PERGAMON

Vision Research 39 (1999) 1991–2015

Vision Research

Biometric, optical and physical changes in the isolated human crystalline lens with age in relation to presbyopia

Adrian Glasser [a,*], Melanie C.W. Campbell [b]

[a] *College of Optometry, University of Houston, Houston, TX, 77204 USA*
[b] *School of Optometry, University of Waterloo, Waterloo, Ont., N2l 3G1, Canada*

Received 18 May 1998; received in revised form 1 October 1998

Abstract

The biometric, optical and physical properties of 19 pairs of isolated human eye-bank lenses ranging in age from 5 to 96 years were compared. Lens focal length and spherical aberration were measured using a scanning laser apparatus, lens thickness and the lens surface curvatures were measured by digitizing the lens profiles and equivalent refractive indices were calculated for each lens using this data. The second lens from each donor was used to measure resistance to physical deformation by providing a compressive force to the lens. The lens capsule was then removed from each lens and each measurement was repeated to ascertain what role the capsule plays in determining these optical and physical characteristics. Age dependent changes in lens focal length, lens surface curvatures and lens resistance to physical deformation are described. Isolated lens focal length was found to be significantly linearly correlated with both the anterior and posterior surface curvatures. No age dependent change in equivalent refractive index of the isolated lens was found. Although decapsulating human lenses causes similar changes in focal length to that which we have shown to occur when human lenses are mechanically stretched into an unaccommodated state, the effects are due to nonsystematic changes in lens curvatures. These studies reinforce the conclusion that lens hardening must be considered as an important factor in the development of presbyopia, that age changes in the human lens are not limited to the loss of accommodation that characterizes presbyopia but that the lens optical and physical properties change substantially with age in a complex manner. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Optics; Presbyopia; Accommodation; Spherical aberration; Crystalline lens; Age

1. Introduction

Age changes in the biometric (e.g. weight, cross-sectional area), optical (e.g. focal length, spherical aberration) and physical properties (e.g. lens thickness, curvatures, hardness) of the human lens are frequently invoked to explain the progression of presbyopia. These include changes in mass, volume, shape, surface curvatures, refractive index distribution, elasticity and hardness of the lens. Given the prevalence of presbyopia, the impact on society and the abundant and disparate theories of its causes, surprisingly few studies have made optical or physical measurements of human lenses to understand the lenticular contribution to presbyopia. Fewer studies include both optical and physical measurements on the same group of human lenses. If the etiology and the causes of presbyopia are to be understood and attempts are to be made to slow its progression or reverse its effects, an essential basic requirement is a comprehensive understanding of the aging of the optical and physical properties of the human lens and an understanding of how these factors may interact during the aging process to contribute to presbyopia.

The lens optical and physical properties are closely related. The crystalline lens focal length and spherical aberration are profoundly influenced by the lens surface curvatures and gradient refractive index. Lens thickness and curvatures change during accommodation with resulting changes in lens power and spherical aberration (Glasser & Campbell, 1998). The substance of the young lens has been described as plastic (Fincham, 1937), purely elastic (Fisher, 1971) or viscoelastic (Beers & Van der Heijde, 1994, 1996) with the lens capsule

* Corresponding author. Tel.: +1-713-7431876; fax: +1-713-7432053; e-mail: aglasser@popmail.opt.uh.edu.

PII: S0042-6989(98)00283-1

serving to mould and alter the shape of the lens during accommodation and relaxation of accommodation. The properties of the lens substance and the role of the capsule are thus integral to the normal optical function of the crystalline lens. Only a few studies have attempted experiments to determine the nature of the interaction between the lens substance and capsule (Fincham, 1937; Fisher, 1969a,b, 1971, 1973); others have speculated on the interaction (Wyatt & Fisher, 1995). The mechanical properties of either the lens substance (human: Pau & Kranz, 1991; cat, rabbit and dog: Kikkawa & Sato, 1963; Ejiri, Thompson & O'Neill, 1969) or the human lens capsule (Krag, Olsen & Andreassen, 1997) have also been studied in isolation. No studies to date have undertaken systematic experiments to determine the optical and functional relationship between the capsule and the lens substance despite their obvious interdependence. Further, it is generally assumed that the capsule serves to mould the young lens into an accommodated form when the zonular forces are removed and that when the capsule is removed the lens takes on an unaccommodated form (Fincham, 1937). Remarkably, these theories are supported with evidence from only one monkey lens (Fincham, 1937) and one human lens (Fisher, 1969a). Not only are these two data points cited frequently, but complex hypotheses have been formulated based on this sparse data (Wyatt & Fisher, 1995). A comprehensive study exploring the relationships between the lens optical and physical properties and the role of the capsule is thus long overdue.

Age changes in the optical properties of the lens have been measured in a variety of cross sectional studies. Sorsby, Benjamin and Sheridan (1961) measured age changes in the eyes of subjects 3–15 years of age. Refractive error measurements, slit-lamp measurements of lens thickness and lens curvatures (to compute axial lengths) were used in conjunction with an age independent equivalent refractive index of the lens. A decrease in hyperopia towards emmetropia with a concurrent increase in axial length, anterior chamber depth and a decrease in lens power was reported for some 1500 subjects, but subsequent follow-up showed considerable individual variation in this generalized pattern (Sorsby et al., 1961). The changes in lens thickness, anterior chamber depth and axial length are essentially in agreement with subsequent ultrasound measurements (Larsen, 1971a,b,c; Zadnik, Mutti, Fusaro & Adams, 1995). Purkinje image photography of infants 3–18 months of age showed a decrease in the calculated equivalent refractive index and power of the lens over this age range (Wood, Mutti & Zadnik, 1996). Brown (1974) showed a decrease in both anterior and posterior lens radii of curvature with age in subjects aged 3–82 years using slit-lamp Scheimpflug photography. Koretz, Kaufman, Neider and Goeckner (1989) showed an increase in the thickness of the unaccommodated lens using ultrasound and Scheimpflug photography in subjects ages 18–70 years. The accuracy of the optical techniques of Purkinje image and Scheimpflug photography are limited by the fact that the calculations of anterior lens surface curvature must be made through the optics of the anterior segment of the eye and in addition the posterior lens surface curvature must be made through the unknown gradient refractive index of the lens itself. While corneal curvature and anterior chamber depth can be measured with some accuracy, age changes in corneal curvature (Saunders, 1982) and the asphericity of the peripheral cornea also represent sources of error for both anterior and posterior lens surface measurements such as those made by Brown (1974).

The anterior and to a lesser extent the posterior lens radii of curvatures are reported to decrease with age (Brown, 1974). Without other changes in the eye this would cause an increase in power of the lens with age resulting in myopia, yet presbyopia actually results in a loss of near vision rather than a loss of distance vision. This has led to the conception of the lens paradox (Koretz & Handelman, 1986, 1988) which has been resolved in theory by the suggestion that the refractive index gradient of the lens changes with age (Koretz & Handelman, 1988; Pierscionek, 1990, 1993a). This change is thought to compensate for increased lens surface curvatures to maintain lens power and emmetropia with age (Koretz & Handelman, 1988; Smith, Atchison & Pierscionek, 1992; Hemenger, Garner & Ooi, 1995; Ooi & Grosvenor, 1995). While studies have shown the theoretical feasibility of age changes in the gradient refractive index of the lens (Smith et al., 1992; Heminger et al., 1995), no empirical studies have shown an actual compensation between surface curvatures and the gradient refractive index of the human lens. This is in part due to the substantial theoretical and practical complexities involved in accurately determining the gradient refractive index distribution of the human crystalline lens. Direct measurements of the refractive index gradient in the human lens along the optical axis, close to the axis most relevant to vision, using an invasive optic fiber sensor have not shown significant age dependent changes (Pierscionek, 1997). Since lens surface curvatures were not measured, no relationships or compensation of curvatures and refractive index were identified (Pierscionek, 1997). Further, accommodative changes that occur when young human lenses are isolated make in vivo and in vitro comparisons difficult. A basic and concrete empirical knowledge of age changes in the lens is an essential first step towards modeling the human lens since the true value of theoretical work is realized only when the theoretical results and the empirical measurements can be shown to agree. This is equally important for schematic eye models which are currently incomplete since they fail to incorporate and predict the optical changes that occur in the human lens with accommodation and with age (Glasser & Campbell, 1998). While the nature of the gradient refractive index distribution of the human crystalline lens and its postulated changes with age and accommodation are complex and difficult to measure directly (Campbell, 1984) and may be beyond the resolution of the invasive optical fiber sensor technique used by Pierscioneck (1997) to measure it, the physical and optical characteristics of the human lens can readily be directly measured in vitro. Systematic measurement of these physical and optical properties in a variety of accommodated states and from a range of ages will inevitably improve our understanding of age changes in the lens and prove beneficial for the development of accommodation and age dependent schematic eye models. If these schematic eye models are ultimately to be of value, they must be able to predict measured lens optical properties. As a step towards this goal we have undertaken measurements of the isolated human lens.

Although a number of studies have made direct measurements of human lens surface curvatures, the variety of methods employed (i.e. in vivo vs. in vitro, frozen vs. fresh, optical vs. physical) and the wide range and somewhat contradictory results obtained (see Brown (1974) vs. Howcroft and Parker (1977) for example) leave no clear consensus. Although more recent studies have undertaken measurements of human lens dimensions in vitro (Pierscionek & Augusteyn, 1991; Pierscionek, 1993b, 1995) these studies have either not reported surface curvatures and focal lengths (Pierscionek & Augusteyn, 1991) or not shown age dependent changes, perhaps through use of too few human lenses to identify age dependent changes (Pierscionek, 1993b, 1995). Despite having described methodology to measure lens surface curvatures (Pierscionek, 1993b, 1995) these studies have not directly compared physical surface curvatures, lens dimensions and optical focal length measurements in the same group of human lenses. Such measurements are necessary since the lens paradox addresses the relationship between the physical (surface curvatures and gradient refractive index) and optical properties (focal length) of the lens, and the lens surface curvatures and gradient of refractive index determine the lens optical properties. Attempts to ascertain lens power in vivo have largely relied on measuring lens surface curvatures in vivo either assuming an equivalent refractive index for the lens (Sorsby, Benjamin, Davey, Sheridan & Tanner, 1957; Sorsby et al., 1961; Mutti, Zadnik & Adams, 1992, 1995) or relating refraction, phakometry measurements and ocular dimensions to extract lens power and then computing equivalent refractive indices for the lens (Mutti et al., 1995; Wood et al., 1996) from lens power and dimensions. These relationships are circular and do not give an unambiguous value of equivalent refractive index (Mutti et al., 1995). Our study has combined direct measurements of lens power, lens surface curvatures and lens thickness in isolated human lenses to facilitate a more direct and accurate determination of the equivalent refractive index of the lens and the relationships between these measures.

Despite a number of studies which have shown hardening of the lens substance with age as a cause of or a contribution to presbyopia, the possibility of lenticular hardening occurring or being a contributing factor in the development of presbyopia has been entirely excluded by some authors (Weale, 1963a, 1989; Nordmann, Mack & Mack, 1974). The belief has even led to the suggestion that the term lenticular sclerosis be removed from the ophthalmic literature (Nordmann et al., 1974). Whatever the interpretation of the term lenticular sclerosis, the fact that no clear consensus exists on how the lens physical properties change with age suggests that further measurements are required to determine the age changes in the physical properties of the lens. Further, numerous disparate theories for the causes of presbyopia abound, many of which exclude the possibility of hardening of the lens substance (see Glasser & Campbell, 1998). Given the historical bias towards lenticular sclerosis as a cause of presbyopia (Gullstrand, 1909; Fincham, 1937), the substantial evidence for hardening of the lens (Fisher, 1971, 1973; Pau & Kranz, 1991), and recent evidence from in vivo studies for changes in lens visco-elasticity (Beers & Van der Heijde, 1996) it is surprising that lenticular causes for presbyopia have been de-emphasized. Since Fisher's lens spinning experiments (Fisher, 1971, 1973) have been criticized as being indirect evidence for hardening of the lens and the results from previous in vitro studies of human lenses (Glasser & Campbell, 1998) do not preclude altered capsular/zonular forces playing a role in presbyopia, we have undertaken more direct, physical measurements of human lenses that allow more concrete conclusions about changes in hardness or visco-elasticity of the human lens and their role in the development of presbyopia.

Previous measurements of age changes in the optical properties of the human lens in relation to accommodation (Glasser & Campbell, 1998) show an age dependent increase in the shortest attainable focal length of the human lens; an age related loss in ability of the human lens to undergo changes in focal length; and changes in spherical aberration of the human lens with age and as a function of accommodative state (Glasser & Campbell, 1998). Here we have used similar methodology to measure the optical and physical properties of a group of isolated, unstretched human lenses as a function of age. The results of this comprehensive study employing direct measurements of multiple parameters on each lens before and after removal of the capsule provides information relating lens physical and optical properties and the progression of presbyopia.

2. Methods

A total of 19 pairs of human eyes ranging in age from 5 to 96 were obtained from the Eye Bank of Canada, Toronto, Ontario. Five donors were female and 14 were male. Besides age, gender, time and cause of death, no other personal information on the donors was known. The tissue handling procedures have been described previously (Glasser & Campbell, 1998, 1999). The eyes were kept on moist gauze in sealed bottles in chilled coolers during transit. In most cases the corneas were removed by the Eye Bank. Upon receipt, the eyes were placed in a dissecting dish filled with a balanced salt solution (human saline) with an osmolarity close to that of the vitreous (290 milliosmoles) of the following composition (g/l) NaCl 8.00, KCl 0.40, Na_2HPO_4 0.10, glucose 1.00, Hepes 2.38, buffered with 8 ml of 0.5 M NaOH to a pH of 7.4. If present, the corneas were removed. The iris was excised and the sclera and anterior ciliary body were separated by running forceps tips around the globe between the sclera and the ciliary body. The anterior sclera was cut away to expose the anterior ciliary body and choroid. The anterior segment of the eye comprising the lens, zonule, ciliary body and ciliary muscle was separated from the remainder of the globe by cutting circumferentially with fine scissors through the choroid and retina at the region of the ora serrata. The ring of ciliary body was opened with a radial cut and the lens removed after cutting all zonular fibers. The wet weight and the resistance to physical deformation were measured in one lens and the surface curvatures and optical properties were measured in the lens from the other eye of each donor. The lens capsule was then removed from each lens as described below and all measurements were repeated on the decapsulated lenses. Lenses were maintained in room temperature saline for 30–45 min before measurement commenced. Lenses were used within an average of 63 h after death with a minimum of 18 h and a maximum of 185 h.

