

Human lens weights with increasing age

Ashik Mohamed,^{1,2,3} Robert C. Augusteyn^{2,3,4}

¹Ophthalmic Biophysics, L V Prasad Eye Institute, Hyderabad, India; ²Brien Holden Vision Institute, Sydney, Australia; ³School of Optometry and Vision Science, University of New South Wales, Sydney, Australia; ⁴Ophthalmic Biophysics Center, Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, FL

Purpose: To evaluate the changes with age in human lens wet and dry weights.

Methods: All procedures were performed by the same person in the same environment. Lenses were extracted from donor eyes within a median post-mortem time of 22 h, blotted dry and weighed within 30 min, immediately placed in fixative for 1 week, and then dried at 80 °C until a constant weight was achieved.

Results: Wet and dry lens weights were obtained from 549 human lenses. Before age 2 years, most of the weight increases are due to a self-limiting process and can be described with logistic equations. The maximum asymptotic wet and dry weights for male lenses are 6.0 and 1.77 mg, respectively, heavier than those for female lenses. After age 3 years, male and female lens weights increase at the same linear rate.

Conclusions: The data support the biphasic growth model for human lenses. Male lenses are significantly larger than female lenses at the conclusion of the prenatal growth mode, but the rate of weight accrual is constant thereafter. Lens weights increase continuously throughout life and can be described with equations that incorporate terms for prenatal and postnatal growth.

Understanding ocular lens changes, such as presbyopia and cataract, which develop with increasing age, requires detailed information on the growth and properties of the human lens. Some of this information can be difficult to obtain. For example, growth could be assessed from in vivo lens dimensions which can be measured with several techniques, but these techniques may not be reliable because of the need to correct for optical or sonic distortion. Furthermore, the dimensions depend on the accommodative state of the eye, as well as the interaction of the lens with other ocular components. Less variable data can be obtained in vitro from isolated lenses where corrections are not required and all lenses are in a uniform state of maximum accommodation, free of zonular tension. However, because of complex shape changes during lens remodeling, in which the thickness decreases while the diameter increases until the late teens [1], dimensions offer little information on lens growth in the young.

More appropriate growth information can be obtained in vitro from changes in the weight of the lens. Several previous studies have measured wet weights [2-12], but the data obtained are highly variable, probably because the weights had been affected by uptake or loss of water during storage and handling [13]. A better measure of growth can be

obtained from the dry weight of the lens which eliminates the variability due to water movements.

Limited data have previously been obtained on human lens dry weights [1,3,4,8]. Analyses of the data indicated that lens growth may be biphasic: rapid and self-limiting (asymptotic) during prenatal development and slow and linear during postnatal life [1,2]. Because the reduced growth rate could be detected in lenses around 2 years of age, it was suggested that the transition took place at or near birth. However, these conclusions were based on limited pooled data obtained from various studies in different laboratories, using different methods for handling, storing, fixing, and drying the lens. Therefore, we undertook to collect data from lenses stored and handled under closely controlled conditions and to use the same fixing and drying protocol throughout the study.

We have previously presented preliminary observations on the growth of adult human lenses and concluded that this is the same in Indian and Western populations [14]. In this communication, we present more comprehensive data covering the age range 0-93 years obtained for 549 lenses.

METHODS

Human donor eyes were obtained from the Ramayamma International Eye Bank, L V Prasad Eye Institute, Hyderabad, India, after the corneas have been removed under sterile conditions for transplantation. Consent to enucleate eyes for the purpose of transplantation, therapy, medical research, or education was obtained by the eye bank in accordance with its practices and procedures. The study protocol was

Correspondence to: Robert C Augusteyn, 30 Melcombe Rd Ivanhoe, Vic 3079, Australia, Phone: +613 9499 1838; email: raugustn@bigpond.net.au

approved by the Institutional Ethics Committee of the L V Prasad Eye Institute and adhered to the tenets of Declaration of Helsinki and the ARVO statement on human subjects.

