Structural Evidence of Human Nuclear Fiber Compaction as a Function of Ageing and Cataractogenesis

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This study was conducted to quantify structural change associated with human nuclear fiber compaction as a function of ageing and nuclear cataract formation. Normal donor lenses in three age ranges, young (15–25 years), middle-aged (36–46 years) and aged (59–81 years) were compared to each other and to age-related nuclear cataracts (55–81 years) surgically removed by extracapsular extraction. Several structural modifications which occurred as a manifestation of fiber compaction were noted. In the fetal nucleus (FN), the average anterior and posterior fiber angles decreased approximately 20% with age. Additionally, there was a reduction in the thickness of both the anterior and posterior segments of fetal fibers with age. On average, the anterior–posterior (A–P) axis in the embryonic nucleus (EN) decreased 33% with age. The average length of EN fibers decreased significantly (37%) as a function of age. This change in EN fiber length was accomplished by effective compaction folds along fiber length. By comparison, in nuclear cataracts the anterior and posterior angles of FN fibers were about 12% smaller than comparably aged normal lenses. Similarly, the A–P axis and the length of EN fibers were 13% smaller than age-matched normals. Nuclear fiber compaction in early adulthood was significant and may contribute to the lens hardening and loss of accommodative ability symptomatic of presbyopia. 3D-CAD reconstructions of fiber compaction show how the reduction in the spacing of lateral interdigitations along fiber length causes an increase in the fiber membrane complexity along the A–P axis in relation to fiber cytoplasm as light passes through lenses. These results may explain, at least in part, how an increase in large particle scatter occurs as light is transmitted through fiber membranes, resulting in reduced lens optical quality as a function of age. By extrapolation, the significantly increased compaction of nuclear fibers in age-related nuclear cataracts may be a contributing factor for excessive scatter in nuclear opacification.

Key words: crystalline lens; ageing; nuclear cataracts; morphology; scanning electron microscopy.

1. Introduction

The structure of lens fibers changes significantly both with maturation and senescence. Recent studies have shown that fibers in the adult and juvenile nuclear regions undergo considerable age-related compaction (Taylor et al., 1996; Al-Ghoul and Costello, 1997). Compaction of the central fibers has also been inferred from the shape of lamellar cataracts with increasing age (Brown, Sparrow and Bron, 1988). It is well established that variations in fiber structure adversely affect the optical properties of the lens (Kuszak, Sivak and Weerheim, 1991; Kuszak et al., 1994, 1999, 2000; Sivak et al., 1994). It follows that substantial senescent alterations in the structure of the embryonic and fetal nuclear fibers would lead to degradation of lens optical quality, especially since those fibers are located entirely within the region defined by the pupillary margin. In fact, clinical observations of aged lenses show increased light scatter even without overt visual impairment (Lerman, 1985).

Light scatter in the lens has been attributed to the interaction of the incident beam with both the cell membranes and the cytoplasmic proteins, producing respectively, large and small particle scatter (Trokel, 1962). It has been suggested that in normal lenses, the majority of light scatter originates from interactions with fiber membranes, which have a higher refractive index as compared to the cytoplasm (Bettleheim, 1985). The cytoplasm is virtually transparent due to the close association of the crystallin proteins which minimizes refractive index fluctuations (Benedek, 1971; Delaye and Tardieu, 1983). Although numerous biochemical modifications have been noted in the cytoplasmic and membrane components of lenses with age-related nuclear cataracts, the sources of excessive light scatter have yet to be definitively identified.

In most age-related nuclear cataracts, opacification begins in the lens center, and enlarges gradually. It has been noted clinically that cataracts often have reduced...
anterio–posterior thickness as compared to age-matched normal lenses (Laursen and Fledelius, 1979). Significant compaction along the anterior–posterior (A–P) axis in the oldest lens fibers could account for the ‘thinning’ of cataractous lenses as well as the typical etiopathology wherein the embryonic
and fetal nuclei display the greatest amount of light scatter. Therefore, the purpose of this investigation was to quantify structural parameters of human lens fibers in the fetal (FN) and embryonic (EN) nuclear regions as a function of age and cataractogenesis. Our results will demonstrate that significant age-related compaction of the oldest fibers occurs, primarily affecting lens thickness by decreasing the length of the A–P axis in the lens nucleus. In addition, age-related nuclear cataracts displayed significantly more compaction than age-matched normal lenses, reflecting the pathological changes which may be responsible for cataractogenesis.