2.1. Scanning laser measurements

The lens focal lengths and spherical aberration were measured using a scanning laser apparatus as described previously (Glasser & Campbell, 1998). In the experiments described here, the isolated lens was placed in a Plexiglas chamber filled with human saline. Each lens was positioned on its equatorial edge in a groove in the base of the chamber with the anterior surface facing the front of the chamber. The lens remained immobile in this position under gravitational force. The laser beam passed into the front of the Plexiglas chamber through a glass window and entered the lens parallel to the optical axis. The laser was moved horizontally by a stepper motor so the beam scanned across the full diameter of the lens in 75 discreet steps (Fig. 1a, b). The lens focal length and spherical aberration measurements have been described in detail previously (Glasser & Campbell, 1998). The intersection point of each entrance beam with its corresponding exit beam provides the principle points. The mean focal length of the lens is the average distance along all exit beams from the principle point to the intersection point with the optical axis (Fig. 1b). Since the diameter of the lens is small relative to the lens focal length, this is essentially equivalent to the average of the axial distance between the principle plane and the intersection with the optical axis. The spherical aberration is given by fitting a 4th order polynomial $I(y)$ through all points describing the position where each exit beam intersects the optical axis as a function of the incident height (y) of the ray on the lens (Fig. 1b) (Glasser & Campbell, 1998). The paraxial focal length is given by the point where the 4th order polynomial crosses the optical axis (as the x value of the polynomial tends towards zero). The scanning laser measurement was repeated four times in the same meridian without moving the lens within 15 min and the results presented are the average of these four scans.

Fig. 1. (*Opposite*) Diagrams showing methods for lens focal length and lens surface curvature measurements. (a) An isolated 63 year old lens is positioned on its equatorial edge in a chamber filled with buffered human saline. A laser beam enters the chamber through a glass window to the left and is refracted and deflected towards the optical axis as it passes through the lens. The laser is scanned horizontally across the diameter of the lens in 75 discreet steps (only 11 steps shown). As an image scale this lens is 7.68 mm in diameter and 3.73 mm thick. (b) A video camera mounted above the chamber allows the laser beams entering and leaving the lens to be digitized at each step in the scan. An entrance and an exit box (black boxes) are drawn to identify the regions in which the entrance and exit beams are to be located. Lens focal length and spherical aberration is calculated from subsequent analysis of the scan as described previously (Glasser & Campbell, 1998). The black horizontal line represents the optical axis. The solid circles (to the left) are the principle points of the lens where the entrance and exit beams intersect. The open circles to the right represent the vertical distance from the optical axis at which each entrance beam is incident on the lens and the horizontal position of the intersection point of each exit beam with the optical axis. A 4th order polynomial (curved line), describing the spherical aberration of the lens, is drawn through these open circles. The single larger black circle on the optical axis is the mean focal length of the lens representing the average of the intersection points of all the open circles. By way of horizontal and vertical scales, the entrance box is 12.13 mm high and 14.49 mm wide. (c) A magnified image of the lens is used to digitize the surface curvatures after the focal length measurement is completed. (d) After digitizing the anterior (circles) and posterior (squares) lens surfaces, the cross sectional area is calculated and parabolas are fitted to the central 4 mm of both surfaces (dark lines) to allow the lens radii of curvature to be calculated. By way of a scale, the lens thickness is 3.73 mm in (c) and (d).

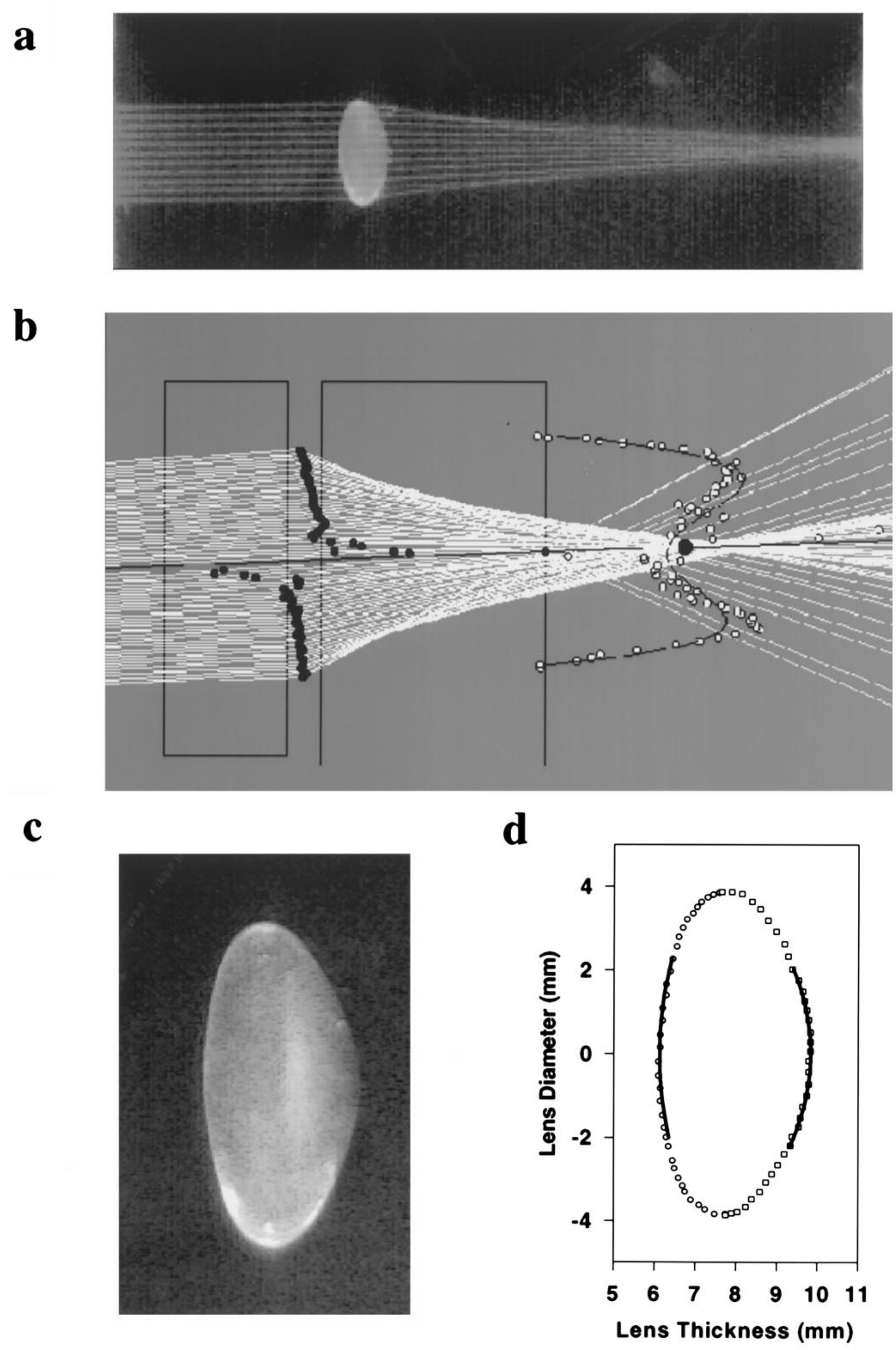

Fig. 1. (*Caption opposite*).

2.2. Lens surface curvature, thickness and cross-sectional area measurements

While the lens remained immobile in solution, the video camera mounted above the Plexiglas chamber (Glasser & Campbell, 1998) was lowered to within 10 cm of the lens to increase the image magnification. An image of millimeter graph paper was digitized at the plane of the human lens to calibrate the video camera magnification. Lens anterior and posterior surface curvatures were digitized using an AT computer and frame grabber board (Oculus 300, Coreco, Canada) (Fig. 1b).

An image of the lens profile was saved to disk and then roughly 35 points were manually marked on the lens images along each of the anterior and posterior surfaces with a mouse cursor. The coordinates of these points were saved to disk for subsequent analysis (Fig. 1c). Lens thickness, equatorial diameter, anterior and posterior surface curvatures, anterior and posterior sagittal thickness, cross sectional area and an ellipticity factor of the ratio of lens diameter to lens thickness (Pierscionek & Augusteyn, 1991) were calculated from the digitized profiles of each lens. Lens cross-sectional area was calculated by integrating the digitized lens surface profiles to calculate the area between the surfaces. The lens was then decapsulated as described below, repositioned in the Plexiglas chamber as before and the surface curvatures remeasured. The video camera was then repositioned and the scanning laser measurements were repeated on the decapsulated lens.

To calculate the lens surface radii of curvature the data points from the central 40% of each lens surface were fitted with parabolic curves of the form:

$$y = a + cx^2 \quad (1)$$

The anterior and posterior lens radii of curvature (r) were calculated from these curves using the formula:

$$r = [1 + (2cx)^2]^{3/2}/2c \quad (2)$$

(Koretz, Handelman & Brown, 1984). In calculating the lens surface radii of curvature, Eq. (2) was solved for each lens using $x = 0.5$ mm. This considers the lens surface radii of curvature for the central 1.0 mm of the lens. The true paraxial lens radii of curvature can be calculated for $x = 0$ where $r = 1/2c$. From these formulae, values for the central anterior lens radius of curvature, central posterior lens radius of curvature, lens thickness and the constant (c) of the fitted parabolas were obtained. The lens surface curvatures could be fitted with circular fits for small values of x, but surface curvatures clearly become aspheric for larger diameters.

2.3. Lens equivalent refractive index calculations

After the anterior and posterior lens curvatures, the lens thickness and the lens paraxial focal length were measured as described above, an equivalent refractive index could be calculated for each lens using the thick lens formula:

$$F_L = F_2 + F_3 - (d_2 \times F_2 \times F_3)/(1000 \times n_3) \quad (3)$$

$$\text{where } F_2 = 1000 \times (n_3 - n_2)/r_2 \quad (4)$$

$$\text{and } F_3 = 1000 \times (n_4 - n_3)/r_3 \quad (5)$$

Where F_L is the focal power of the lens in diopters, F_2 is the anterior lens surface power (in diopters), F_3 is the posterior lens surface power (in diopters), d_2 is the lens axial thickness (in mm), n_2 and n_4 are the refractive indices of the surrounding media (1.3333), r_2 and r_3 are the radii of curvature of the anterior and posterior lens surfaces, respectively (in mm) and n_3 is the unknown lens equivalent refractive index (Bennett & Rabbetts, 1989). Eq. (3) was solved for the equivalent refractive index value n_3.

2.4. Spherical aberration

As shown previously (Glasser & Campbell, 1998) the spherical aberration of the lenses can be well described by forth order polynomials. From the scanning laser measurements all intersection points of the exit beams with the optical axis are fitted with 4th order polynomials (Fig. 1b). These 4th order polynomials describe the change in the distance of the focal points from the lens as a function of the distance of the entrance beam from the optical axis. The 4th order polynomials also give the extent of the spherical aberration (SA) of the lens from the equation:

$$\text{SA} = n'/l'_y - n'/l'_0 \quad (6)$$

where n' is the refractive index of the human saline (1.333), l'_0 is the intersection of the polynomial with the optical axis, and l'_y is the solution of the polynomial at a height y from the optical axis (Bennett & Rabbetts, 1989).

2.5. Measurements of lens resistance to physical deformation

A custom built squeezing apparatus was designed to measure the resistance of the lenses to a mechanical compressive force applied along the optical axis of the lens (Fig. 2). This provides a measure of the axial compressibility of the whole lens. A Plexiglas sleeve was fitted around a pressure transducer (Grass Instruments) to create a fluid filled well. Each lens was placed anterior surface down on the pressure transducer diaphragm in the fluid well. The pressure transducer was attached to a Grass Instruments balanced bridge amplifier through which the baseline and sensitivity were set. The output of the pressure transducer was fed to an AT computer and recorded at a frequency of 10 Hz. The pressure transducer was bolted firmly beneath a plunger attached to a stepper motor. The plunger contacted the upper (posterior) lens surface with a beveled tip approximately matching the curvature of the posterior lens surface. The plunger could be moved up or down in 75 μm steps under the control of the stepper motor. The plunger was initially moved down until it first contacted the lens. This was determined by the first visible incremental step in the output of the pressure

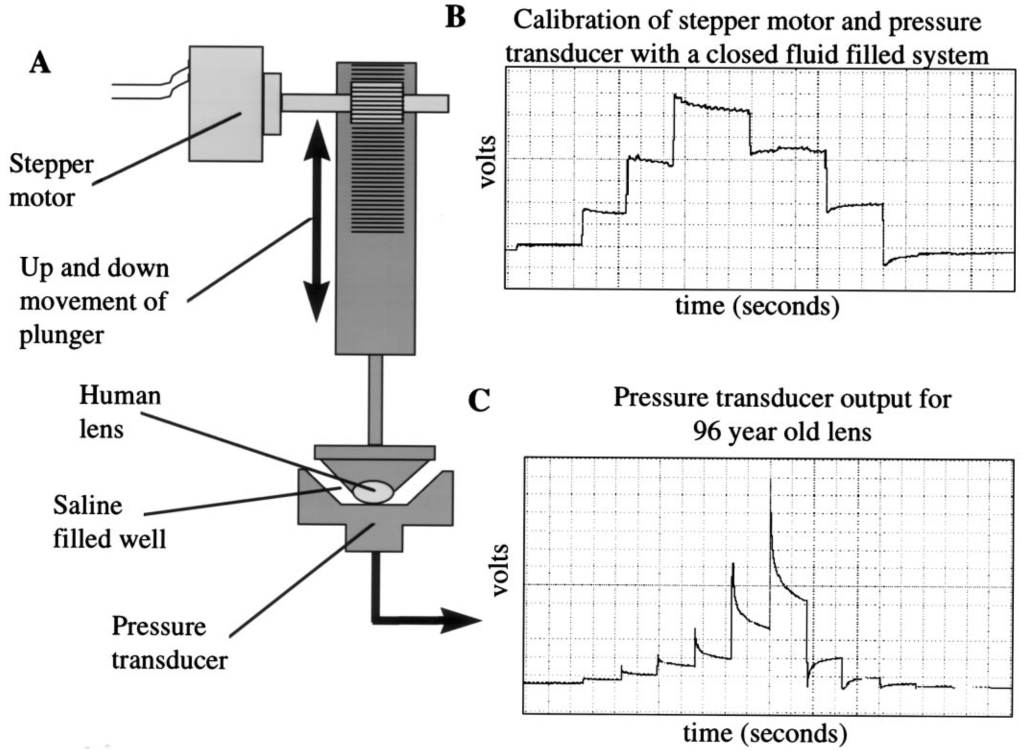

Fig. 2. Diagram of apparatus used to measure resistance of human lenses to mechanically applied compressive forces. (A) The lens is placed anterior surface down in a well of human saline on the diaphragm of a pressure transducer (Grass Instruments). A plunger with a beveled tip to match the posterior lens surface is moved up and down by means of a stepper motor in 75 μm steps to apply or to reduce the compressive force on the lens. The output of the pressure transducer is recorded by an AT computer after amplification. (B) Calibration of the pressure transducer using a closed fluid-filled system. The plunger was driven down onto the plunger of a 1 cc syringe attached to the pressure transducer via a closed fluid-filled tube. Each downward step of the plunger increases the fluid pressure in the system and causes an increase in the output voltage from the pressure transducer. When the direction of the stepper motor is reversed, the plunger moves upwards at each step, reducing the pressure in the closed system and the pressure transducer output returns to zero. (C) An example of the pressure transducer output from a 96 year old human lens is shown. In contrast to the measurements from the closed fluid filled system, the older human lens shows a biphasic response with a sharp initial spike followed by a transient decay at each step of either increasing or decreasing force. The output of the pressure transducer is in volts with each grid square being 0.3 V. The time scale is 2 s per square in the upper trace and 6 s per square in the lower trace.

transducer. The plunger was then stepped down onto the lens in five further successive steps at 10 s intervals and then up again in six successive steps back to the initial starting position. This process was repeated four times on each lens. The output of the pressure transducer was recorded via an AT computer with a data acquisition module (Fig. 2). This data was subsequently analyzed to determine the maximum change in output voltage from the pressure transducer at the sixth step of the plunger onto the lens.