Age, gender, time and cause of death, time of enucleation, and time of use were recorded. The iris was excised, and the lens was extracted with a lens spoon after the zonules were cut with Vannas scissors. The lens was then carefully blotted dry and weighed using an analytical balance (KERN ABS 120–4, Kern & Sohn GmbH, Balingen-Frommern, Germany). Lenses with obvious cataracts and capsular or other damage were discarded. The lens was then fixed for 1 week in 5 ml of 5% buffered formalin, after which the lens was dried at 80 °C in a hot air oven (Sisco, Thane, India) until constant weight was achieved. This typically took around 2 weeks. The constant weight was recorded as the dry weight of the lens. Data were obtained for 549 lenses, 365 from male donors and 184 from female donors, collected between 2009 and 2018. The ages of the donors ranged from 0 to 93 years, and the median post-mortem time (duration between time of death and time of use) was 22 h (inter-quartile range, 17–27 h).

Lens weights were examined as a function of age, and regression analyses (linear and logarithmic) were used to fit the data using Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA). Multiple regression analysis was performed using STATA v 11.0 (StataCorp, College Station, TX) taking

age and gender as independent variables and wet or dry weight as the dependent variable. When two lenses from the same donor were available, the data were averaged for the analyses. Any data more than three standard deviations from the mean at that age were eliminated. This resulted in 36 wet weight and 23 dry weight values being eliminated. Because lens growth commences early in gestation [15], unless otherwise indicated, all ages cited are since closure of the lens vesicle (A_v , approximately 38 days since conception). As in previous reports [1,2], postnatal ages are designated as A_b .

RESULTS

A total of 549 lenses, 365 from male donors and 184 from female donors, provided 509 wet weights and 523 dry weights. Of these weights, 14 wet and 16 dry weights were obtained from donors younger than 1 year postnatal. The plots of all weights, as a function of age, are presented in Figure 1. The time since vesicle closure (A_v) was used as the age of the lens, rather than the previously used time since conception (A_c) [14], because vesicle closure more closely approximates the time from which lens weight accumulates. Unless otherwise stated, all ages quoted refer to the time since vesicle closure. Wet and dry weights increase rapidly in the 2 years since vesicle closure and then appear to increase linearly thereafter. Regression analysis of the averaged data from 3 to 93 years confirmed that weight accumulation was linear at around 1.30 and 0.49 mg/year, respectively.

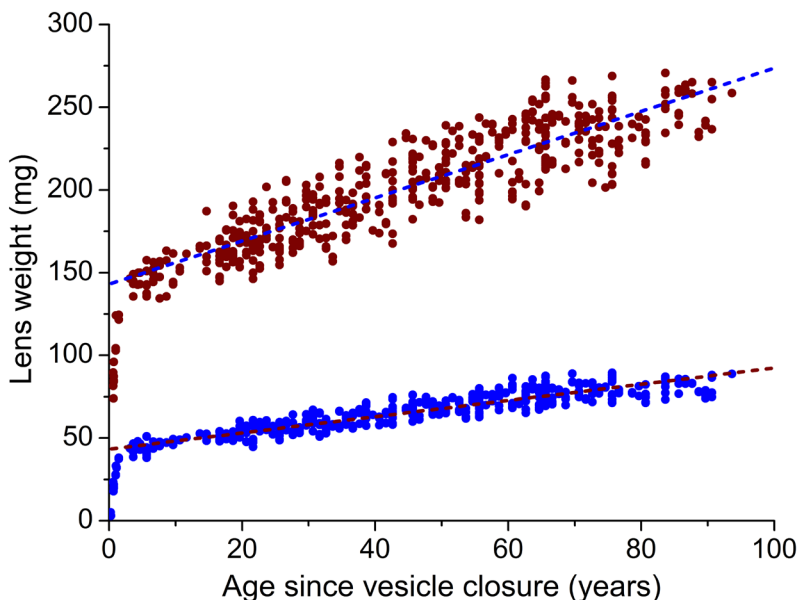


Figure 1. Human lens Wet (brown symbols) and Dry (blue symbols) weights as a function of age. Regression analysis of the averaged data in the range of 3–93 years yielded the relationships; Wet weight= $142.96 (\pm 1.78) + 1.31 (\pm 0.04) * A_b$ ($R^2=0.82$, $p<0.0001$; $n=296$) and Dry weight= $43.29 (\pm 0.57) + 0.49 (\pm 0.01) * A_b$ ($R^2=0.86$, $p<0.0001$; $n=302$), where A_b is the time since birth. The linear regression fits are included as broken lines.