2. Materials and Methods

Lenses

Non-cataractous, normal human lenses from donor eyes were obtained via the National Disease and Research Interchange (Philadelphia, PA, U.S.A.). These lenses were divided into three age groups: young (15–25 years, n = 5), middle-aged (36–46 years, n = 9), and aged (59–81 years, n = 13). Human age-related nuclear cataracts (55–81 years, n = 9) obtained from the Duke Eye Center (Durham, NC, U.S.A.) following extracapsular extraction, were used in this investigation. All normal and cataractous lenses were obtained following the tenets of the Declaration of Helsinki.

Specimen Preparation

All lenses were fixed for 24 hr at room temperature in 10% neutral buffered formalin (in 0.1 M phosphate buffer), then for 3–5 days in 2.5% glutaraldehyde (in 0.12 M sodium cacodylate buffer, pH 7.2) at room temperature with fresh fixative changes daily. After overnight washing in 0.2 M sodium cacodylate buffer, the equatorial and A–P axial dimensions were measured under a dissecting microscope (Zeiss, New York, NY, U.S.A.), then dissected as previously described (Kuszak et al., 1988). Briefly, a cylindrical core encompassing the lens diameter at birth, was removed using a 6 mm trephine, then the anterior and posterior disks were detached using fine forceps. This process was continued until the ‘Y’ suture pattern, indicative of the fetal nucleus, was exposed. The remaining lens core, containing the fetal and embryonic fibers, was split along the A–P axis. In addition, age-related nuclear cataracts displayed significantly more compaction than age-matched normal lenses, reflecting the pathological changes which may be responsible for cataractogenesis.

Dissected lenses were post-fixed in 1% aqueous osmium tetroxide at 4°C overnight, washed in 0.2 M sodium cacodylate buffer, then dehydrated through a graded ethanol series. After overnight dehydration in 100% ethanol, the ethanol was replaced through a graded ethanol/freon 113 series to pure freon 113. Specimens were critical point dried in Freon 23 (Dupont, Wilmington, DE, U.S.A.) in a Balzers CPD 020 (Balzers, Hudson, NH, U.S.A.), secured on aluminum stubs with silver paste, sputter coated with gold and examined in a JEOL JSM 35c scanning electron microscope (JEOL USA, Peabody, MA, U.S.A.) at 15 kV. Electron micrographic magnification series’ were taken (from 30 to 3000 x) for each specimen.

Morphometry

To determine appropriate parameters to measure for this study, we considered which structural features of fetal and embryonic fibers would be affected by compaction of the lens along the A–P axis. Since embryonic fibers are oriented parallel to the optic axis, both the length of individual fibers and the A–P axis of the EN would be affected. In addition, the surface morphology of EN fibers could be greatly altered during compaction. In the FN, crescent-shaped fibers extend toward the anterior and posterior sutures forming one half of an asymmetric ellipse. Both the elliptical angles described by the anterior and posterior segments of fibers and the thickness of individual fiber ends would be modified by compaction along the A–P axis. Fiber end thickness would be reflected in the average number of fibers in a given length.

In consideration of the above factors, seven quantifiable structural parameters were defined. Measurements were made directly from micrographs as indicated in Fig. 1 and numbered as follows: (1) the ellipsoid angle described by anterior portions of fetal fibers; (2) the ellipsoid angle described by posterior portions of fetal fibers; (3) the average number of FN fibers in an anterior radial cell column per 10 μm length; (4) the average number of FN fibers in a posterior radial cell column per 10 μm length; (5) the A–P axis of the EN in μm; (6) the average length of embryonic fibers in μm; and (7) the average number of compaction folds along embryonic fibers per 20 μm. It should be noted that due to variations in the dissection process, it was not possible to measure every parameter in all lenses.