The output characteristics of the pressure transducer were determined and the pressure transducer calibrated prior to lens experiments being performed. The plunger of the squeezing apparatus was attached to the plunger of a firmly clamped 1 cc syringe which was connected via a sealed fluid-filled system to the pressure transducer. Each 75 μm step in the stepper motor infused or withdrew a fixed volume of fluid to the pressure transducer. The output from the pressure transducer in response to four forward steps followed by four backward steps of the stepper motor are shown in Fig. 2.

2.6. Lens decapsulation

After the initial measurements were completed on each lens (laser scanning and lens surface curvature measurements on one lens and physical resistance to deformation on the other), the lens capsule was removed. To do this, each lens was placed in a dissecting dish filled with human saline. The lens was held positioned on its equatorial edge beneath the shanks of curved forceps lightly touching the uppermost equatorial edge of the lens. The point of the lower blade of fine scissors was used to perforate the capsule along the equator of the lens between the shanks of the forceps. Once the capsule was perforated, it was lifted to stretch it up off the lens cortex with the lower blade of the scissors and was cut through. The capsule was cut half way around the diameter of the lens while supporting the lens on its equatorial edge. The opened capsule was then peeled off the remainder of the lens. In general, decapsulation was accomplished without touching the lens surfaces and with little or no visible damage to the surfaces of the lens. In a few instances, some lens fibers remained adhered to the anterior or posterior capsule and were pulled from the lens surfaces as the capsule was removed. In no cases were more than a few lens fibers removed with the capsule. Following removal of the capsule, all measurements were repeated on the lenses.

2.7. Lens weight

The wet weight was measured for each of the lenses used to measure the resistance to physical deformation. The lens was weighed before squeezing, after squeezing with the lens capsule intact, again after the capsule had been removed, and again after squeezing the decapsulated lens.

3. Results

The results will be presented to show (1) the age dependent changes in the physical and optical properties of the lens with the capsule intact; (2) the relationships between the physical and optical properties of the lens with the capsule intact; (3) the changes in the optical properties that occur when the capsule is removed; (4) results relating to the equivalent refractive index of the lens; and (5) results describing the lens resistance to deformation.

3.1. Age dependent relationships in the lens with the capsule intact

3.1.1. Age changes in the biometric properties of the lens

For the group of 19 lenses used in this study there is a significant linear increase in lens weight with age (Fig. 3a). No significant differences in lens weight were found with or without the capsule or before and after squeezing the lenses. Lens cross sectional area, as calculated from the digitized lens profiles, showed a significant linear increase with age (Fig. 3b). Since lens weight and lens cross sectional area are each linearly correlated with age, it follows that lens cross sectional area and lens weight are linearly correlated ($r^2 = 0.620$; $P < 0.001$). Lens equatorial diameters tend to increase up to age 70 and then decrease beyond this age ($P = 0.003$ for 2nd order polynomial regression).

Isolated lens thickness tends to increase with age, especially in the older lenses. No significant correlation exists as a function of age for all lenses (linear: $r^2 = 0.162$, $P = 0.088$; 2nd order $r^2 = 0.417$, $P = 0.013$; 3rd order $r^2 = 0.424$; $P = 0.036$).

We find that the change in central anterior lens surface radius of curvature as a function of age is well fitted by two linear regression lines, selected arbitrarily for lenses less than and for lenses greater than 65 years of age (Fig. 4a). There is a significant linear increase in anterior lens surface radius of curvature up to age 65 and then a significant linear decrease after age 65 of the form:

$$< 65{:}\ y = 0.068x + 4.324 \quad (7a)$$

$$> 65{:}\ y = -0.182x + 22.926 \quad (7b)$$

The significant relationship between posterior lens surface radius of curvature and age (Fig. 4a) is given by the equation:

$$\text{Post. rad. of curvature} = -3.143 - (0.0536 * \text{Age}) + (4.173 \times 10^{-4} * \text{Age}^2) \quad (8)$$

Since this is similar to, although flatter than, the inverted age versus focal length relationship, this implies

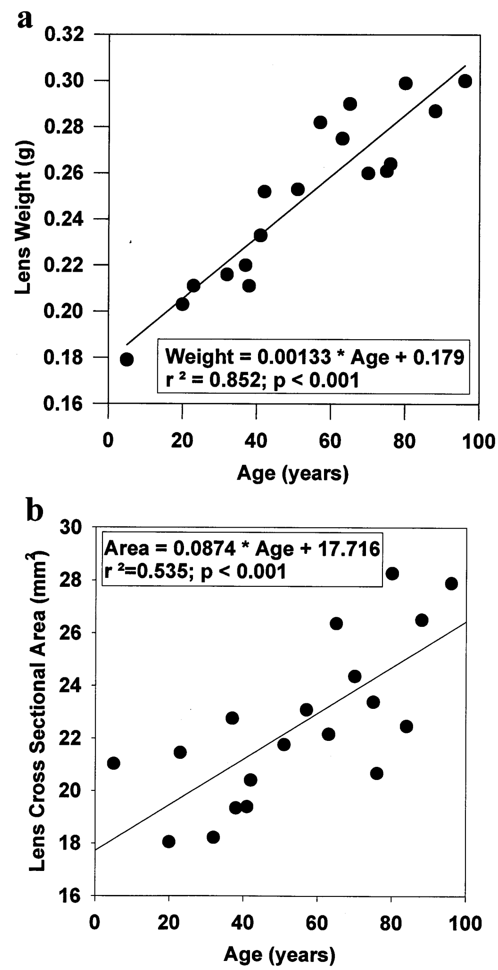

Fig. 3. (a) The weight of the human lens increases linearly with age throughout the normal life span. (b) The cross sectional area of the human lens as measured from digitized images of the lens profile show a linear increase with age over the normal life span. There is a non-significant tendency for lens thickness to increase with age (Fig. 10). Lens cross sectional area, however, is a function of the lens thickness and lens equatorial diameter and thus has a relationship that differs from lens thickness.

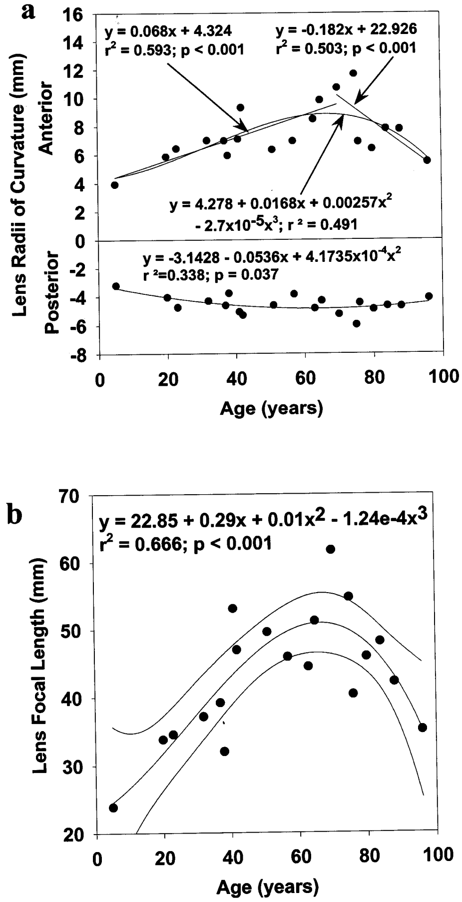

Fig. 4. (a) Lens anterior radius of curvature increases nearly linearly up to age 65 and then decreases nearly linearly beyond this age. The relationship shown here is essentially identical to data from several studies replotted by Wyatt and Fisher (1995) who show a regression line of $y = 0.083x + 4.9$ and suggest that the age of 65 separates the younger near-accommodated lenses from the older far-accommodated lenses. The data are well fitted by two linear regression lines below and above 65 years of age. This data is also well fitted with a third order polynomial. The lens posterior radii of curvature show only a small change with age without any obvious inflection point and is fitted with a second order polynomial. (b) There is a linear increase in lens focal length with age from five to roughly 65 years of age and thereafter a decrease in lens focal length with age for isolated lenses. The initial linear increase in lens focal length between 5 and 65 years has a slope not statistically different ($t = 1.0231$, $P > 0.1$; df = 25), but an intercept that differs (intercept: 23.94 here 34.67 previously, $t = 4.95$, $P < 0.001$, df = 25) from data from a different group of lenses measured under slightly different circumstances (Glasser & Campbell, 1998). The decline in lens focal length in the older age groups shown here may be due to the increased number of older lenses and a the higher incidence of lenses with early stages of cataract being used in this study. The youngest lenses have short focal lengths because they take on an accommodated form in the absence of external outwards directed zonular forces. This tendency is reduced towards 55 years of age by which time the accommodative amplitude reaches zero due to the progression of presbyopia. The upper and lower limits of the 95% confidence interval is also shown.

that an inverse linear correlation exists between posterior lens radii of curvature and lens focal length (as will be shown below).

3.1.2. Age changes in the optical properties of the lens

For the group of isolated lenses measured in this study, average lens focal length increases linearly up to age 65 ($r^2 = 0.750$; $P < 0.001$), and then decreases. A third order polynomial provides a significant fit to the average focal length of all these isolated lenses as a function of age (Fig. 4b).

3.1.3. Age changes in spherical aberration

In the isolated lenses with the capsule intact there is a significant linear correlation between the extent of the spherical aberration across the full diameter of the lens and the age of the lens. This relationship is of the form:

$$\text{Spherical aberration} = 0.387 \times \text{Age} - 10.160 \quad (9)$$

with $r^2 = 0.556$ and $P < 0.001$, where spherical aberration is defined by Eq. (6). This relationship is not statistically different (slope: $t = 0.0012$, df = 17; intercept: $t = 1.088 \times 10^{-5}$, df = 17) from the spherical aberration versus age relationship shown previously (Glasser & Campbell, 1998) in which spherical aberration was measured in unstretched human lenses prior to applying stretching tension through the intact zonule.

3.2. Physical and optical relationships of the lens with the capsule intact

3.2.1. Relationships between lens curvatures and focal length

From measurements of average focal length and surface radii of curvature we find that, independent of the age of the lens, anterior and posterior surface curvatures are significantly linearly correlated with focal length. There is a significant linear relationship between average focal length and anterior radius of curvature (measured across the central 1 mm) (Fig. 5a) and a significant inverse linear relationship between average focal length and posterior radius of curvature (Fig. 5b). Since both the anterior and the posterior lens surface radii of curvature are correlated with focal length, it follows that anterior and posterior lens surface radii of curvature are themselves linearly related. We find a significant inverse linear relationship between anterior radius of curvature and posterior radius of curvature (Fig. 5c). There is, among all the lenses measured, a greater range of lens anterior radii of curvature compared to the range of lens posterior radii of curvature.

3.2.2. Relationships between lens equatorial diameter and focal length

We find a significant linear correlation between lens focal length and lens equatorial diameter for lenses of all ages (Fig. 6a). A higher regression coefficient is seen ($r^2 = 0.813$ vs. $r^2 = 0.726$ as shown) if lenses older than 75 are excluded, but without altering the regression equation. Because of the linear relationships between lens anterior radius of curvature and focal length (Fig. 5a) and lens posterior radius of curvature and focal length (Fig. 5b), we also find good linear correlations between lens anterior radius of curvature and equatorial diameter (Fig. 6b) and lens posterior radius of curvature and equatorial diameter (Fig. 6c).

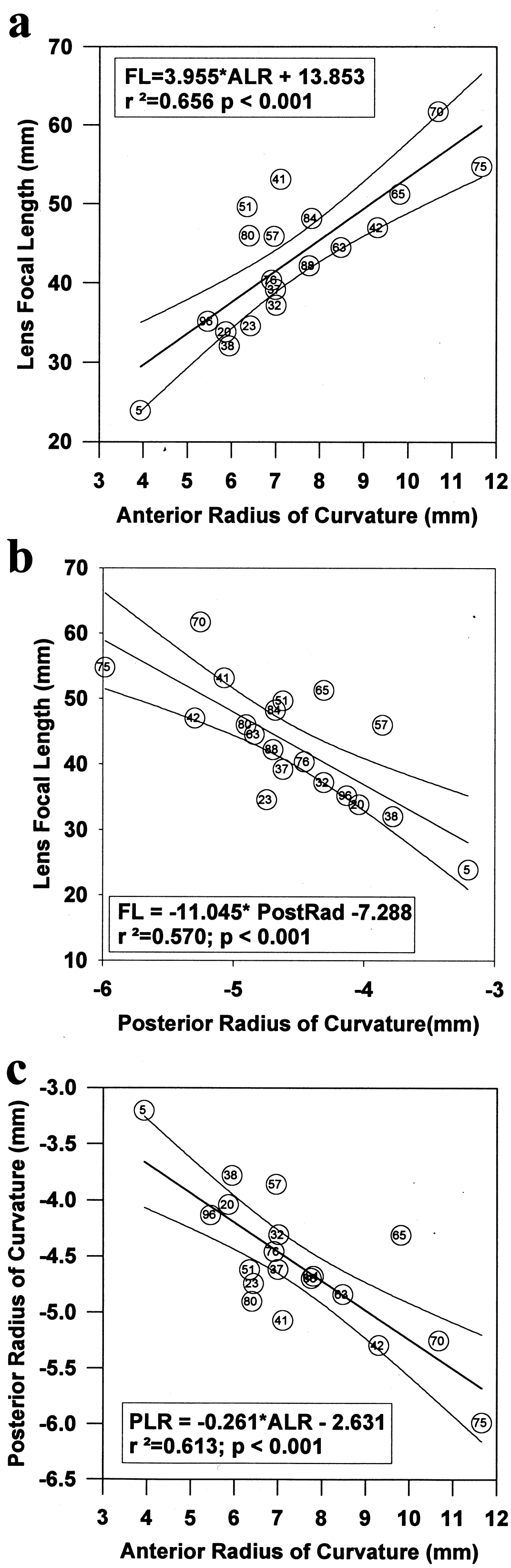

Fig. 5. There are good linear correlations between lens anterior radius of curvature and average lens focal length (a) and lens posterior radius of curvature and average lens focal length independent of the ages of the lenses (b). In addition, the anterior lens radius of curvature is well correlated with the lens posterior radius of curvature (c). Thus, average lens focal length can be well predicted from the lens surface curvatures alone and in addition, either the lens anterior surface curvature or the lens posterior surface curvature can be predicted reasonably well from the other. It should be noted that the youngest lenses are in a maximally accommodated state in the absence of any zonular forces on them while the older presbyopic lenses which are unable to undergo any changes in focal length (Glasser & Campbell, 1998) most likely maintain the same shape and focal length as in vivo. In this and subsequent figures the numbered symbols represent the age of each lens.

3.2.3. Lens paraxial properties

The lens paraxial focal lengths are significantly linearly correlated with the average focal length ($y = 1.046^* \times -3.305$; $r^2 = 0.791$; $P < 0.001$). The slope greater than unity and the intercept less than zero for this relationship reflects the negative spherical aberration of the younger lenses and the positive spherical aberration of the older lenses as mentioned above and in Glasser & Campbell (1998).

