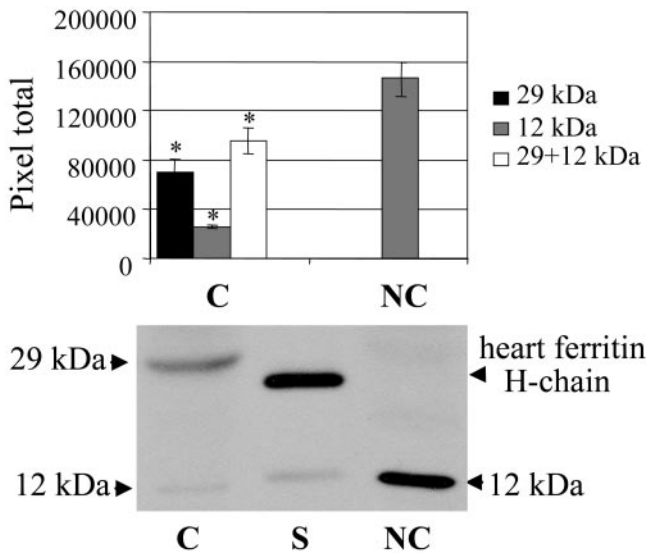

FIGURE 2. Western blot analysis and densitometric quantitation of ferritin H-chain in fiber cells of cataractous (C) and noncataractous lenses (NC). The blot shown is from a representative experiment. Proteins from lens fiber homogenates (50 μg/sample) were separated by 15% SDS-PAGE under reducing conditions and blotted onto nitrocellulose membranes. Ferritin H-chain was identified with anti–ferritin H-chain antibodies. Results are expressed as mean ± SEM for single lens homogenates from four different dogs used in each group (*$P <$ 0.05; significantly different from value in noncataractous lenses). Standard: S, canine heart ferritin H-chain.

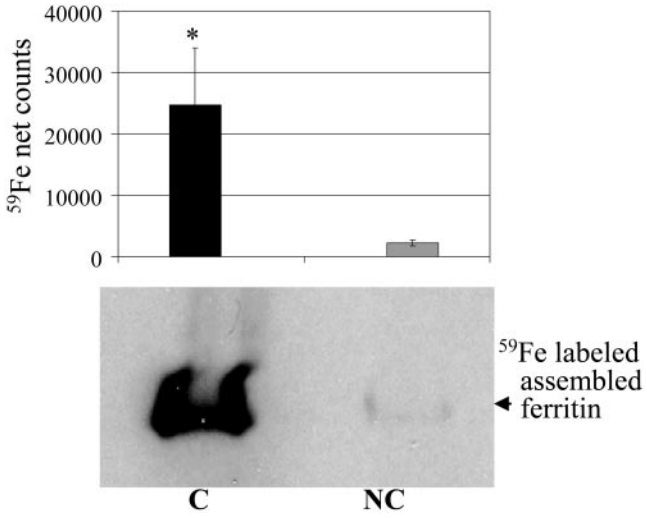

FIGURE 3. Comparison of in vitro binding of ^{59}Fe to lens fiber cell ferritin from cataractous (C) and noncataractous (NC) lenses. Lens fiber cell homogenates containing the same amount of protein were incubated with $^{59}FeCl_3$. Labeled proteins were separated by 8% SDS-PAGE under nonreducing conditions. 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 ± SEM for single lens homogenates from four different dogs used in each group (*$P < 0.05$; significantly different from value in noncataractous lenses).

Analysis of Subunit Makeup of Assembled Ferritin from Fiber Cells of Noncataractous 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 Methods. 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 noncataractous lenses contained the 29-kDa modified H-

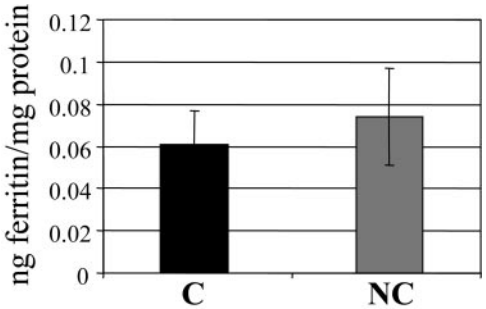

FIGURE 4. Comparison of assembled ferritin content in lens fiber cells of cataractous (C) and noncataractous (NC) lenses as measured by ELISA using goat anti–horse ferritin antibodies. Results are expressed as mean ± SEM for single lens homogenates from four different dogs used in each group.