Fig. 1. Scanning electron micrographs, demonstrating methods for morphometry of nuclear lens fibers. (A) An aged normal human lens nucleus split along the optic axis exposes fibers in the EN and FN. The arcs (dotted lines) denote the ellipsoidal angles described by fetal fibers at 1 mm from the lens center: No. 1 = anterior segment, no. 2 = posterior segment. Ellipse templates were used to make measurements directly from micrographs. (B)–(C) Left and right insets from (A) of, respectively, anterior (no. 3) and posterior (no. 4) radial cell columns. The number of fibers in a 10 μm radial cell column was used to calculate the average thickness of anterior and posterior fiber segments. (D) Central inset from (A), showing fibers in the EN. The EN optic axis (no. 5) was measured from the anterior to posterior pole (unbroken line). The average EN fiber length (no. 6) was determined using measurements of intact EN fibers (dashed line). (E) Inset from (D). The number of compaction folds in a 20 μm length (no. 7) was measured along the broad face of EN fibers.
The ellipsoid angles of fetal nuclear fibers were measured at 1 mm from the lens center, with the major axis of the ellipse equal to 1 mm along the equatorial axis of the lens. Ellipse templates (Alvin & Company Inc., Windsor, CT, U.S.A.) were utilized to obtain direct measurements of the anterior and posterior elliptical angles [Fig. 1(A), no. 1 and no. 2]. To measure the average number of fibers per 10 μm in an anterior or posterior radial cell column, fibers at 1 mm from the lens center (along the equatorial axis) were followed to their anterior or posterior segments [Fig. 1(A), small insets]. From four to six measurements were made from micrographs at approximately 2000 × magnification to obtain average values [Fig. 1(B), no. 3 and Fig. 1(C), no. 4].

Since, by definition, the embryonic fibers do not form sutures, the A–P axis of the EN was measured from the initiation of fetal suture planes at the anterior and posterior poles [Fig. 1(D), no. 5]. To obtain the average length of embryonic fibers in a given lens, six to 15 fibers were measured. Only fibers for which both ends could be unequivocally identified were measured [Fig. 1(D), no. 6]; fibers broken during specimen preparation were excluded. Finally, to obtain the average number of compaction folds per 20 μm in embryonic fibers, four to six measurements per lens were made at approximately 3000 × magnification [Fig. 1(E), no. 7].

Scaled 3-dimensional computer-assisted drawings (3-D CADs) of lens growth and embryonic fiber compaction were made using average dimensions of whole fixed lenses and measurements of fiber length from SEMs, respectively. 3-D CADs were constructed and animated in 3-D Studio Max version 2.5 (Kinetix, San Francisco, CA, U.S.A.) supplemented with Scalpel version 1.0 (Trinity Animation, Lee’s Summit, MO, U.S.A.) on a Pentium III pc platform.

### Statistical Analysis

As specified above, lenses were divided into four sample groups according to age and cataract state. For each variable, descriptive statistics were compiled in order to reveal trends. Non-parametric statistical analyses were utilized to determine statistical significance of results due to the small number of specimens in some groups. A Kruskal–Wallis test was used to determine whether the three age groups of normal lenses were significantly different (P ≤ 0.05). Three two-sided Mann–Whitney tests using a Bonferroni correction factor were performed (for each variable) to determine which pairs were significantly different (P ≤ 0.017). Finally, the cataractous lenses were compared to the age-matched normal lenses for each variable. A Mann–Whitney test was performed with significance for P-values ≤ 0.05.

### 3. Results

#### Morphological Changes as a Function of Ageing

SEM examination of lens cores demonstrated that the dissection process routinely exposed the fetal and embryonic fibers along the optic axis in lenses of various ages [compare Figs 2(A), 3(A) and 4(A), from the young, middle and aged normal lens groups, respectively]. At low magnification, lens cores displayed triangular suture planes indicative of the fetal nucleus. At medium magnification, the EN was unequivocally identified by the lack of suture planes and the fiber orientation, which was parallel to the A–P axis [Figs 2(B), 3(B) and 4(B)].

Young lenses had the largest average fetal fiber elliptical angle measurements of all the normal lenses examined (see Table I for a summary of morphometric data). In general, both the anterior and posterior elliptical angles decreased gradually with increasing age, for an overall reduction of approximately 20 %.

The average number of anterior FN fiber ends per 10 μm increased 35 % overall as a function of age. This corresponds to a decrease in average fiber thickness with ageing from 2.35 μm in young lenses to 1.82 μm in middle-aged lenses and 1.73 μm in aged lenses. It is apparent that the majority of the decrease in fiber end thickness occurred between the young and middle-aged groups; only a slight (5 %) change was noted from the middle-aged to aged lenses. Similarly, the average number of posterior FN fiber ends per 10 μm increased 26 % from the young to middle-aged lenses, while virtually no change was found between the middle-aged and aged lenses. Specifically, average posterior fiber end thickness decreased as a function of age from 2.49 μm in young lenses to 1.97 μm in middle-aged lenses and 1.96 μm in aged lenses. The average thickness of posterior fiber ends, as expected, was slightly greater than anterior ends in all age groups because the lens is an asymmetric spheroid.