From Eq. (2) it is clear that paraxial lens radii of curvature are inversely proportional to the constant of the x^2 term of the parabolas describing the lens surface curvatures.

$$r_{\text{para}} = 1/2c$$

Because of the significant linear relationships between lens focal lengths (paraxial or average) and lens radii of curvature for the central 1 mm of the lens surface, the significant linear relationships between lens average focal length and the constant (c) of the parabolas describing anterior and posterior lens surfaces (Fig. 7a, b) would be expected.

3.3. Effects of decapsulating the human lens

3.3.1. Changes in focal length with decapsulation

There is an age dependent decrease in the scanning laser measured lens power (increase in average lens focal length) when the capsule is removed from the lens (Fig. 8a). This relationship is similar in form to the age dependent change in lens power when human lenses are mechanically stretched through the intact zonule (Glasser & Campbell, 1998). The two curves are shown together in Fig. 8a and although they follow the same general tendency, they are statistically different. Thus, when the capsule is removed from young lenses they tend to flatten and undergo an increase in focal length (a decrease in power), while older lenses tend to undergo little or no systematic changes in lens curvature or lens focal length. The form of the change in lens power (from the average focal length) (ΔLP) as a function of age due to decapsulation is given by the equation:

$$\Delta LP = 14.211 - 0.150 \times Age - 8.805 \times 10^{-3} \times Age^2 + 1.715 \times 10^{-4} \times Age^3 - 8.201 \times 10^{-7} \times Age^4 \quad (10)$$

with $r^2 = 0.818$ and $P < 0.001$.

The focal lengths of the decapsulated lenses from this study are plotted together with the stretched focal lengths of lenses from our previous study (Glasser & Campbell, 1998) in Fig. 8b. Although removing the lens capsule results in an increase in focal length of the younger lenses, there remains a considerable age dependent variation in focal lengths of the decapsulated lenses. Decapsulating the young lenses does not increase their focal lengths enough to match the longest focal lengths of either the stretched lenses (Glasser & Campbell, 1998) or the older lenses around the age of 65. This relationship is similar for both the mean focal length and the paraxial focal length since there is a significant linear correlation between the two measures of focal length as mentioned above.

3.3.2. *Changes in lens surface curvatures with decapsulation*

The systematic increase in lens focal length with decapsulation in the younger lenses is due to the combined effects of a flattening of the anterior lens surface, flattening of the posterior lens surface and a decrease in lens thickness. Although we find no significant correlations between age and changes in any of these three parameters with decapsulation, each of them change to some degree in a relatively nonsystematic manner to contribute to a systematic change in lens power. 68% of all the lenses show a flattening of the anterior lens surface, 95% show a flattening of the posterior lens surface and 79% become thinner with decapsulation. A signed rank *t*-test shows that there is a significant change in overall posterior lens radius of curvature with decapsulation (*t*-test: $t = 2.471$; df = 36; $P = 0.018$), but there is no systematic change in anterior radius of curvature with decapsulation (*t*-test: $t = 1.233$; df = 36; $P = 0.226$) or lens thickness ($t = 1.133$; df = 36; $P = 0.265$) for all lenses. *T*-tests on the younger pre-presbyopic lenses (arbitrarily selected as 55 years and younger,

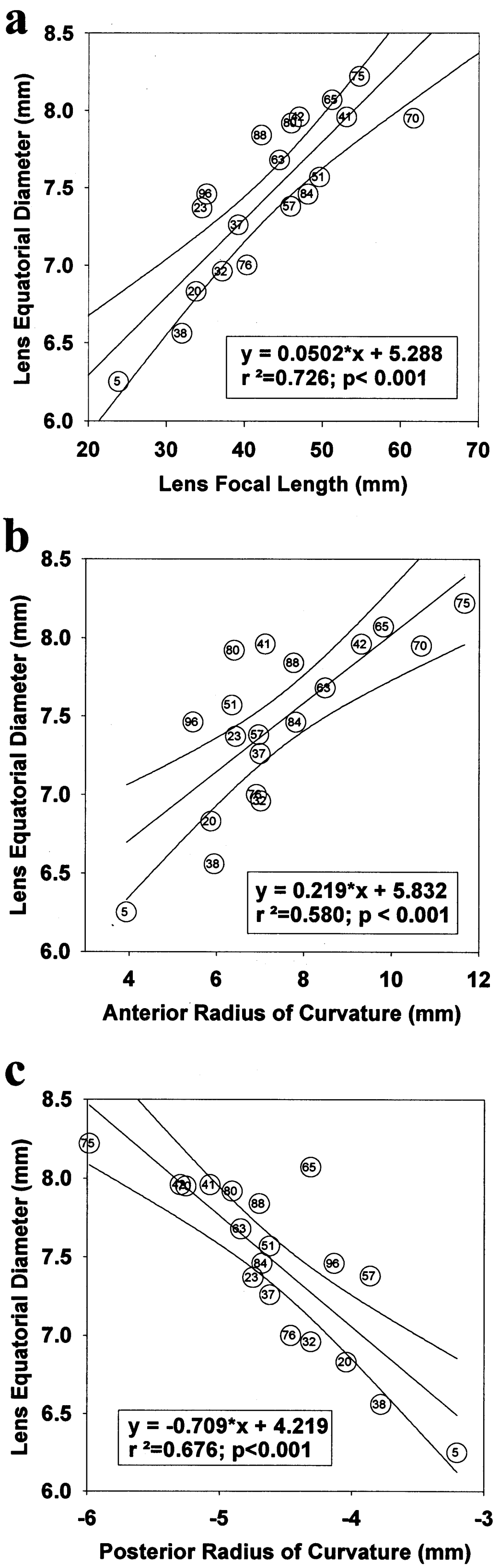

Fig. 6. There is a good linear correlation between lens equatorial diameter and average lens focal length independent of the age of the lens. This correlation is improved by omitting lenses over 75 years of age (a). Because of the good correlation between the anterior and posterior radii of curvature and the average lens focal length (Fig. 5a), it follows that lens equatorial diameter is also linearly correlated with anterior (b) and posterior (c) radii of curvature.

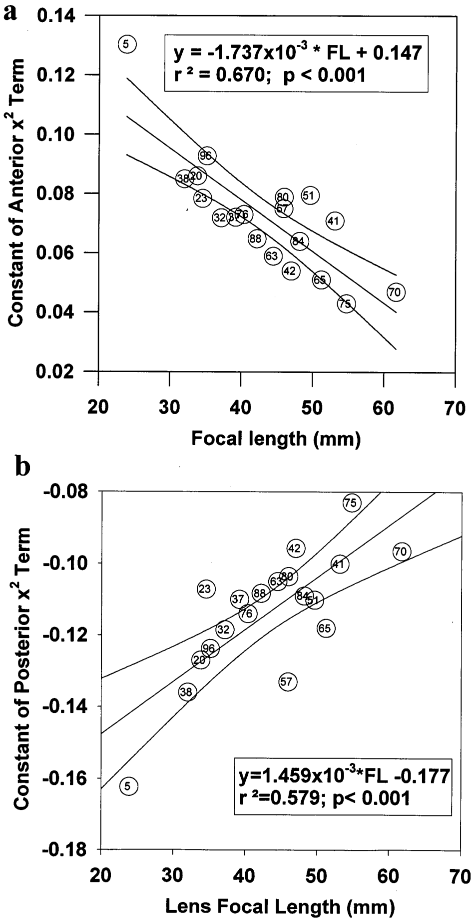

Fig. 7. There are good inverse linear and linear correlations between the constant (c) of the second order term of the parabolas (Eq. (2)) used to describe the lens anterior (a) and the lens posterior (b) surfaces respectively with the average lens focal length. The constant (c) in turn is inversely proportional to the paraxial radius of curvature. From Fig. 5 and this figure the lens focal length can be predicted from the radii of curvature calculated from any lens surface diameter up to 1 mm.

$n = 8$) reveal a statistically significantly flatter posterior radius of curvature after removal of the capsule ($t = -2.631$; df = 16; $P = 0.018$), but not for anterior radius of curvature ($t = 1.878$; df = 16; $P = 0.072$) or lens thickness ($t = 1.823$; df = 16; $P = 0.087$). Lens thickness decreases in 90% of lenses less than 55 years of age.

After decapsulation, there remains a reasonably good correlation between the lens focal length and the anterior curvature and a good correlation between the lens focal length and the posterior curvature (Fig. 9a, b). Further, after decapsulation there still exists a good correlation between anterior and posterior lens curvatures (Fig. 9c).

3.3.3. Changes in shape with decapsulation

In an attempt to identify other possible systematic changes in lens shape with decapsulation as a function of age we have compared the thickness of the anterior and posterior halves of the lens before and after removing the capsule. We find constant age independent relationships for anterior lens thickness, posterior lens thickness and the ratio between anterior and posterior thickness (Fig. 10a). When the lenses are decapsulated,

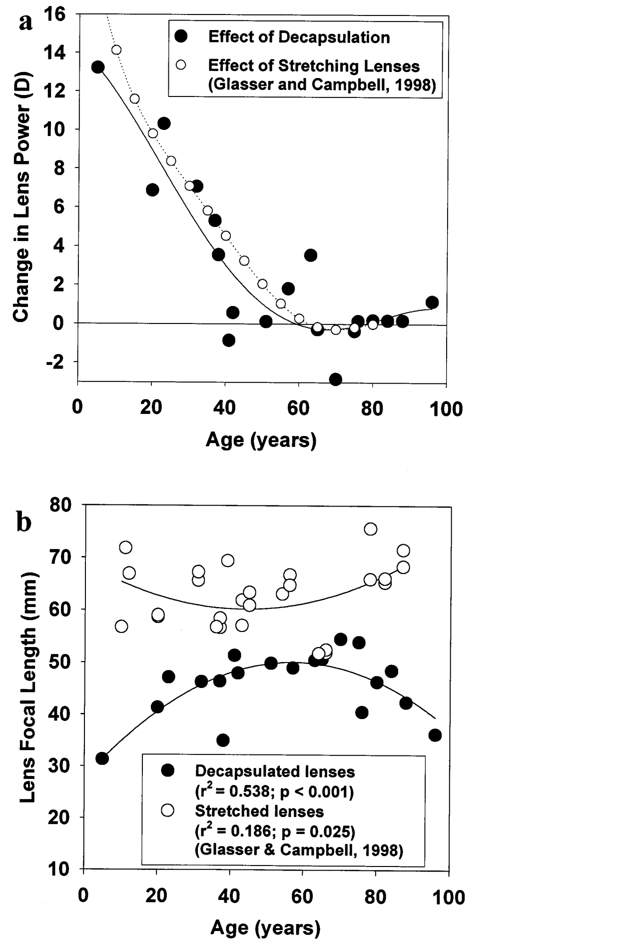

Fig. 8. (a) The *change* in lens power that occurs with the removal of the lens capsule (solid symbols and solid line) closely matches the change in lens power that occurs when the lens is mechanically stretched through the intact zonule (open symbols and dotted line) (Glasser & Campbell, 1998). The change in lens power with decapsulation is similar to the change in lens power with stretching. This age dependent change in focal length with decapsulation is likewise, similar in form to the age dependent loss of accommodative amplitude with the development of presbyopia (Duane, 1912; Glasser & Campbell, 1998). The change in lens power after removal of the lens capsule is significantly fitted with the forth order polynomial given in Eq. (10) (solid line). There is more variability of change in lens focal length (as evidenced by the scatter) among the older lenses. Despite this, lenses older than about 55 years of age do not undergo any systematic change in focal length with removal of the capsule. (b) Despite the similarities in the change in focal length with stretching and decapsulation, the mean focal length of the decapsulated lenses in this study are different from the maximal mean focal length obtained by stretching the lenses in a previous study (Glasser & Campbell, 1998). For the stretched lenses, the change in maximal focal length with age is insignificant ($P = 0.025$) while for the decapsulated lenses it is significant ($P < 0.001$). The difference between the two situations is greatest for the youngest and oldest lenses.

the anterior and posterior thickness decrease in the younger lenses while the anterior and posterior thickness of the older lenses remain unchanged. As a result, after decapsulation there is a significant increase in anterior lens thickness ($P < 0.003$) and a significant increase in posterior lens thickness ($P < 0.005$) with age, but no age change in the ratio of these two parameters, thus demonstrating that, with decapsulation, they increase by equivalent amounts (Fig. 10b). We have also calculated a shape factor or ellipticity constant for each lens (Pierscionek & Augusteyn, 1991). The ellipticity constant is the ratio of lens equatorial diameter to lens saggital diameter (thickness). There are small age dependent changes in the lens diameter to lens thickness ratio (the ellipticity constant) with the capsule on and after the capsule is removed (Fig. 10c) and there is a systematic change in the ellipticity with decapsulation of the form:

Change in ellipticity with decapsulation

$$= -0.00441 * \text{Age} + 0.358 \quad (11)$$

with $r^2 = 0.537$; $P < 0.001$. A larger change in ellipticity constant is seen with decapsulation for the younger lenses than for the older lenses. Small or no change is seen for lenses over age 60 (Fig. 10c). We find no significant correlations between age and any of the following parameters; lens thickness, decapsulated lens thickness, change in lens thickness with decapsulation, decapsulated lens diameter, change in lens diameter with decapsulation.

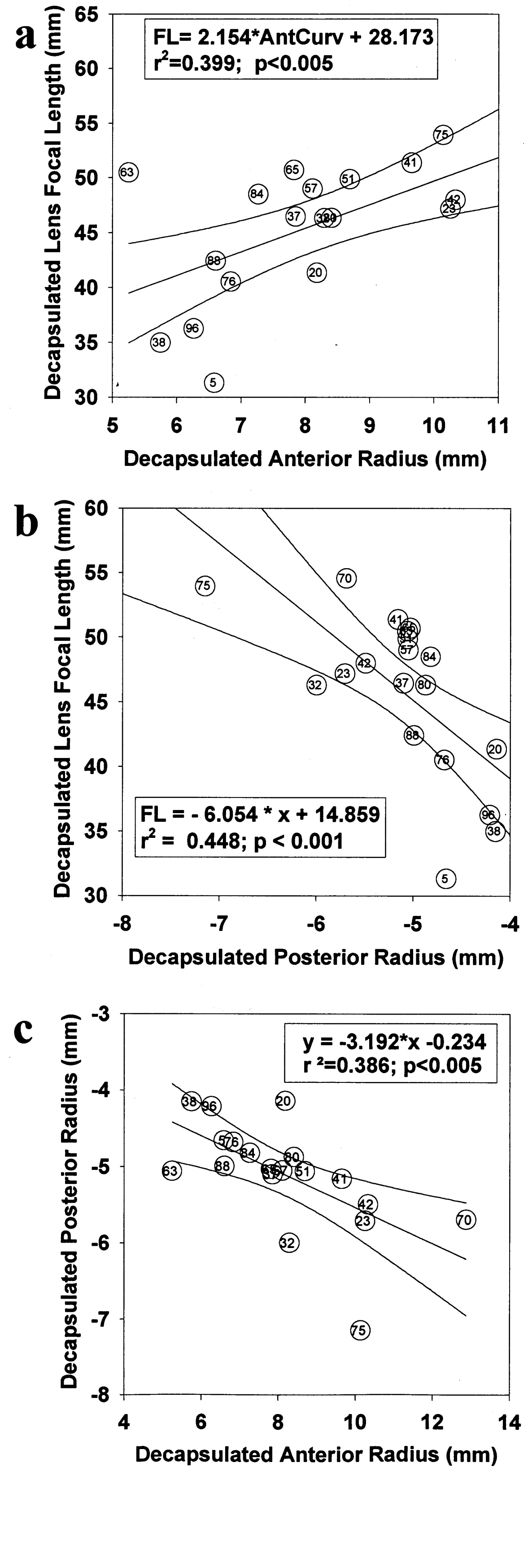

Fig. 9. After the lens capsule is removed, there are still reasonably good correlations between lens focal length and anterior radii of curvature (a) and between lens focal length and lens posterior radius of curvature (b) although the regressions are not as good as before the capsule is removed. The decrease in these correlations after decapsulation is anticipated because the nonsystematic changes in anterior lens curvature, posterior lens curvature and lens thickness with decapsulation. As in lenses with the capsule intact, there is still a reasonably good linear correlation between the anterior and posterior radii of curvature of the decapsulated lenses (c).