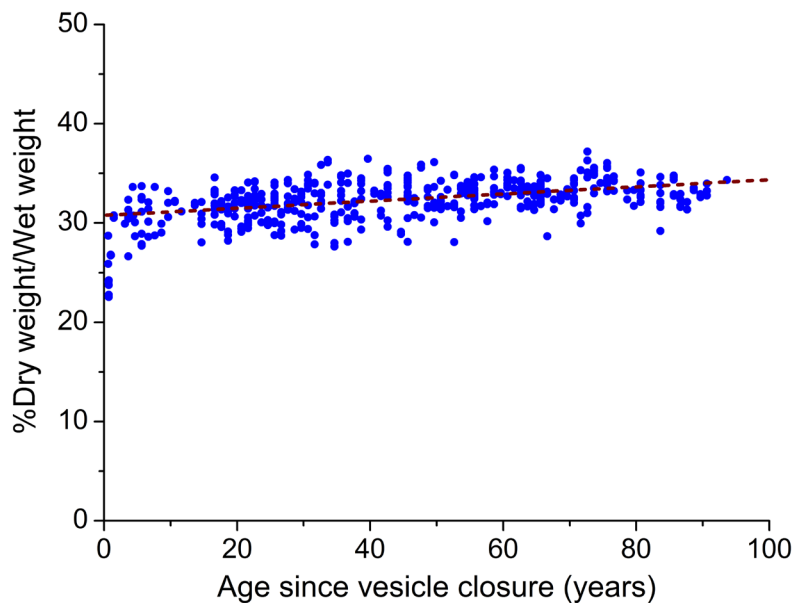


Figure 2. The Dry weight percentage of Wet weight as a function of age. The data from lenses older than 2 years since vesicle closure can be described by the equation, %Dry weight/Wet weight = $30.77 (\pm 0.20) + 0.036 (\pm 0.004) \cdot A_b$ ($R^2=0.22$, $p<0.0001$; $n=286$). For lenses <2 years, the equation is %Dry weight/Wet weight = $18.9 (\pm 1.38) + 7.9 (\pm 1.51) \cdot A_b$ ($R^2=0.71$, $p=0.0003$). The broken line represents the linear regression fit for >3 years.

The ratio of dry weight/wet weight, expressed as a percentage, is shown in Figure 2. The dry weight percentage increases rapidly from 16.1% to 30.8% during the early years. After 3 years since vesicle closure, the dry weight increases slowly at 0.036%/year, indicating continued slow compaction, and reaches 34.30% by age 90, a 10% increase. After age 3, the 0.377 ratio of the rates at which dry and wet weights accumulate (0.49 and 1.3 mg/year) is higher than the lens dry weight/wet weight ratio (0.309–0.343), consistent with the slow and continuous compaction increase.

Similar conclusions can be reached using allometric analysis. A plot (Figure 3) of \ln [dry weight] against \ln [wet weight] yields slopes (compaction constants) of 1.38 and 1.08 for the less than 2-year-old lenses and the 3- to 93-year-old lenses, respectively. The positive allometry shows that dry weight increases at a greater rate than wet weight, resulting in continuous compaction of the lens contents. The analysis also shows that the compaction is faster in the early years.

The difference in the rate of weight accumulation between lenses younger than 2 years and those older is consistent with the suggestion that growth is biphasic [2]. Semilogarithmic plots were used to examine this more closely. They are presented in Figure 4. The semilogarithmic plots of the lens weight against \ln [Age] (Figure 4) confirmed that there are distinct growth phases. The young data (<2 years) lie close to a straight line while the older data follow a more complex curve, indicating the logarithm of a straight

line relationship. Backward extrapolation of the older data suggests the young and old relationships intersect around 0.6 on the \ln [A_b] scale, corresponding to a time of 1.8 years since lens vesicle closure.