In the lens center, measurements of the A–P axis length of the EN revealed a marked and progressive decrease with ageing. Specifically, the average length of the A–P axis was approximately 20 % shorter in middle-aged lenses as compared to young lenses, and showed an additional 15 % reduction in length in aged lenses. Consistent with the decrease in the length of the A–P axis, the average length of embryonic fibers sustained a comparable decrease as a function of ageing. EN fibers were 37 % shorter in aged lenses than in young lenses. These measurements reflected a change in the embryonic nuclear shape from a prolate spheroid in young lenses [Fig. 2(B)] to an oblate spheroid in middle-aged [Fig. 3(B)] and aged lenses [Fig. 4(B)]. Primary fiber organization appeared disordered at all ages examined, as documented previously in various species (Kuszak, Bertram and Rae, 1986; Kuszak et al., 1989; Taylor et al., 1996;...
## Table I

Summary of morphometric data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Young normals</th>
<th>Middle-aged normals</th>
<th>Aged normals</th>
<th>Age-related nuclear cataracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$ ± SD (Minimum and Maximum)</td>
<td>$\bar{x}$ ± SD (Minimum and Maximum)</td>
<td>$\bar{x}$ ± SD (Minimum and Maximum)</td>
<td>$\bar{x}$ ± SD (Minimum and Maximum)</td>
</tr>
<tr>
<td>Anterior FN Elliptical Angles (°) [1]</td>
<td>31 ± 2 (29,31)</td>
<td>27 ± 4 (23,32)</td>
<td>25 ± 2 (23,29)</td>
<td>22 ± 3 (18,26)</td>
</tr>
<tr>
<td>Posterior FN Elliptical Angles (°) [2]</td>
<td>34 ± 2 (32,38)</td>
<td>29 ± 4 (25,34)</td>
<td>27 ± 2 (25,30)</td>
<td>24 ± 3 (20,28)</td>
</tr>
<tr>
<td># Anterior FN Fibers/10 μm [3]</td>
<td>4.26 ± 0.09 (4.25-4.38)</td>
<td>5.48 ± 0.77 (4.30-6.63)</td>
<td>5.77 ± 0.44 (4.9-6.63)</td>
<td>5.77 ± 0.82 (4.6-6.63)</td>
</tr>
<tr>
<td># Posterior FN Fibers/10 μm [4]</td>
<td>4.02 ± 0.12 (3.83-4.13)</td>
<td>5.07 ± 0.75 (4.15-6.20)</td>
<td>5.09 ± 0.88 (4.75-6.73)</td>
<td>4.98 ± 0.58 (4.19-5.94)</td>
</tr>
<tr>
<td>EN A-P Axis Length (μm) [5]</td>
<td>211 ± 18 (200.232)</td>
<td>167 ± 20 (146.195)</td>
<td>141 ± 12 (120.160)</td>
<td>123 ± 9 (110.132)</td>
</tr>
<tr>
<td># EN Fiber Folds/20 μm [7]</td>
<td>0.97 ± 0.50 (0.5-1.5)</td>
<td>3.75 ± 1.58 (1.5-6.17)</td>
<td>5.40 ± 1.19 (2.75-6.67)</td>
<td>6.26 ± 0.48 (5.83-7.06)</td>
</tr>
</tbody>
</table>
FIG. 2. Scanning electron micrographs of young donor lenses. (A) Fetal fibers described average elliptical angles of 31° for the anterior segments and 34° for the posterior segments. Age = 20 years. (B) The EN was a prolate spheroid in lenses from this sample group, having an average A–P axis length of 211 μm. Age = 15 years in (B)–(D). (C) EN fibers displayed abundant lateral interdigitations (arrows), while broad (asterisks) and narrow faces of fibers were relatively flat. Lenses in this sample group had an average of only 0.97 compaction folds per 20 μm, although none were present in the young lens depicted here. (D) Polygonal domains of furrowed membranes were conspicuous on the oldest fibers (arrowheads).
FIG. 3. Scanning electron micrographs of middle-aged donor lenses. (A) Low magnification overview of a split lens showing the reduction in elliptical angles in these lenses (27° for anterior, 29° for posterior) as compared to young lenses. Age = 45 years. (B) The shape of the EN was an oblate spheroid in this age group, reflecting the decrease in the length of the A–P axis (x̄ = 167 μm). Age = 38 years in (B)–(D). (C) Most EN fibers exhibited low-amplitude accordion-like compaction folds along their length, however, regions of flat membranes were also present (asterisks). On average, middle-aged lenses had 3.75 compaction folds per 20 μm. (D) At high magnification, the furrowed membrane domains and lateral interdigitations were comparable to those in young lenses. Arrows denote compaction folds.
The surface morphology of EN fibers was profoundly altered as a consequence of ageing. In young lenses, fibers in the EN had numerous lateral edge processes [Fig. 2(C), arrows], were variable in width, and had relatively flat broad and narrow faces [Fig. 2(C), asterisks]. Surprisingly, at high magnification, EN fibers in young lenses displayed the prominent polygonal domains of furrowed membranes [Fig. 2(D), arrowheads] which have been previously associated with membrane senescence (Kuszak et al., 1988). In middle-aged lenses, EN fibers exhibited accordion-like folds along their length [Fig. 3(C) and (D)]. In this sample group, the folds were of relatively low-amplitude and areas of flat membrane were present [Fig. 3(C), asterisks]. In contrast, EN fibers of aged lenses were characterized by numerous, prominent accordion-like folds along their length [Fig. 4(C) and (D)]. Quantification of the average number of fiber folds per 20 μm demonstrated that their frequency increased more than 450% (or approximately 5.6 times) with age. Additionally, most aged lenses appeared to have higher-amplitude folds than middle aged lenses [compare Figs 3(D) and 4(D)], however, this feature was not quantified. Furrowed membrane domains were present, as in young lenses, on fibers in both middle-aged and aged lenses [Figs 3(D) and 4(D)].