3.3.4. Changes in spherical aberration with decapsulation

Although decapsulating the lenses causes a change in spherical aberration as a function of age as seen from the change in slope (from 0.387 to 0.254) and the change in intercept (from −10.160 to −3.787), these changes are not significant (slope: $t = 1.588$, df = 17, $0.05 < P < 0.1$; intercept: $t = -1.260$, df = 17, $0.05 < P < 0.1$). The change in slope and intercept of the spherical aberration versus age relationship as a consequence of decapsulation are of the same sign and magnitude as those that occur with mechanical stretching (change in slope: decapsulation = 0.133 and stretching = 0.096 and change in intercept: decapsulation = 6.373 and stretching = 5.91) reported previously (Glasser & Campbell, 1998). Thus, although statistical significance of the change in spherical aberration with decapsulation is not attained due to the low correlation, the overall effect on spherical aberration of removing the capsule tends to be the same as for stretching the lenses.

3.4. The equivalent refractive index of the human lens

To determine how well measured lens surface radii of curvature and lens thickness are able to predict the scanning laser measurement of the focal length of each lens, we used lens curvatures and lens thickness values together with an age independent Gullstrand–Emsley lens equivalent refractive index value ($n_3 = 1.4160$) in Eq. (3) to *calculate* the paraxial power of each lens (Bennett & Rabbetts, 1989). For comparison, the *measured* paraxial power of each lens can be obtained from the point where the 4th order spherical aberration polynomials from the scanning laser measurements cross the optical axis. This allows a comparison of measured and calculated paraxial lens power and we find a significant linear relationship (Fig. 11a). Thus, by assuming an age *independent* Gullstrand–Emsley equivalent refractive index value for all lenses together with the measured lens curvatures and lens thickness we can predict, with reasonably good accuracy, the paraxial scanning laser measurements of lens power. A good linear relationship also exists between this calculated paraxial lens power and the scanning laser measurements of total mean lens power (i.e. considering all beams passing through the full diameter of the lens, not just the paraxial rays) (Fig. 11b). There is also a good linear relationship between the paraxial lens focal length and the measured total mean lens focal length (Fig. 11c). This relationship shows that for this group of lenses the paraxial focal length is on average 3 mm shorter than the mean focal length.

Since we have measured lens curvatures, thickness and focal length, from Eqs. (3)–(5) we are able to iteratively back calculate an equivalent refractive index for each lens. This calculated equivalent refractive index ensures that the lens power to the left of the equality sign in Eq. (3) is exactly determined from the lens surface curvatures and the lens thickness values to the right of the equality sign. Computing the mean equivalent refractive index using the paraxial focal lengths gives us a value of 1.4257 (± 0.0163 S.D.) which is not

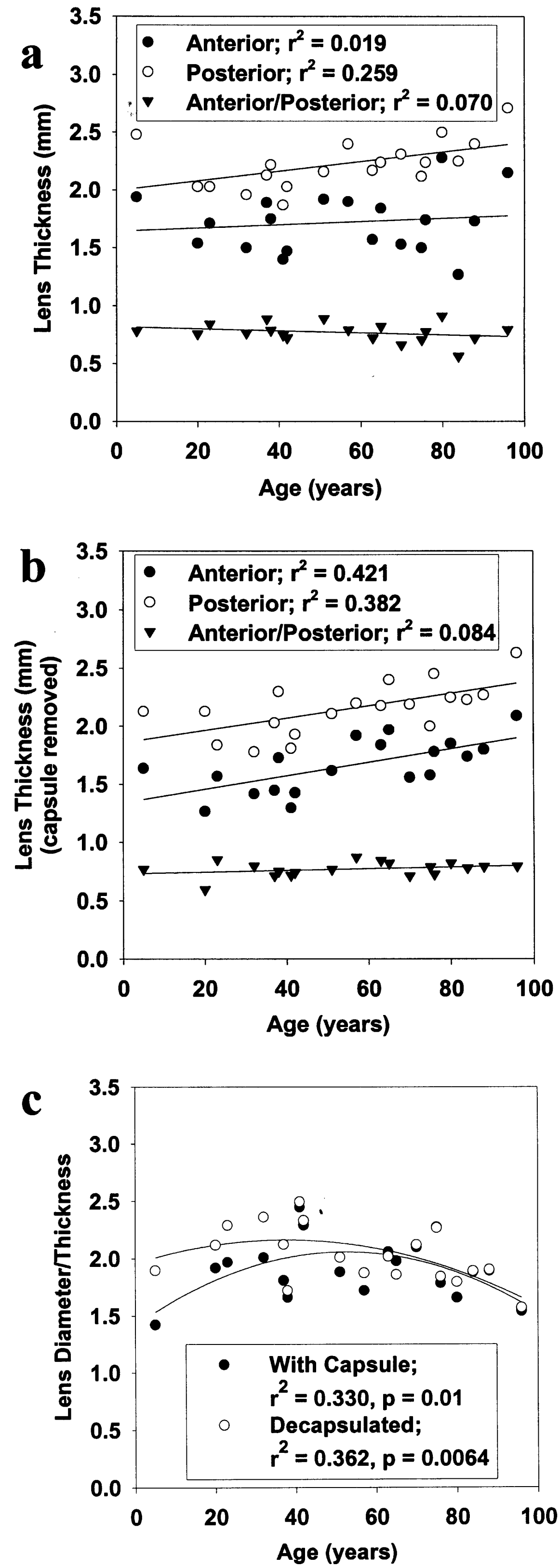

Fig. 10. The thickness of the anterior and posterior halves of the lens and the ratio between these two measures (anterior/posterior) are relatively constant and do not undergo any significant age dependent change with the capsule intact (a). After the capsule is removed, however, there results a significant increasing thickness of the anterior ($r^2 = 0.421$, $P < 0.003$) and the posterior ($r^2 = 0.382$, $P < 0.005$) halves of the lens as a function of age. This change in the anterior and posterior halves is of approximately equal extent resulting in no significant change in the ratio of the two with increasing age (b). This change is a consequence of a decrease in thickness of both the anterior and the posterior halves of the younger lenses with decapsulation without a change in the older lenses. The ratio of the lens equatorial diameter to lens thickness (the ellipticity constant) are best fitted with second order equations suggesting age dependent changes (with capsule: $r^2 = 330$, $P = 0.01$; capsule removed: $r^2 = 362$, $P < 0.0064$) (c).

statistically different from the Gullstrand–Emsley value of 1.416 (Mann–Whitney Rank Sum Test: $P = 0.169$). The mean equivalent refractive index value calculated using the lens focal length measured across the entire lens diameters (i.e. non-paraxial) is 1.4216 (± 0.0102 S.D.). These values are not statistically different from each other ($t = 1.790$; $P = 0.090$) or from the Gullstrand–Emsley value (Mann–Whitney Rank Sum Test: $P = 0.169$). We find no age correlation of these calculated lens equivalent refractive index values (linear: $r^2 = 0.150$; $P = 0.101$; second order: $r^2 = 0.306$, $P = 0.054$). The paraxial equivalent refractive index calculated for the *decapsulated* lenses is 1.4248 (± 0.0109). This is not statistically different from the paraxial refractive index calculated for the lenses with the capsule intact ($t = -1.346$; $P = 0.195$). The equivalent refractive index of the decapsulated lenses also shows no significant age correlation ($r^2 = 0.203$, $P = 0.053$).

3.5. The lens resistance to mechanical deformation

For each step increase in the compressive force applied to the lens, there is a corresponding increase in the output voltage from the pressure transducer (Fig. 12a). The magnitude of this response is considerably smaller for the young lenses than for the older lenses. In the younger lenses there is a square step increment in the output from the pressure transducer for each increase in force on the lens. The response remains constant over the subsequent ten second inter-step interval after each increment in compressive force. The lens resistance to compressive forces changes considerably with age. In the older lenses a distinctive biphasic response occurs with each increase in compressive force. An initial transient spike (a fast phasic response) is followed by a slower secondary decay back down towards a steady state (a slower tonic response). As the age of the lens increases, the peak amplitude of the initial phasic response increases. In the oldest lens, the secondary decay in the output from the pressure transducer at each step does not reach a steady state or asymptote during the following 10 s inter-step interval as it does in the younger lenses. As the plunger is stepped up off the lens

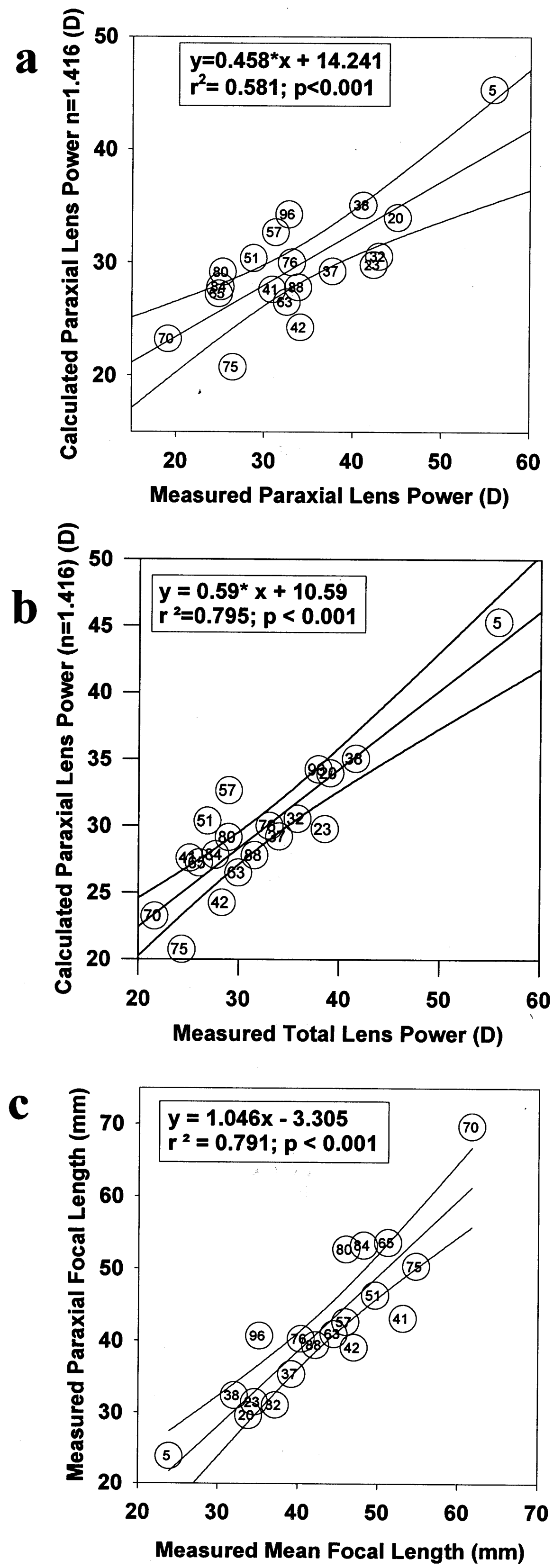

Fig. 11. The measured paraxial lens power obtained from the 4th order spherical aberration polynomials shows a linear correlation with the paraxial lens power calculated using the lens surface curvatures, lens thickness and an age independent Gullstrand–Emsley equivalent refractive index for the lens ($n = 1.4160$) (a). This suggests that there is no age change in the equivalent refractive index of the isolated human lens. There is also a good linear correlation between the paraxial lens power calculated from the lens biometric measurements as described above and the *total* lens power which is the average focal length obtained from all the rays passing through the full diameter of the lens (b). The linear correlations seen in (a) and (b) indicate that a good linear correlation also exists between the measured paraxial lens power (obtained from the spherical aberration polynomials) and the measured total lens power obtained from the scanning laser measurements across the full lens diameter (c).

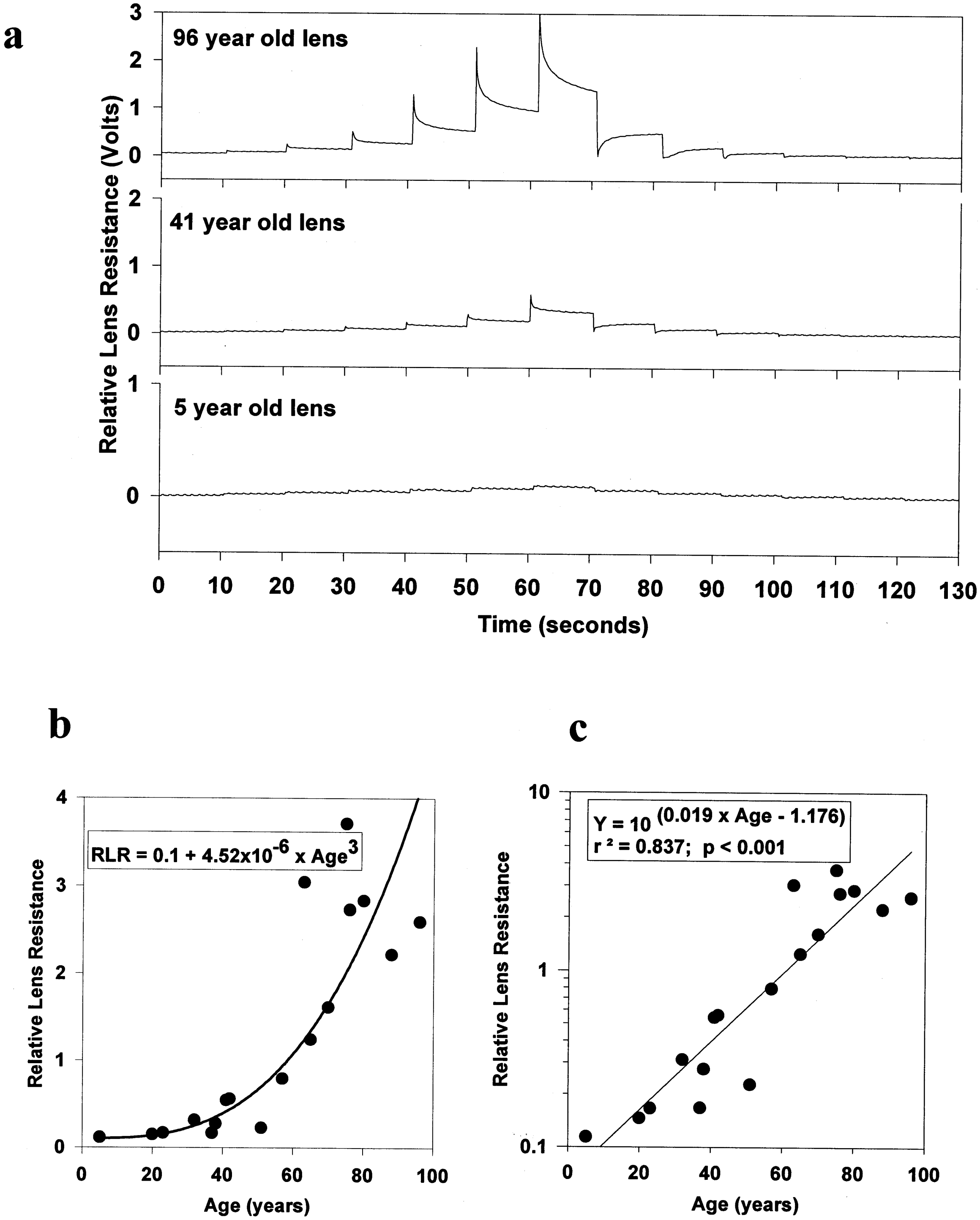

Fig. 12. (a) Changes in pressure transducer output voltage as increasing compressive forces are applied to three human lenses of different ages (a). Each lens is subjected to six successive steps of increasing compressive force and then six successive steps of decreasing force. The 5 year old lens (lower trace) shows a square staircase-like function of low amplitude. A 41 year old lens (middle trace) starts to show signs of a more biphasic response consisting of an initial phasic spike and a secondary tonic decay at each step. This biphasic response is accentuated in a 96 year old lens (top trace) with a greater amplitude spike and a larger decay following each spike. The amplitude of each step of the 96 year old lens is considerably increased relative to the 5 year old lens and there is an asymmetry between the rising and the falling phases in the output from the 96 year old lens that is not seen in the output from the 5 year old lens. Note that the voltage scale increases from the 5 to the 41 to the 96 year old. (b) The maximum amplitude of the largest spike at the 6th step (relative lens resistance) increases as a function of the age for all lenses. (c) If this same relative lens resistance is plotted on a logarithmic scale a good linear relationship is seen as a function of age.