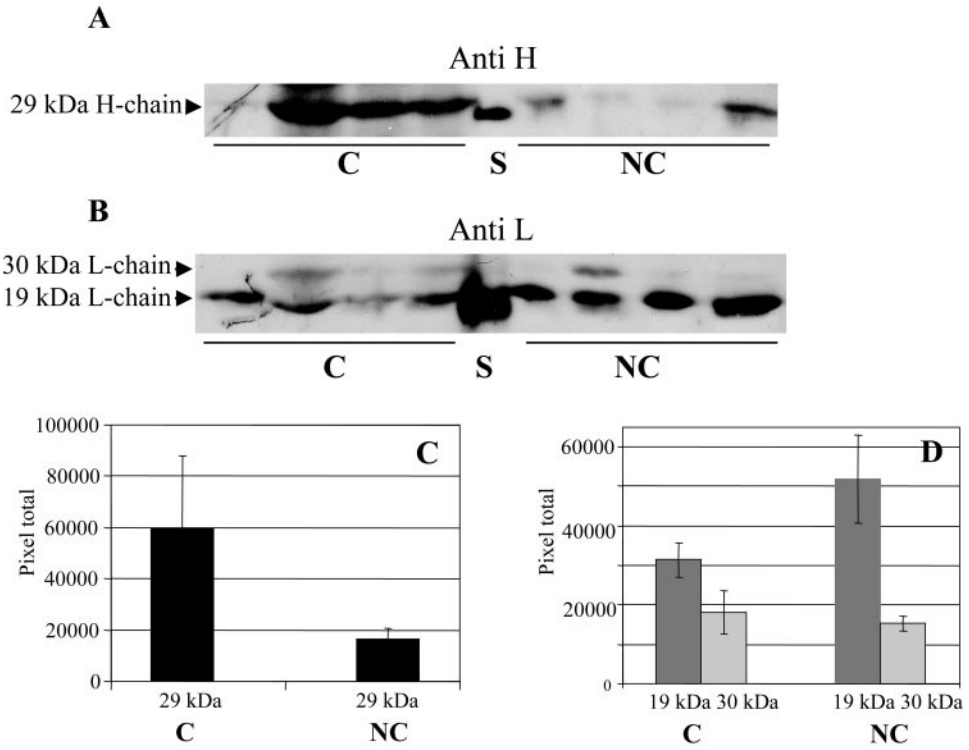

FIGURE 5. Comparison of H-chain (**A**) and L-chain (**B**) makeup of assembled ferritin from four cataractous (C) and four noncataractous (NC) lenses. Lens fiber cell proteins were separated by 8% SDS-PAGE under nonreducing conditions. Location of assembled ferritin was identified based on the mobility pattern of colored holoferritin standard. Gel pieces containing assembled ferritin were excised and 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. Images were digitized and quantitated with gel software (**C**, **D**).