**Morphological Changes as a Function of Cataractogenesis**

Low magnification SEM demonstrated that the morphology in the nuclear cores of age-related nuclear cataracts was comparable to that in normal aged donor lenses [compare [Figs 4(A) and 5(A) and (B)]]. Consistent with previous investigations, there was no evidence of major disruptions of fiber structure in opaque regions (Costello, Oliver and Cobo, 1992; Al-Ghoul and Costello, 1996; Al-Ghoul et al., 1996).

Morphometric analysis revealed that nuclear cataracts had increased compaction along the A–P axis as compared to age-matched normal lenses. The average anterior elliptical angles of cataractous lenses were 12% smaller than those in aged normals, (see Table I for morphometric results). Similarly, the average posterior elliptical angles decreased by 11% in cataracts as compared to aged normals. There was virtually no difference in the average number of anterior or posterior fiber ends per 10 μm in cataracts as compared to aged normal lenses. On average, anterior fiber ends in cataracts were 1.73 μm thick, while posterior fiber ends were 2.00 μm thick. In the EN of cataractous lenses, both the average length of the A–P axis and the average fiber length were 13% smaller than age-matched normals. The frequency of folds along EN fibers increased by a comparable percentage as expected. Cataracts had approximately 16% more fiber folds per 20 μm than aged normal lenses. EN fibers in age-related nuclear cataracts displayed mostly high-amplitude folds [Fig. 5(C) and (D)], as in normal aged lenses. Large undulations of fibers were also present in the EN of many cataractous lenses [Fig. 5(C), arrows].

It is important to note that the occurrence of accordion-like fiber folds was not limited to the primary fibers. Early secondary fibers in nuclear cataracts exhibited prominent, high-amplitude folds across their broad faces [Fig. 6(A)–(C)]. These fiber folds were present in early secondary fibers of both middle-aged and aged normal lenses as well (data not shown).

**Statistical Analysis**

**Comparisons between normal donor lenses.** The Kruskal–Wallis test for the three age groups showed significant differences for all seven variables measured in this study (all \( P \) values were ≤0.05; see Table II). For the paired Mann–Whitney tests, the results varied for each parameter. The anterior elliptical angles were significantly different only for young vs aged lenses (\( P ≤ 0.001 \)); young vs middle-aged lenses and middle-aged vs aged lenses were not significantly different (\( P = 0.060 \) and 0.292, respectively). Similarly, for the posterior elliptical angles, a significant difference was found only between young and aged lenses (\( P ≤ 0.001 \)). The number of anterior FN fibers per 10 μm was significantly different between young vs middle aged (\( P ≤ 0.003 \)) and young vs aged (\( P ≤ 0.001 \)) lenses, but not between middle-aged vs aged lenses (\( P = 0.336 \)). In a comparable manner, the number posterior FN fibers per 10 μm in young lenses was significantly different from middle-aged (\( P ≤ 0.002 \)) and aged (\( P ≤ 0.001 \)) lenses: middle-aged and aged lenses were not statistically different from each other (\( P = 0.916 \)).