to *reduce* the compressive force in six successive steps, there is a corresponding decrease in the output of the pressure transducer. The characteristics of this output also show age dependent differences. In general, the response to decreasing compressive force is essentially an inverse of the response to increasing compressive forces on the lens. In the younger lenses, at each decrease in the compressive force there is a small, abrupt square step-like decrease to a new steady-state level. In contrast, in the older lenses there is an initial, relatively larger, rapid phasic decrease in compressive force, followed by a slower secondary tonic increase towards a new steady state. The 96 year old lens shows considerable asymmetry in the increasing and decreasing phases (Fig. 12a) relative to the young lenses (Fig. 12c). When decreasing the compressive force on the older lens, the pressure transducer output returns to zero by the forth step in the 96 and 41 year old lenses whereas in the 5 year old lens the zero position is attained only after the sixth step. There is an age dependent increase in the maximum output voltage recorded from the pressure transducer at the sixth step increase in force (the point of maximum compression) applied to each lens (Fig. 12b). This demonstrates that the physical resistance of the lenses increases with increasing age. A significant linear relationship between the age of the lens and the logarithm of the pressure transducer output exists (Fig. 12c).

4. Discussion

4.1. Summary of results

The results presented here consider age changes in the optical and physical properties of isolated human lenses. The results show that the human lens grows throughout life and becomes heavier and larger in cross sectional area. The equatorial diameter of this group of isolated lenses increases up to age 70 and then decreases, anterior lens surface radii of curvature increase up to age 65 and then decrease and the posterior lens surface curvature has a tendency to flatten with increasing age. The thickness shows no significant age dependence, although it has a tendency to increase. This implies that, beyond the age of 70, cross-sectional area is increasing primarily in the mid-periphery of the lens. Focal length changes with age in proportion to surface curvatures, first lengthening with age and then shortening in the oldest lenses. The equivalent refractive index of the lens shows no age dependence and the human lens becomes increasingly resistant to compressive forces throughout life.

4.2. Use of human tissues

We have previously addressed the problem of time-after-death and the scarcity of young human eyes for human lens studies (Glasser & Campbell, 1998, 1999). Since these issues represent possible sources of variability in the present study, we will address them again. As in the previous study (Glasser & Campbell, 1998), human tissues were maintained and transported under ideal conditions, chilled, in humidified, sealed jars and were maintained in solution at room temperature for 30–45 min during the dissection prior to any measurements being made. The optical quality of organ cultured animal lenses assessed with similar methodology to that used here is maintained for weeks without significant deterioration (Weerheim & Sivak, 1992). In the present study, three human lenses (ages 38, 57 and 65) were used more than 71 h after death, the longest being 182 h. The data from only one of those three lenses, that of the 57 year old, appeared as an outlier on any of the graphs with a frequency greater than that of any other lenses used. A reanalysis of the data excluding this 57 year old lens did not significantly alter the regressions or the level of significance of any of the data shown. Reanalysis of our previous results (Glasser & Campbell, 1998, 1999) indicates that excluding lenses older than 72 h did not change the form of the relationships or the significance of the results found. Weale (1983, 1985) has shown that the optical properties of post mortem human lenses are maintained (Glasser & Campbell, 1999). While time after death may cause subtle changes in the optical characteristics of the human lens (Weale, 1983), the maximal possible change in focal length that can be expected at 182 h for a 23.4 D female lens, for example, would be about 2.3 D (Weale, 1983). Since the age dependent changes in lens focal length reported here are substantially larger (35 D between shortest and longest), any post mortem changes in focal length must be considered as relatively insignificant. Since our lenses remained in situ in the eye under ideal conditions until used, rather than in castor oil (Weale, 1983), it is likely that any possible post mortem changes would be minimized. The scientific merit in actually doing experimental studies on human lenses far outweighs possible compromises introduced by difficulties in obtaining fresh donor tissues. Other influences on the lenses such as the effects of temperature variations between body and room temperature and the influence of gravity on the isolated lenses, while possible, are unlikely. Such factors are not likely to significantly alter the results obtained. Possible differences between lens pairs from the same donors are also unlikely, given the similarities between lens pairs found previously (Glasser & Campbell, 1998). Because of the limited and unequal number of female donors, the absence of gender matched lenses, and the absence of any other personal information it is impossible to relate the data to any other personal or biometric parameters of the donors.

4.3. Lens size and mass

The continued linear growth in mass and volume of the human lens after the age of 5 years and throughout the remainder of the normal life-span has been well documented (Scammon & Hesdorffer, 1937; Weale, 1963b). Our measurements agree well with established rates of increase in lens mass, but our lenses were roughly 30 mg (8–10%) heavier than previous reports which have either used fixed lenses or provided few details of the tissue handling procedures (Scammon & Hesdorffer, 1937; Weale, 1963a; Willekens, Kappelhof & Vresen, 1987). The tissues we obtained were kept under ideal conditions by the eye bank (chilled and on moist cotton in sealed bottles) to maintain optimal hydration. This may account for the slight increased mass of our lenses relative to previous studies which may not have maintained the hydration of the lenses after death. Although we cannot directly compare our measures of cross sectional area with previous measurements of lens volume (Scammon & Hesdorffer, 1937), both measures show an increase with age. Our measurements of lens thickness are not significantly correlated with age with or without the presence of the capsule, but both conditions show a tendency for an increased lens thickness with age. It is of interest to note that while the lens weight increases linearly throughout life, this is in marked contrast to the age related changes in focal length and anterior lens curvature which show a change in the sign of the slope at about age 65 years of age for this group of lenses. The decrease in anterior lens radius of curvature and lens focal length after age 65 in this group of lenses is, therefore, independent of the continued growth of the lens and not an indication of a change in growth rate.

4.4. Lens focal length

For isolated lenses younger than 75 years of age, the relationship between lens focal length and age (Fig. 4) is similar to human lens focal lengths measured previously in unstretched human lenses (Glasser & Campbell, 1998). The lenses older than 75 years of age that were used in this study have progressively shorter focal lengths with increasing age. The lenses over 75 years of age used in the previous study (Glasser & Campbell, 1998) fell along the same linear increase in lens focal length as the younger lenses. A statistical comparison between the older lenses in the two studies is inappropriate due to the limited number of lenses in the older age groups. The age dependent changes in focal length presented here are consistent with cross-sectional measurements of resting refraction which show a gradual increase in hyperopia and then a progression towards myopia from the age of about 65 or 70 (Slataper, 1950; Saunders, 1981). Slataper (1950) suggested that this senile progression towards myopia was due to senile changes in the lens associated with early cataract with resulting changes in the refractive index distribution of the lens, rather than changes in globe length. It is likely that the present study incorporated more human lenses with visible signs of early cataract than did our previous study (Glasser & Campbell, 1998) in an effort to include more older lenses. Lenses with opacities preventing a laser scan across the full lens diameter were excluded. Some lenses with mild cortical opacities were used, but the frequency with which older lens pairs could not be used due to cataract in one or both lenses or surgically implanted intraocular lenses being found in the donor eyes bears witness to the high prevalence of cataract in these older eyes. However, it seems unlikely that the decreasing focal length among the older lenses can be associated with changes in the refractive index distribution as suggested by Slataper (1950), since our results show that lens surface curvature and focal length remain linearly correlated even among these older lenses. In spite of the differences in focal length between the lenses from this and the previous study (Glasser & Campbell, 1998), the results are similar in that we still see a drift towards longer focal lengths in the lenses up to about age 75. This again identifies continued age related changes in the already presbyopic lenses (between the ages of 45 and 75) beyond those contributing to a loss of accommodative amplitude.

Our results of anterior lens radius of curvature as a function of age for the isolated lenses show a remarkable similarity to the data shown by Wyatt and Fisher (1995). They show a regression line of approximately $y = 0.083x + 4.90$ for ages 0 to 65 years. Neither the slope nor the intercept of our relationship shown in Fig. 4b and given in Eq. (6) are significantly different from this relationship (slope: $t = 1.55$, $P > 0.05$, df = 10; intercept: $t = 2.3$, $P > 0.01$, df = 10). Other more recent studies which have made direct measurements on isolated human lenses have either not measured lens curvature (Pierscionek & Augusteyn, 1991) or have measured curvatures in too few lenses (two or five lenses) making it impossible to identify any age related trends (Pierscionek, 1993b, 1995).

4.5. The role of the lens capsule in accommodation and presbyopia

Much debate has centered around the role of the lens capsule in accommodation and presbyopia. The lens capsule serves as the site of attachment of the zonular fibers to the equatorial edge of the lens. In a young eye, resting zonular tension serves to maintain the lens in a flattened and unaccommodated state. It is generally recognized that the natural state of the isolated young lens is accommodated (Helmholz, 1909) and this is supported by the shorter focal lengths we have measured in the younger of the isolated lenses. The act of accommodation reduces the resting zonular tension and the young lens is allowed to round up under elastic recovery to become accommodated. Fincham (1937) demonstrated the role of the lens capsule in maintaining the young lens in an accommodated form by photographing the profile of a young lens before and after removing the capsule. Removing the capsule increased the lens equatorial diameter, flattened the lens and increased the anterior lens surface radius of curvature (Fincham, 1937; Fisher, 1969b; Wyatt & Fisher, 1995). Fincham (1937) believed that in the young eye the lens substance was plastic and the capsule provided the elastic molding pressure to accommodate the lens and that presbyopia was due to an inability of the capsule to mold the hardened lens substance. The measurements presented here generally confirm that the lens capsule draws the young lens into a more accommodated form since decapsulation results in flattening and an increase in focal length of the younger lenses, although with other nonsystematic effects. The age dependent change in lens focal length that occurs when the capsule is removed also closely matches the expected accommodative amplitude for the age of the lens (Glasser & Campbell, 1998). Older lenses do not undergo systematic changes in focal length (Fig. 8a) or lens shape (Fig. 10) when the capsule is removed. This suggests either that (1) the lens substance is hardened to the point where the capsule is unable to exert an influence on the overall lens shape and upon removal of the capsule the hardened lens substance is unable to flatten out as in the younger lenses or that; (2) the capsule does not exert any force on the older lenses at all and the lens shape is thus not changed when the capsule is removed. The former hypothesis is consistent with the increased resistance of the older lenses to compressive forces and with our previous demonstration that older lenses do not undergo changes in focal length with stretching forces applied through the zonule (Glasser & Campbell, 1998). Our previous results also reduce the likelihood of the second hypothesis since neither maximally stretching or releasing zonular tension results in changes in focal length of the older lenses (Glasser & Campbell, 1998). The more than three-fold increase in resistance of the human lens to compressive forces over the human life-span provides evidence for the hardening of the lens substance as a major factor in the loss of accommodative amplitude with the development of presbyopia. Fisher (1969a) showed that the lens capsular elasticity decreased with age and suggested this as a possible contributing factor in presbyopia (Fisher, 1969b). More recent work, however, has shown that the elastic stiffness of the capsule actually increases up to age 35 resulting in a less extensible and more brittle capsule with increasing age (Krag et al., 1997). Evidence for changes in capsular elasticity with age is thus contradictory and without knowledge of the capsular forces on the lens substance, it is difficult to draw any specific conclusions as to the role of the capsule in the development of presbyopia. The results presented here together with our previous results (Glasser & Campbell, 1998) suggest that irrespective of the presence or absence of the capsule or changes in capsular elasticity, the lens substance undergoes an increase in hardness ultimately to a point well beyond which the capsule is able to exert any influence on it.

4.6. Effects of decapsulation and the role of the capsule

Decapsulation results in changes in lens power which closely match the changes in lens power that occur when the lens is mechanically stretched through the intact zonule (Fig. 8), which in turn closely match the accommodative amplitude for all ages and the well characterized loss of accommodative amplitude with increasing age (Duane, 1912; Glasser & Campbell, 1998). However, the other effects of decapsulation on the lens are not so systematic. Although removing the capsule causes changes in thickness, ellipticity and posterior surface curvature with age, the extent of the changes do not show consistent age correlations. Four lenses actually get thicker with decapsulation (*ages 65*, *63*, *76* & *38*), in six lenses the anterior radius of curvature decreases and the lens diameter actually gets smaller in 50% of lenses. Of the nine prepresbyopic lenses less than 55 years of age, eight undergo an increase in anterior radius of curvature, nine an increase in posterior radius of curvature, seven an increase in focal length and eight become thinner. These are the changes expected with the relaxation of accommodation. The anterior surface curvature of all decapsulated lenses show no age dependence and do not agree with in vivo Scheimpflug slit-lamp measurements of unaccommodated anterior lens surface curvatures (Lowe & Clarke, 1973; Brown, 1974). Sorsby et al. (1957) has given data for lens surface curvatures only for young subjects 7–16 years of age and although Purkinje image photography has been used to measure lens surface curvatures, no data from cross-sectional studies encompassing the full human life-span are available for comparison. We also find that decapsulation causes nonsystematic changes in both the anterior and posterior surface curvatures, unlike accommodation which has been thought to produce systematic changes to the lens anterior, and to a smaller extent the posterior, radius of curvature (Brown, 1973). No previous attempts have been made to systematically measure the effects of decapsulation on the human lens.