chain (Fig. 5A). Neither ferritin H-chain of the proper size (21 kDa) nor the 12-kDa modified ferritin H-chain, which was detected in total homogenates of noncataractous and cataractous lenses (Fig. 2), was found as a subunit of assembled ferritin. Fiber cells of cataractous lenses contained more of the 29-kDa H-chain than noncataractous lenses (Fig. 5C, 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, than did ferritin from the noncataractous lenses (Fig. 5D; Table 1). There was no difference in content of modified 30-kDa L-chain in assembled ferritin between noncataractous and cataractous lenses (Fig. 5D; Table 1). The ratio (H/L) of heavy (29 kDa) to light (30 + 19 kDa) chains in assembled ferritin, based on quantitated images, was significantly higher for cataractous lenses than for noncataractous lenses. The higher ratio resulted from both the higher content of the H-chain and the 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 noncataractous and cataractous lenses with respect to the ferritin size variants found in fiber cells, we examined the distribution of ferritin H- and L-chain sections of cataractous and similarly aged noncataractous dog lenses using immunofluorescence 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 noncataractous 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 μm from the epithelium, as measured from the equator (Figs. 6A, 6B, 6D, 6E, 6G–J). Ferritin L-chain signals in these outermost cortical fiber cells were generally lower in cataractous lenses than in noncataractous lenses (Figs. 6B, 6E, 6H, 6J), consistent with Western blotting results (Fig. 1). Interestingly, additional brighter bands of ferritin H-chain–specific signals were detected in fiber layers further toward the center, but only in cataractous lenses (Figs. 6D, 6G, 6I). These bands were never observed in noncataractous lenses; instead, ferritin H- and L-chain signals were restricted to the outer cortex (Figs. 6A, 6B). The unique bands of ferritin H-chain labeling extended up to 1.8 mm from the epithelium, as measured from the equator, and 200 μm 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 cata-

TABLE 1. Comparison of Subunit Makeup of Assembled Ferritin from Noncataractous and Cataractous Lenses

	L-Chain		H-Chain		
	19 kDa	30 kDa	12 kDa	29 kDa	H/Total L
Noncataractous	100	100	—	100	100
Cataractous	60.6 ± 8.3	118.7 ± 36.1	—	358.3 ± 157.0	465.8 ± 165.8

Values obtained for noncataractous lenses were used as 100% reference points.