Paired Mann–Whitney tests of the A–P axis length in the EN revealed that all three age groups were significantly different from each other (0.009 ≤ \( P \) ≤ 0.012). Similarly, all three pairwise tests for EN fiber length resulted in \( P \) values ≤ 0.012, indicating they were statistically different (Table II). Finally, statistical analysis of the number EN fiber folds per 20 μm demonstrated that all three age groups were significantly different from one another for this parameter (0.009 ≤ \( P \) ≤ 0.012).

**Comparisons between normal aged lenses and nuclear cataracts.** Mann–Whitney tests indicated that the cataracts were statistically different from age-matched normal lenses for four of the seven parameters measured. Both the anterior and posterior FN elliptical angles of cataracts were significantly different from the analogous elliptical angles in normal aged lenses (\( P ≤ 0.010 \) and 0.045, respectively). However, the number FN fibers per 10 μm was not different in cataracts vs normal aged lenses for either
the anterior or the posterior regions ($P = 0.75$ and $0.916$, respectively). In the EN, the A–P axis was significantly shorter in cataracts than in aged normals ($P < 0.008$). EN fiber length in cataracts was, as expected, also significantly shorter than in aged normal lenses ($P < 0.008$). Although the number of EN fiber folds per 20 $\mu$m was greater in cataracts than in aged normals, this difference was not statistically significant ($P = 0.113$).

## 4. Discussion

The size and shape of the human lens changes dramatically during development and maturation. Assessments of human lens growth have established that the equatorial dimension of lenses increases at a greater rate than the polar (A–P axis) dimension (Duke-Elder and Wybar, 1961; Worgul, 1982; Kuszak and Brown, 1994). Using measurements of human lenses at various ages, 3-dimensional computer-assisted drawings (3-D CADs) were constructed to illustrate the non-proportional changes in equatorial and polar dimensions over time (Fig. 7). Although new fiber growth results in an increase in both the equatorial and A–P axes, the increase in the A–P axis is offset by compaction [Fig. 7(C) and (D)].

This is the first study to quantify the type and extent of structural changes which accompany lens fiber compaction. The results demonstrate that while significant compaction of nuclear fibers occurred along the A–P axis with ageing, an even greater degree of compaction occurred in nuclear cataract formation. In normal lenses, every parameter measured showed statistically significant age-related changes overall (when young lenses were compared to aged lenses). However, the rate of compaction was not constant. Morphometric analysis indicated that, in general, more compaction occurred between young and middle-aged lenses than between middle-aged and aged lenses. Although initially surprising, this finding is temporally consistent with the onset of presbyopia near age 40. It is likely that condensation and compaction of nuclear fibers in early adulthood contribute to lens hardening and the loss of accommodative ability which characterize presbyopia.