Our results show the highly variable responses of individual human lenses to decapsulation. In spite of this variation, the changes in spherical aberration as a result of decapsulation are not significantly different from the changes in spherical aberration induced by stretching the lenses (Glasser & Campbell, 1998). This and the similarity of the change in lens power with stretching (Fig. 8) suggests that decapsulation has, on average, a similar effect to that of stretching the lens (Glasser & Campbell, 1998). However, the focal lengths of the decapsulated lenses are not as long as that of the maximally stretched lenses. Wyatt and Fisher (1995) have speculated that the anterior radius of curvature of the isolated (decapsulated) lens substance falls between the accommodated and the unaccommodated lens anterior radii of curvature while Fincham (1937) postulated that the decapsulated lens is equivalent to an unaccommodated lens. Our results make it clear that it is impossible to draw such generalized extrapolations from the data of just one or two lenses. The apparent differences between accommodation and decapsulation may be due to many other factors including in vivo vs in vitro methods used to measure anterior lens surface curvature, nonsystematic effects of decapsulation due to the mechanical manipulation required to remove the capsule, anatomical differences between the anterior and posterior lens capsule (differences in capsular thickness for example), the orientation of the zonular forces and the presence of the vitreous force in vivo, for example. Further in vivo measurements and measurements of lens shape changes with stretching will be necessary to definitively identify what the relationships are between in vivo unaccommodated, in vitro stretched and decapsulated lenses. We can neither definitively confirm or dismiss that the decapsulated lens is similar to an unaccommodated lens in vivo.

4.7. The lens paradox and equivalent refractive index

Previous Scheimpflug slit-lamp measurements made by Brown (1974) show increases in lens surface curvature (i.e. decrease radii of curvature) with age in the unaccommodated eye. The lens paradox stems from the progressive *loss* of near vision that occurs with presbyopia rather than a shift towards myopia as should be predicted from increasing lens surface curvatures (Koretz & Handelman, 1986, 1988). It has been postulated that the expected myopic shift does not occur because of compensatory changes in the gradient refractive index distribution of the lens with age (Koretz & Handelman, 1988; Pierscionek, 1993b; Hemenger et al., 1995; Ooi & Grosvenor, 1995). Such compensation would necessarily also cause a change in the equivalent refractive index with age. The measurements we report here are for isolated lenses without any forces on them. Under these conditions the younger lenses are maximally accommodated while the older presbyopic lenses, which are unable to undergo any accommodative changes at all (Glasser & Campbell, 1998), remain much as they were in the eye. We are thus unable to directly compare our lens curvature measurements of isolated lenses with intact capsules with Brown's (1974) Scheimpflug measurements of unaccommodated eyes. However, we find no age dependence of surface curvatures for the decapsulated lenses. Focal lengths of the decapsulated lenses are directly proportional to the anterior and inversely proportional to the posterior lens radius of curvature providing no evidence for a paradox between lens focal length and lens surface curvatures. On the contrary, we find that independent of the age of the lenses or the presence or absence of the capsule, lens focal length is directly proportional to the anterior lens surface radius of curvature and inversely proportional to the posterior lens surface radius of curvature. Brown's (1974) Scheimpflug slit-lamp measurements were necessarily made through the optical surfaces preceding those being measured. The anterior lens surface measurements are influenced by the cornea and the anterior chamber depth and the posterior lens surface measurements are additionally influenced by the optics of the lens itself. Although Brown (1974) undertook corrections for the corneal curvature, the peripheral asphericity of the cornea through which the slit-lamp imaging is done and possible age dependent changes in corneal curvature and anterior chamber depth could be sources of systematic errors in the anterior lens surface curvature measurements. The posterior lens surface measurements, for which Brown (1974) provided no corrections, are influenced by the unknown gradient refractive index of the lens. If there is no lens paradox for accommodated lenses as suggested by our results from isolated lenses, but there is for unaccommodated lenses, the equivalent refractive indices of the unaccommodated and accommodated lenses must differ.

Although we have no direct measurements of unaccommodated lens radii of curvature (other than for the decapsulated lenses which may differ from unaccommodated lenses), we can estimate unaccommodated lens radii of curvature from our previous results (Glasser & Campbell, 1998). In that study, unaccommodated (stretched) lens focal lengths were given by the equation:

$$\text{Unaccommodated focal length} = 68.86 - (0.394 \times \text{Age}) + (0.00447 \times \text{Age}^2) \quad (12)$$

We have established in the present study that isolated lens focal length is correlated with anterior surface curvature (Fig. 5a) independent of the age of the lenses. If we assume that this remains true for the previous group of lenses (Glasser & Campbell, 1998) and we assume that lens equivalent refractive index does not change with accommodation (as is supported by data from the decapsulated lenses), we can estimate unaccommodated anterior lens surface curvature from Fig. 5a and Eq. (12) above. Unaccommodated anterior lens surface radius of curvature (ALC) as a function of age is then given by:

$$\text{ALC} = 11.88 - (0.07 \times \text{Age}) + (7.91 \times 10^{-4} \times \text{Age}^2) \quad (13)$$

In sharp contrast to the results from Brown (1974) this produces a shallow U-shaped function with little age change. Over the age range 1–100 years this function has a minimum (the steepest anterior surface curvature) of 10.3 mm at age 44 years and a maximum of 12.8 mm (flattest anterior lens surface) at 100 years. The anterior lens radius of curvature for the unaccommodated lenses from Brown (1974) falls linearly from roughly 17.0 mm at age 10 to 9.0 mm at age 80. Despite measurements of lens curvature in a large population using Purkinje image photography (Sorsby et al., 1957) no raw lens curvature data are given and no other cross sectional studies of Purkinje image measurements are known to the authors, thus preventing a comparison of Brown's measurements, our measurements and those from Purkinje image photography.

The lens paradox has been addressed through in vitro study of isolated human lenses (Pierscionek & Augusteyn, 1991) where, although surface curvatures were not measured, the ratio of lens equatorial diameter to lens thickness was used and was expected to decrease if the curvatures of the lens surfaces increase without a change in lens diameter. Accommodation, for example, would cause a decrease in this ratio as lens thickness increases and equatorial diameter decreases. This ratio is constant with age for the lenses used by Pierscionek and Augusteyn (1991) and so no predictions about shape changes with age could be made. Our data are better fit by second order equations demonstrating some age dependent changes (Fig. 10c). However, this analysis is complicated by the fact that young isolated lenses are accommodated, while old ones are not, so the state of accommodation as well as age will influence the ratio. This was perhaps not evident to Pierscionek and Augusteyn (1991) since they made no focal length measurements and did not discuss it, but is evident here and previously (Glasser & Campbell, 1998) since the focal lengths are shortest in the youngest, unstretched lenses and increase towards that of the unchanging, older lenses by mechanical stretching (Glasser & Campbell, 1998). Although the ratio of lens equatorial diameter to lens thickness (the ellipticity constant) is well correlated with both anterior and posterior lens radii of curvature, the relationship between lens curvatures and age is more clearly evident from direct plots of lens curvatures (Fig. 4b) than from this ratio (Fig. 10c). Clearly, the relationship between lens thickness, diameter and surface curvature is also influenced by lens asphericity and the inherent variability dictates that age changes in lens curvature are best determined by direct curvature measurements. In order to make direct comparisons with in vivo measurements of unaccommodated lens curvatures, future in vitro measurements need to be extended to unaccommodated lenses.

The results from the present study also show no age dependent change in the equivalent refractive index of the isolated human lens. The mean equivalent refractive index value obtained from all 19 lenses (1.4257) is very close to (and not significantly different from) that assumed for schematic eye model calculations such as the Gullstrand–Emsley schematic eye ($n = 1.416$) and is closer still to the equivalent refractive index value of 1.422 given by Bennett and Rabbetts (1989) for a proposed new schematic eye which is consistent with more recent biometric ocular measurements on emmetropic eyes. Although we find no change in equivalent refractive index over the age range covered by the lenses used, Wood et al. (1996) have shown that a higher equivalent refractive index value (1.49) is required to translate surface curvature data into unaccommodated lens power in children aged 3–18 months. Their value of 1.49 is significantly higher than the values we calculated (Mann–Whitney Rank Sum Test; $P < 0.001$). The age range Wood et al. (1996) consider is not covered by our study since the youngest lens we used was that of a 10 year old. Mutti, Zadnik, Fusaro, Friedman Sholtz and Adams (1998) show an age dependent change between 6 and 14 years in the form of a shallow U-shaped curve with a minimum at age 10 years.

We also see no change in equivalent refractive index with decapsulation and we found that the equivalent refractive index of the decapsulated lenses was independent of age. This is significant because removing the lens capsule results in decreases in focal length of the younger lenses resembling that of accommodation. If optical changes on decapsulation are found to mimic changes on accommodation, then the results from the present investigation will cast doubt on the existence of the lens paradox from two points of view: (1) the decapsulated lenses do not have systematically increasing anterior and posterior curvatures as in vivo Scheimpflug slit-lamp measurements suggest (Lowe & Clarke, 1973; Brown, 1974); and (2) we find no age change in the equivalent refractive index of the decapsulated human lens. It remains possible that there are changes in the form of the gradient refractive index distribution of the human lens with accommodative state and with age which may account for age changes in spherical aberration as shown in this study and previously (Glasser & Campbell, 1998). However, such changes in spherical aberration are also profoundly influenced by changes in lens surface curvatures and aspheric surface shapes, potentially even by the small shape changes with age shown in Fig. 10c.

4.8. Physical and optical relationships of the lens

A significant and unexpected linear correlation found is that between lens focal length and lens equatorial diameter (Fig. 6a). This relationship is not significantly different from the same relationship derived from lenses of all ages and in different (multiple) stretch states from our previous study (Glasser & Campbell, 1998), shown here for the first time:

$$\text{Lens equatorial diameter} = 0.047 * \text{Focal length} + 6.195 \quad (14)$$

with ($n = 98$; $r^2 = 0.391$; $P < 0.001$). In that study lens diameter is equivalent to the horizontal extent that the scanning laser apparatus traverses the laser across the lens diameter. Our measurements from the present study show that lens surface curvatures and lens thickness used together with the Gullstrand–Emsley equivalent refractive index for the lens, allow good predictions of lens focal length, but it is not clear why lens focal length and lens equatorial diameter are related. As the lens ellipticity factor is fairly constant with age, the overall shape of the lens may also be relatively constant, in which case, the diameter and thickness of the lens will determine the surface curvature. This might predict a better correlation between lens equatorial diameter and surface curvatures than between lens equatorial diameter and focal length. Since the correlation for the latter is among the highest we have found, this may provide important information for understanding the physical and optical changes in the lens that occur with accommodation since the change in zonular force with accommodation directly affects the lens equatorial diameter.

4.9. Lens resistance to mechanical compressive deformation

We have found an age dependent increase in the resistance of the human lens to axially applied compressive forces. Interestingly, the age-course of this relationship over the life span is exponential rather than a more nearly linear relationship which might match the age dependent decline of accommodation with the development of presbyopia. Although there is a progressive increase in hardness from birth, the predominant increase in lens hardness as measured here occurs after age 50 after which accommodation is already completely lost. The consistent increase in hardness that occurs from birth to age 50 may well provide sufficient increasing resistance to the accommodative mechanism to account for the loss of accommodation. The continued growth, increased volume and thickness of the lens has been thought to alter the geometric relationship between the lens and zonular fibers (Koretz & Handelman, 1986, 1988), but in the face of a hardening of the lens substance, the altered geometry, if it exists, must be a concurrent effect (Glasser & Campbell, 1998). We have been unable to identify functional relationships between any of the other optical or biometric measures made and the increasing resistance of the lens to mechanical compressive forces.

Our measurements of the effects of mechanical compressive forces were intended to identify the role of hardening of the lens in the development of presbyopia. Caution should be employed in attempting to relate the mechanical squeezing to the accommodative forces on the lens. The flattening of the lens that occurs through capsular forces with the relaxation of accommodation in vivo is undoubtedly very different from the mechanical squeezing we have employed. Although the direction of forces may both be along the optical axis, squeezing a lens between two incompressible plates cannot and is not meant to try to simulate the forces that occur with relaxation of accommodation. To understand the nature of the forces on the lens during accommodation, one would need to consider how changes in zonular forces influence both the optical pole and the equatorial edge of the lens, few details of which are known with any degree of certainty. Our measurements were undertaken to attempt to characterize changes in the hardness of the lens with age since this has long been considered as an influential factor in the development of presbyopia yet has been de-emphasized more recently in favor of alternative theories. Our measurements are thus a simple demonstration that the hardness of the human lens increases with age. This is in good agreement with previous studies which show an age dependent increase in resistance to penetration (Heyningen, 1972; Pau & Kranz, 1991) or an increased resistance to lens flattening during spinning (Fisher, 1971). The increasing resistance of the lens to compressive forces with age is also in good agreement with our previous observation that there is an age dependent decline in the ability of the lens to undergo changes in focal length with stretching (Glasser & Campbell, 1998).

We have previously argued for a predominantly lens based explanation for presbyopia (Glasser & Campbell, 1998). Our mechanical stretching experiments (Glasser & Campbell, 1998) cast doubt on various alternative theories of presbyopia such as the geometric theory (Koretz & Handelman, 1988), the disaccommodation theory (Bito & Miranda, 1989) and Schachar's theory (Schachar, Black, Kash, Cudmore & Schanzlin, 1995). These have been discussed in detail previously (Glasser & Campbell, 1998) and since the present study is concerned with isolated lenses, little more can be added to that discussion. We have confirmed the continued growth of the lens throughout life which has been implicated as a causal factor in presbyopia (Koretz & Handelman, 1988; Pierscionek & Weale, 1995). The increasing resistance of the human lens to mechanical compressive forces shown here provides further evidence for a direct lens based explanation of presbyopia. The increasing lens resistance to mechanically applied compressive forces provides direct evidence for hardening or sclerosis of the lens (Fincham, 1937; Pau & Kranz, 1991) which, although only one of many age changes in the accommodative apparatus, must necessarily be considered as major factor. In the absence of lenticular hardening, any number of other age changes may serve as a contributing factors to presbyopia. However, evidence for hardening of the lens cannot be discounted as a major factor and further, in the absence of other age related changes, could fully account for the accommodative loss with increasing age. Previous suggestions of alternative causes for presbyopia which exclude the possibility of lenticular hardening (Weale, 1963a; Koretz & Handelman, 1988; Weale, 1989; Pierscionek & Weale, 1995; Schachar et al., 1995) must be questioned given direct evidence for hardening of the lens.