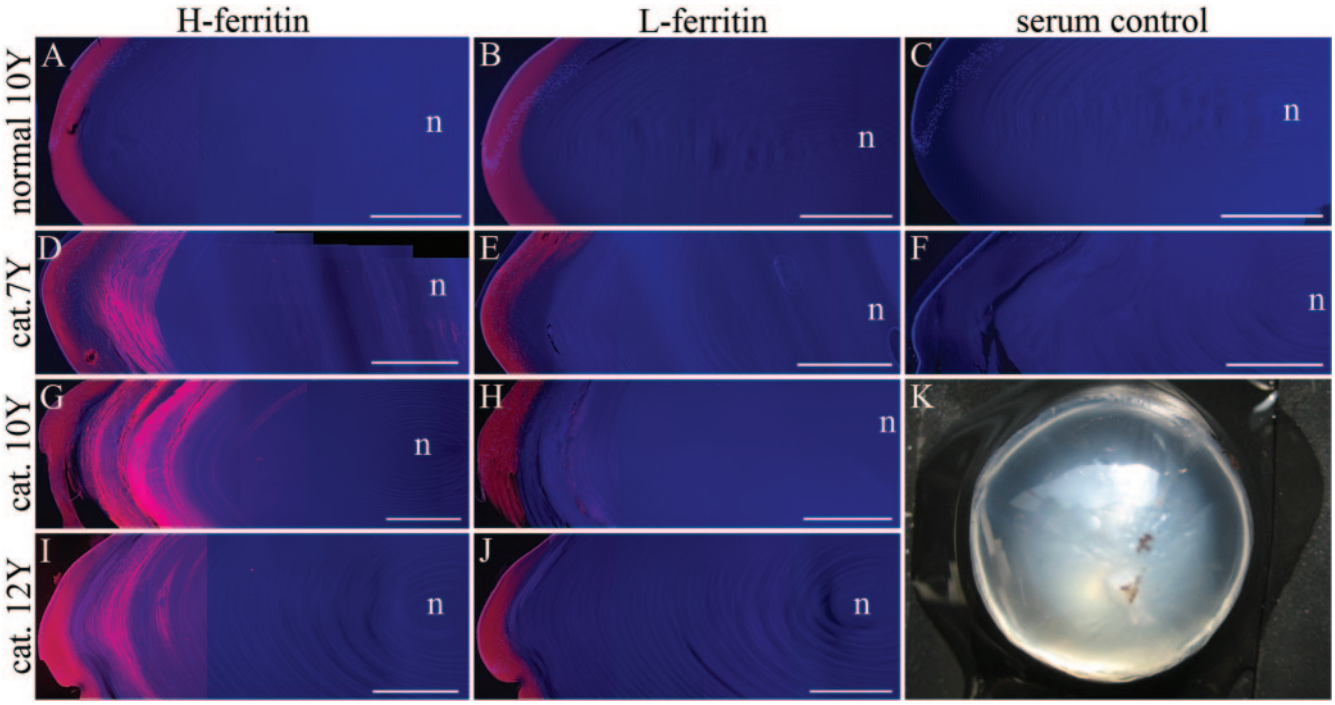

FIGURE 6. Distribution of ferritin H- and L- chains in noncataractous and cataractous canine lenses. Serial longitudinal sections (cut parallel to the visual axis) from one noncataractous (**A–C**) and three cataractous (**D–F**, **G–H**, and **I–J**) lenses were incubated with ferritin H- or L-chain–specific antibodies or normal rabbit serum, followed by fluorescent secondary antibodies (*red*) and DAPI staining (*blue*). 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, centermost nuclear region of each lens section. Scale bars, 1 mm.

ractous lenses. These gaps were also observed between inner ferritin H-chain bands (Figs. 6G, 6I). 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 (Figs. 6C, 6F).

DISCUSSION

This is the first comparative study to characterize ferritin H- and L-chains in fiber cells of noncataractous 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. Cataractous lens fiber cells, however, contained significantly less (threefold) modified L-chain than noncataractous lenses of similar age (Fig. 1). The proper size L-chain was found only in assembled ferritin (Fig. 5B), and the amount of the chain was lower (by 40%) in cataractous lenses than in noncataractous lenses (Table 1). The concentration of assembled ferritin in lens fiber cells from both lens types was low compared with 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 a small amount of the 30-kDa modified L-chain (Fig. 5D), the content of which was similar in noncataractous 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 cells.[12] We previously did not detect the presence of the 30-kDa L-chain in lens epithelial cells, which contained only normal-sized (19-kDa) L-chain.[12] Therefore, based on our previous[12] 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-sized L-chain remained in these differentiated fiber cells, most likely as a subunit of assembled ferritin rather than as a free chain (Figs. 5B, 5D). There was a further decrease in the content of normal-sized L-chain associated with the formation of nuclear cataract (Table 1). Because the major role of the L-chain is the long-term storage of iron,[17] 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 unable to detect proper-sized (21-kDa) ferritin H-chains in fiber cells either as free chain in total homogenates or in assembled ferritin. This was true for noncataractous 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 noncataractous canine lenses as young as 3 months.[12] 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 noncataractous 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 the H-chain undergoes different posttranslational modifications in cataractous lens fiber cells than it does in noncataractous lens fiber cells, but it is also possible that the 29-kDa protein is a

dimer of 12-kDa H-chains because 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, cataractogenesis, or both. 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, 1998). In this scenario, the 29-kDa H-chain would be synthesized de novo in the 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 (Figs. 6 D, G, I) may reflect such accumulation. It is known that macromolecules can move between lens fiber cells.[20] Given that 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 only 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 the lower content of proper-sized L-chain in cataractous lenses increased the H/L ratio of assembled ferritin more than fourfold compared with assembled ferritin from noncataractous 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 noncataractous 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 because only H-chain has ferroxidase activity.[17] 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 because there was no difference in the concentration of assembled ferritin between noncataractous and cataractous lenses (Fig. 4).

The results of this investigation revealed significant differences in ferritin L- and H-chain characteristics, subunit makeup of assembled ferritin, and distribution of ferritin chains between noncataractous and cataractous canine lenses. There is evidence that human cataractous lenses have higher concentrations of iron and, therefore, increased ability to generate hydroxyl radicals,[11] which has been implicated in nuclear cataractogenesis.[21] 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 its major antioxidant, reduced glutathione, from the metabolically active cortex to the lens nucleus.[6] 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 for full understanding of the possible role of ferritin in the prevention of lens opacification caused or exacerbated by iron-catalyzed oxidative damage.

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