The average length of primary fibers was less than the average EN A–P axis length in both the normal and cataractous lenses. This may be due to two factors. First, the length of primary fibers showed considerable variation within individual lenses such that not all fibers extended to the anterior or posterior surface of the EN. This data is consistent with previous investigations which showed EN fiber length variations in several species including tadpole/frog (Kuszak et al., 1986, 1989), chick (Kuszak, 1995; Shestopalov and Bassnett, 2000) and human (Taylor et al., 1996).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P values*: Kruskal-Wallis tests</th>
<th>P values**: Mann-Whitney paired tests for normal lenses</th>
<th>P values*: Mann-Whitney tests for normal aged vs cataracts</th>
</tr>
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<tr>
<td>Anterior FN Elliptical Angles</td>
<td>0.008</td>
<td>young vs middle: 0.060</td>
<td>young vs aged: 0.001</td>
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<tr>
<td></td>
<td></td>
<td>middle vs aged: 0.292</td>
<td>middle vs aged: 0.292</td>
</tr>
<tr>
<td>Posterior FN Elliptical Angles</td>
<td>0.004</td>
<td>young vs middle: 0.029</td>
<td>young vs aged: 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>middle vs aged: 0.292</td>
<td>middle vs aged: 0.292</td>
</tr>
<tr>
<td># Anterior FN Fibers/10 $\mu$m</td>
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<td>young vs aged: 0.001</td>
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<td></td>
<td></td>
<td>middle vs aged: 0.336</td>
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<tr>
<td># Posterior FN Fibers/10 $\mu$m</td>
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<td>young vs middle: 0.002</td>
<td>young vs aged: 0.001</td>
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<tr>
<td></td>
<td></td>
<td>middle vs aged: 0.916</td>
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<tr>
<td>EN A–P Axis Length ($\mu$m)</td>
<td>0.003</td>
<td>young vs middle: 0.012</td>
<td>young vs aged: 0.009</td>
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<tr>
<td></td>
<td></td>
<td>middle vs aged: 0.011</td>
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<td>EN Fiber Length ($\mu$m)</td>
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<td>young vs middle: 0.012</td>
<td>young vs aged: 0.009</td>
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<tr>
<td># EN Fiber Folds/20 $\mu$m</td>
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<td>young vs aged: 0.009</td>
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<td>middle vs aged: 0.011</td>
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* P values $\leq 0.05$ indicate that the medians are statistically different.
** P values $\leq 0.017$ indicate that the medians are statistically different (to maintain an overall error ratio of 0.05).
Fig. 4. Scanning electron micrographs of aged donor lenses. (A) Lenses in this sample group had the smallest average anterior and posterior elliptical angles of all the donor lenses (anterior = 25°, posterior = 27°). Age = 71 years. (B) Medium magnification of this specimen shows approximately one quarter of the EN, which was broken during dissection. The average EN A–P axis length was 141 μm, or 33% shorter than in young lenses. Age = 59 years in (B)–(D). (C) Fibers displayed numerous compaction folds (x = 5–40 folds per 20 μm) along virtually all of their length. (D) Compaction folds appeared to be mostly high-amplitude as compared to those in middle-aged lenses.
Fig. 5. Scanning electron micrographs of lenses with age-related nuclear cataracts. (A) and (B) Low magnification overviews of split lenses from 76 year old (A) and 55 year old (B) patients. The average elliptical angles described by fetal fibers were significantly smaller than those in age-matched normal lenses (anterior \( \approx 22^\circ \), posterior \( \approx 24^\circ \)). Inset in (B) depicts the embryonic and initial fetal fibers split along the A–P axis from the same lens. The EN is an extremely flattened oblate spheroid due to pathological compaction in this relatively young cataractous lens. On average, the A–P axis was 123 \( \mu \text{m} \) long, a 13% reduction as compared to aged normal lenses. (C) and (D) Medium and high magnification of the EN fibers from the same lens in (B) demonstrate the increased number of compaction folds (6-26 folds per 20 \( \mu \text{m} \)), as well as the presence of large fiber undulations (arrows).
Second, peripheral fibers in the EN are substantially shorter than those at or near the polar axis, since they fill the spheroid edges but do not extend to the poles. Together these two considerations account for the disparity between the above parameters, since only primary fibers situated on the A–P axis and extending to both poles would be equal in length to the A–P axis.

Polygonal domains of furrowed membranes were present on primary and early secondary fibers of all lenses in this investigation (from 15 to 81 years old). In primate lenses, this feature was shown to be most prominent in the oldest fibers and absent from cortical fibers (Kuszak et al., 1988), leading to its association with membrane senescence. However, in this study, the ultrastructure of furrowed membrane domains was comparable in all age groups, that is, the furrowed membranes did not become more prominent with age. This data indicates that furrowed membrane domains are a feature of specific developmental regions of the lens, rather than a consequence of senescent membrane changes.

The process of nuclear fiber compaction is probably multifactoral. The most obvious structural change is the formation of accordion-like folds, which account for much of the compaction along the A–P axis. These folds begin in early adulthood and increase in both frequency and amplitude with age. The early onset of structural changes may be due to controlled modifications in the cytoskeletal (Kuwabara, 1968; Allen et al.,