The age dependent alterations in the response of the human lens to compressive forces provides some possible clues to the kinds of physical changes that may be occurring with age that contribute to presbyopia. The initial sharp phasic spike and the secondary, slower tonic decay at each increase in compressive force that is seen in the older lenses, but not in the younger lenses suggests that the lens substance not only becomes harder with increasing age, but that the properties of the lens substance that allow it to undergo alterations in shape (elasticity and viscosity) also change (Fig. 12a). After the compressive force on the older lens is increased at each step, the initial sharp phasic response reflects a direct conduction of the force of the plunger to the diaphragm of the pressure transducer through the lens substance. Over the subsequent 10 s interval, the gradual decrease in the output reflects a slow reorganization of the lens substance to gradually relieve some of the compression on the pressure transducer. Similarly, when the plunger is stepped up off the older lens, there is an initial phasic response as the pressure transducer responds to the reduced compressive force on the lens. This is followed by a slower secondary phase during which the lens substance gradually expands to again begin applying an increasing force to the pressure transducer. It is clear that this is not a characteristic of the transducer itself because this response is not observed with the younger lenses or during calibration of the pressure transducer (Fig. 2). The sharp phasic response seen in the older lenses may reflect an age dependent increase in lens hardness or viscosity which would result in an increased conduction of the mechanical force and a slower subsequent reorganization of the lens substance. The youngest lenses which show a relatively smaller response overall, no initial phasic spike and no subsequent slower tonic recovery, must thus be relatively softer with lower viscosity. The response properties of the closed fluid-filled system used to calibrate the pressure transducer may also provide information about the young lenses. The fluid-filled system is incompressible with low viscosity and an increasing pressure causes a large amplitude, square step response from the pressure transducer. The response from the young lenses, similarly shows a square step response, but of low amplitude, reflecting a low viscosity and high compressibility of these young lens. Thus, not only does the lens become more resistant to axially applied compressive forces with increasing age, but there is also a slower reorganization of the lens substance in the older lenses. This provides an explanation for a reduction in the rate at which the human lens can respond to an accommodative effort or the relaxation of an accommodative effort (Beers & Van der Heijde, 1996) and is entirely consistent with a largely lenticular basis for presbyopia (Glasser & Campbell, 1998).

The decline in accommodative amplitude with age has been attributed to changes in the visco-elasticity of the lens (Beers & Van der Heijde, 1996). These authors suggest that the elastic properties of the lens depend on the interaction between the inherently elastic lens fibers and the elastic lens capsule and that the viscous nature of the lens is due to the resistance to movement of cytoplasm in individual lens fibers (Beers & Van der Heijde, 1996). The elasticity of the lens would determine the ability of the lens to change shape on compression and to return to its original shape after a compressive force is released. The viscosity of the lens would determine the rate at which the lens changes shape initially and returns to its original shape. In our experiments, the pressure transducer output from the younger lenses matches that expected from a lens with high elasticity and low viscosity. High elasticity would dictate that each increasing compressive force applied to the lens would result in a relatively small compressive force being conducted to the transducer and low viscosity would allow rapid reorganization of the lens substance with a rapid square step response and symmetry of the increasing and decreasing phases. The biphasic response seen at each step in the older lenses is perhaps more characteristic of a relatively inelastic body which would produce the high amplitude initial phasic response and a highly viscose body which would cause the slower tonic recovery at each step and the asymmetry of the increasing and decreasing phases as the lens substance is slower to reorganize. These results suggest that the human lens may loose elasticity (i.e.

become more brittle rather than becoming more flaccid) and increase viscosity with age and that these changes may significantly contribute to the loss of accommodative amplitude with the progression of presbyopia. Although previous authors have suggested that age changes in the accommodative mechanism can be accounted for by changes in the elasticity and visco-elasticity of the lens, these authors have been reluctant to speculate on the direction of these changes (Beers & Van der Heijde, 1996).

5. Summary

(1) Despite the increasing mass of the human lenses with increasing age, average focal length of the isolated lens increases linearly up to age 65 and then decreases again after this age. The senile increase in lens power seen in this group of lenses may be attributable to precataractus changes.

(2) Independent of the age or the presence of the capsule on the isolated human lenses, the focal length is directly proportional to the anterior lens curvature and inversely proportional to the posterior lens curvature.

(3) Decapsulating isolated human lenses results in decreases in power of the younger lenses, but is without systematic effects on the lens power of the older lenses. Despite the relatively systematic effects on lens power and the spherical aberration of the younger lenses which mimic the effects of stretching and of accommodation, there are a variety of other effects on lens curvatures, thickness and equatorial diameter which are unlike those observed in accommodation.

(4) We have found no evidence in support of the lens paradox in isolated human lenses or in decapsulated human lenses. Lens focal length is well predicted by the lens surface curvatures independent of the age of the lenses. Further, calculated lens equivalent refractive index shows no age dependence and agrees well with values used in schematic eye models. Using the age independent Gullstrand–Emsley equivalent refractive index together with measured lens thickness and surface curvatures allows an accurate prediction of the measured lens focal lengths.

(5) The human lens shows an exponential increase in resistance to mechanical deformation with age from birth. Even though the predominant increase in hardness occurs after the age at which accommodation is completely lost, the increasing resistance demonstrates increased hardness of the human lens which can account for the loss of accommodation. The age dependent changes in the responses of lenses to mechanical deformation suggests that the human lens may loose elasticity and increase viscosity with age and that this may account for the loss of accommodation with the development of presbyopia.

Acknowledgements

This work was supported by a Natural Sciences and Engineering Research Council (NSERC) of Canada International Postdoctoral Fellowship to AG and an NSERC grant to MCWC. We are indebted to Dr M. Schneider and Mr S.M. Hasany of the Eye Bank of Canada, Toronto, for procuring human eyes. Thanks also to Robin Jones for machine shop assistance, Andrew Nowinski for electronics assistance and Andy Lankin for technical assistance.

References

Bito, L. Z., & Miranda, O. C. (1989). Accommodation and presbyopia. In R. D. Reinecke, *Ophthalmology annual* (pp. 103–128). New York: Raven Press.

Beers, A. P. A., & Van der Heijde, G. L. (1994). In vivo determination of the biomechanical properties of the component elements of the accommodative mechanism. *Vision Research*, *34*, 2897–2905.

Beers, A. P. A., & Van der Heijde, G. L. (1996). Age-related changes in the accommodation mechanism. *Optometry and Vision Science*, *73*, 235–242.

Bennett, A. G., & Rabbetts, R. B. (1989). *Clinical visual optics* (2nd ed.). (pp. 255–256, 338–342). Toronto: Butterworths.

Brown, N. (1973). The change in shape and internal form of the lens of the eye on accommodation. *Experimental Eye Research*, *15*, 441–459.

Brown, N. P. (1974). The change in lens curvature with age. *Experimental Eye Research*, *19*, 175–183.

Campbell, M. C. W. (1984). Measurement of refractive index in an intact crystalline lens. *Vision Research*, *24*, 409–415.

Duane, A. (1912). Normal values of the accommodation at all ages. *Journal of the American Medical Association*, *59*, 1010–1013.

Ejiri, M., Thompson, H. E., & O'Neill, W. D. (1969). Dynamic visco-elastic properties of the lens. *Vision Research*, *9*, 233–244.

Fincham, E. F. (1937). The mechanism of accommodation. *British Journal of Ophthalmology Supplement*, *8*, 5–80.

Fisher, R. F. (1969). Elastic constants of the human lens capsule. *Journal of Physiology*, *201*, 1–19.

Fisher, R. F. (1969). The significance of the shape of the lens and capsular energy changes in accommodation. *Journal of Physiology*, *201*, 21–47.

Fisher, R. F. (1971). The elastic constants of the human lens. *Journal of Physiology*, *212*, 147–180.

Fisher, R. F. (1973). Presbyopia and the changes with age in the human crystalline lens. *Journal of Physiology*, *228*, 765–779.

Glasser, A., & Campbell, M. C. W. (1998). Presbyopia and the optical changes in the human crystalline lens with age. *Vision Research*, *38*, 209–229.

Glasser, A., & Campbell, M. C. W. (1999). Reply: On the potential causes of presbyopia. *Vision Research*, *39*, 1267–1272.

Gullstrand von, A. (1909) Appendix IV. Mechanism of accommodation. In H. H. Helmholz von, (1909) *Handbuch der Physiologischen Optik* (pp. 383–415). New York: Dover (J. P. C. Southall, Trans.; Helmholz's treatise on physiological optics; original work published 1962).

Helmholz von, H. H. (1909). *Handbuch der Physiologischen Optik* (pp. 143–172). New York: Dover (J. P. C. Southall, Trans.; Helmholz's treatise on physiological optics; original work published 1962).

Hemenger, R. P., Garner, L. F., & Ooi, C. S. (1995). Change with age of the refractive index gradient of the human ocular lens. *Investigative Ophthalmology and Visual Science*, *36*, 703–707.

Heyningen, R. (1972). The human lens. III. Some observations on the post-mortem lens. *Experimental Eye Research*, *13*, 155–160.

Howcroft, M. J., & Parker, J. A. (1977). Aspheric curvatures for the human lens. *Vision Research*, *17*, 1217–1223.

Kikkawa, Y., & Sato, T. (1963). Elastic properties of the lens. *Experimental Eye Research*, *2*, 210–215.

Koretz, J. F., & Handelman, G. H. (1986). The lens paradox and image formation in accommodating human eyes. *Topics in Aging Research in Europe*, *6*, 57–64.

Koretz, J. F., & Handelman, G. H. (1988). How the human eye focuses. *Scientific American*, *259*, 92–99.

Koretz, J. F., Handelman, G. H., & Brown, N. P. (1984). Analysis of human crystalline lens curvature as a function of accommodative state and age. *Vision Research*, *24*, 1141–1151.

Koretz, J. F., Kaufman, P. L., Neider, M. W., & Goeckner, P. A. (1989). Accommodation and presbyopia in the human eye-aging of the anterior segment. *Vision Research*, *29*, 1685–1692.

Krag, S., Olsen, T., & Andreassen, T. T. (1997). Biomechanical characteristics of the human anterior lens capsule in relation to age. *Investigative Ophthalmology and Visual Science*, *38*, 357–363.

Larsen, J. S. (1971). The sagittal growth of the eye. I. Ultrasonic measurement of the depth of the anterior chamber from birth to puberty. *Acta Ophthalmologica*, *49*, 139–262.

Larsen, J. S. (1971). The sagittal growth of the eye. II. Ultrasonic measurement of the lens and anterior segment from birth to puberty. *Acta Ophthalmologica*, *49*, 427–440.

Larsen, J. S. (1971). The sagittal growth of the eye. III. Ultrasonic measurement of the posterior segment (axial length of the vitreous) from birth to puberty. *Acta Ophthalmologica*, *49*, 441–453.

Lowe, R. F., & Clarke, B. A. J. (1973). Radius of curvature of the anterior lens surface: correlations in normal eyes and in eyes involved with primary angle-closure glaucoma. *British Journal of Ophthalmology*, *57*, 471–474.

Mutti, D. O., Zadnik, K., & Adams, A. J. (1992). A video technique for phakometry of the human crystalline lens. *Investigative Ophthalmology and Visual Science*, *33*, 1771–1782.

Mutti, D. O., Zadnik, K., & Adams, A. J. (1995). The equivalent refractive index of the crystalline lens in childhood. *Vision Research*, *35*, 1565–1573.

Mutti, D. O., Zadnik, K., Fusaro, R. E., Friedman, N. E., Sholtz, R. I., & Adams, A. J. (1998). Optical and structural development of the crystalline lens in childhood. *Investigative Ophthalmology and Visual Science*, *39*, 120–133.

Nordmann, J., Mack, G., & Mack, G. (1974). Nucleus of the human lens III. Its separation, its hardness. *Ophthalmic Research*, *6*, 216–222.

Ooi, C. S., & Grosvenor, T. (1995). Mechanism of emmetropization in the aging eye. *Optometry and Vision Science*, *72*, 60–66.

Pau, H., & Kranz, J. (1991). The increasing sclerosis of the human lens with age and its relevance to accommodation and presbyopia. *Graefe's Archive for Clinical and Experimental Ophthalmology*, *229*, 294–296.

Pierscionek, B. K. (1990). Presbyopia-effect of refractive index. *Clinical and Experimental Optometry*, *73*, 23–30.

Pierscionek, B. K. (1993a). What we know and understand about presbyopia. *Clinical and Experimental Optometry*, *76*, 83–90.

Pierscionek, B. K. (1993b). In vitro alteration of human lens curvatures by radial stretching. *Experimental Eye Research*, *37*, 629–635.

Pierscionek, B. K. (1995). Age-related response of human lenses to stretching forces. *Experimental Eye Research*, *60*, 325–332.

Pierscionek, B. K. (1997). Refractive index contours in the human lens. *Experimental Eye Research*, *64*, 887–893.

Pierscionek, B. K., & Augusteyn, R. C. (1991). Shapes and dimensions of in vitro human lenses. *Clinical and Experimental Optometry*, *74*, 223–228.

Pierscionek, B. K., & Weale, R. A. (1995). Presbyopia—a maverick of human aging. *Archives of Gerontology and Geriatrics*, *20*, 229–240.

Saunders, H. (1981). Age-dependence of human refractive errors. *Ophthalmic and Physiological Optics*, *1*, 159–174.

Saunders, H. (1982). Corneal power and visual error. *Ophthalmic and Physiological Optics*, *2*, 37–45.

Scammon, R. E., & Hesdorffer, M. B. (1937). Growth in mass and volume of the human lens in postnatal life. *Archives of Ophthalmology*, *17*, 104–112.

Schachar, R. A., Black, T. D., Kash, R. L., Cudmore, D. P., & Schanzlin, D. J. (1995). The mechanism of accommodation and presbyopia in the primate. *Annals of Ophthalmology*, *27*, 58–67.

Slataper, F. J. (1950). Age norms of refraction and vision. *Archives of Ophthalmology*, *43*, 466–481.

Smith, G., Atchison, D. A., & Pierscionek, B. K. (1992). Modeling the power of the aging human eye. *Journal of the Optical Society of America*, *9*, 2111–2117.

Sorsby, A., Benjamin, B., Davey, J. B., Sheridan, M., & Tanner, J. M. (1957). Emmetropia and its aberrations. Medical Research Council Special Report Series, 293, London: HMSO.

Sorsby, A., Benjamin, B., & Sheridan, M. (1961). Refraction and its components during the growth of the eye from the age of three. Medical Research Council Special Report Series, 301, London: HMSO.

Willekens, B., Kappelhof, J., & Vresen, G. (1987). Morphology of the aging human lens. *Lens Research*, *4*, 207–230.

Weale, R. A. (1963). *The lens. In the aging eye* (pp. 69–102). New York: Harper and Row.

Weale, R. A. (1963). New light on old eyes. *Nature*, *198*, 944–946.

Weale, R. A. (1983). Transparency and power of post-mortem human lenses: variation with age and sex. *Experimental Eye Research*, *36*, 731–741.

Weale, R. A. (1985). The post-mortem preservation of the transmissivity of the human crystalline lens. *Experimental Eye Research*, *41*, 655–659.

Weale, R. (1989). Presbyopia towards the end of the 20th century. *Survey of Ophthalmology*, *34*, 15–30.

Weerheim, J. A., & Sivak, J. G. (1992). Scanning laser measure of the optical quality of the cultured crystalline lens. *Ophthalmic and Physiological Optics*, *12*, 72–79.

Wood, I. C. J., Mutti, D. O., & Zadnik, K. (1996). Crystalline lens parameters in infancy. *Ophthalmic and Physiological Optics*, *16*, 310–317.

Wyatt, H. J., & Fisher, R. F. (1995). A simple view of age-related changes in the shape of the lens of the human eye. *Eye*, *9*, 772–775.

Zadnik, K., Mutti, D. O., Fusaro, R. E., & Adams, A. J. (1995). Longitudinal evidence of crystalline lens thinning in children. *Investigative Ophthalmology and Visual Science*, *36*, 1581–1587.