Fig. 6. Scanning electron micrographic magnification series of FN fibers in a 73 year old age-related nuclear cataract. (A) At low magnification, the presence of a suture branch (arrow) is indicative of the FN. (B) and (C) Fibers exhibited numerous, high-amplitude compaction folds along their length, demonstrating that early secondary fibers undergo compaction in a similar manner to primary fibers since they are situated essentially parallel to the A–P axis.
1987; Sandilands et al., 1995a, b) and crystallin (McFall-Ngai et al., 1985; Li, Roy and Spector, 1986; Bours et al., 1990; Garland et al., 1996) proteins which accompany fiber cell maturation and are probably necessary for long term maintenance of fibers. In the fourth through eighth decades, cumulative age-related changes, such as, water and protein loss (Bours, Fodisch and Hockwin, 1987; Horwitz, Ding and Cheung, 1983), modifications to membrane lipids (Borchman, Byrdwell and Yappert, 1994; Borchman and Yappert, 1998) and protein modifications (see Zigler, 1994 for review) could result in the progressive increase in compaction folds (and the corresponding reduction of the elliptical angles of fetal fibers) noted in this investigation. The further increase in nuclear fiber compaction in age-related nuclear cataracts is consistent with the extensive protein modifications (see Andley, Liang and Lou, 2000 for review), dehydration (Bettelheim et al., 1986) and lipid peroxidation (Bhuyan, Bhuyan and Podos, 1986; Babizhayev, Deyev and Linberg, 1988; Babizhayev and Costa, 1994) known to occur in human cataracts.

The precise mechanism of fiber cell compaction is unknown. The major factor influencing compaction is most likely the loss of cytoplasmic water which necessarily results in the loss of cell volume without reduction in cell surface area. The driving force for the loss of water may be the reported tendency of the crystallins to self-associate into larger aggregates with time causing the nuclear cytoplasm to have a reduced osmolarity (Tardieu et al., 1992; Kenworthy et al., 1994). Such changes are essential for the high concentrations of proteins in nuclear cytoplasm to exist adjacent to cortical fiber cells with relatively high water content. Further changes in the proteins and membrane lipids during cataract formation, specifically by oxidative damage (Truscott, 2000), may result in more extensive condensation of cytoplasmic proteins, as well as loss of protein and membrane fragments that lead to increased fluctuations in refractive index at cellular interfaces and increased light scattering.

Excessive senescent changes in the morphology of the nuclear lens fibers are likely to be most detrimental to lens optics, since these fibers are located directly along the visual axis. The changes in primary fiber structure that occur during A–P axis compaction have been depicted using 3D-CADs rotated through 90° (Fig. 8). The formation of compaction folds as a function of age results in an almost 50% decrease in fiber length [Fig. 8(A) and (B)]. Thus, fiber compaction also alters the spacing of fiber interdigitations
Fig. 8. A series of scaled 3D-CADs demonstrating how the variable amount of fiber compaction can contribute to an alteration in the optical elements of human lenses as a function of age and pathology (nuclear cataract). (A) The top fiber depicts an equatorial segment of an embryonic fiber from a normal, young lens (15–25 years old). By comparison, the middle and lower fibers depict the same fiber, respectively, in its compacted state from a normal, aged lens (59–81 years old), and from an age-related nuclear cataractous lens (55–81 years old). (B) Note, if this fiber could be serially-sectioned at its different ages, approximately one-half of this segment would be contained in five equal consecutive serial-sections from a young lens. In contrast, virtually the entire fiber segment from a nuclear cataract would be contained in the same five consecutive serial-sections. When these successive serial-sections are rotated through (C) 22.5°, (D) 45°, (E) 67.5°, and (F) 90°, it is apparent that as light was transmitted through the lens, it encountered an increasing amount of membrane (yellow color), aligned at more complex angles, relative to cytoplasm (tan color), as a function of age and pathology. As a result, this changing ratio of membrane, a principal component of large particle scatter, to cytoplasm, a principal component of small particle scatter, may explain, at least in part, structural alterations that correlate with the significant increase in nuclear scatter characteristic of age-related cataracts.
[Fig. 8(C)–(F)], causing an increase in fiber membrane complexity in relation to fiber cytoplasm as light passes along the lens visual axis. The 3D-CAD analysis is consistent with the increased complexity of interlocking devices seen by thin-section TEM in human nuclear cataracts as compared to age-matched normals (Al-Ghoul and Costello, 1996; Gilliland et al., 1998).

According to Trokel (1962), large particle scatter results from diffraction and reflection of the incident light beam by lens membrane pairs. Therefore, age-related fiber compaction resulting in an increase in the membrane complexity along the light path may be a source of increased large particle scatter and ultimately, reduced lens optical quality with age. In nuclear cataracts, the significantly increased fiber compaction may be one of the factors contributing to the excessive scatter in nuclear opacification.

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