Data from male and female lenses were examined separately. Plots of the weights as a function of age are shown in Figure 5. The regression analyses suggested that there may be a small difference between the two sexes with male wet weight apparently increasing at 1.33 mg/year compared to 1.30 for female wet weight and male dry weight increasing at 0.50 mg/year with female dry weight at 0.49. There also appear to be differences in the intercepts of around 4 and 1 mg for wet and dry weights, respectively.

The possible difference was examined using multiple regression analysis of the data older than 3 years. The coefficient terms for gender were statistically significant at -5.93 ± 1.74 mg ($R^2=0.83$; $p=0.001$) for wet weight and -1.78 ± 0.56 mg ($R^2=0.84$; $p<0.0001$) for dry weight. The analyses yielded the following equations: wet weight = $145.4 + 1.32 \times A_b$ (-5.93 for female lenses) and dry weight = $44.03 + 0.50 \times A_b$ (-1.78 for female lenses). A similar relationship was found by Smith [10] for the wet weights of 156 male lenses, but the wet weights of seven female lenses were up to 70 mg lighter.

The equations derived from the data in Figure 1 do not truly represent the two growth modes because postnatal growth does not start until at least 0.64 years after vesicle closure and because of overlap in the early years of life. To

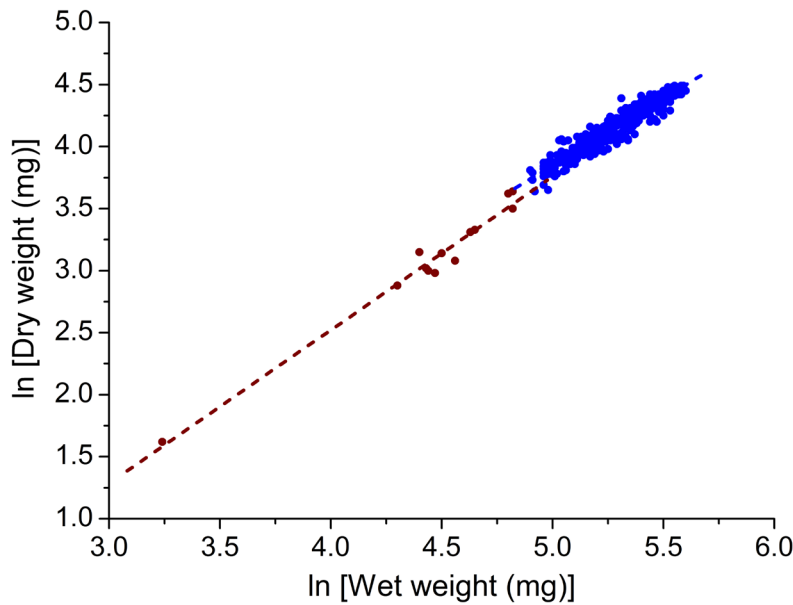


Figure 3. Allometric analysis of the relationship between wet and dry weights. Data from all lenses less than 2 years old can be described with the equation $\ln [\text{dry weight}] = 2.42 (\pm 0.28) + 1.24 (\pm 0.06) \times \ln [\text{wet weight}]$ ($R^2=0.97$; $p<0.0001$; $n=13$); for all ages 3–93, the equation is $\ln [\text{dry weight}] = 1.56 (\pm 0.08) + 1.08 (\pm 0.02) \times \ln [\text{wet weight}]$ ($R^2=0.91$; $p<0.0001$; $n=480$).

determine the true growth parameters, linear mode weights for postnatal ages were predicted using the equations derived from the data in Figure 1. Subtraction of these values from the observed postnatal data yielded residual weights corresponding to the prenatal growth mode. These data could

be described with a logistic equation that was then used to predict the weights generated in the prenatal growth mode. These data were subtracted from the observed data to yield residual values corresponding to the linear growth. Regression analyses provided descriptions of the linear growth mode

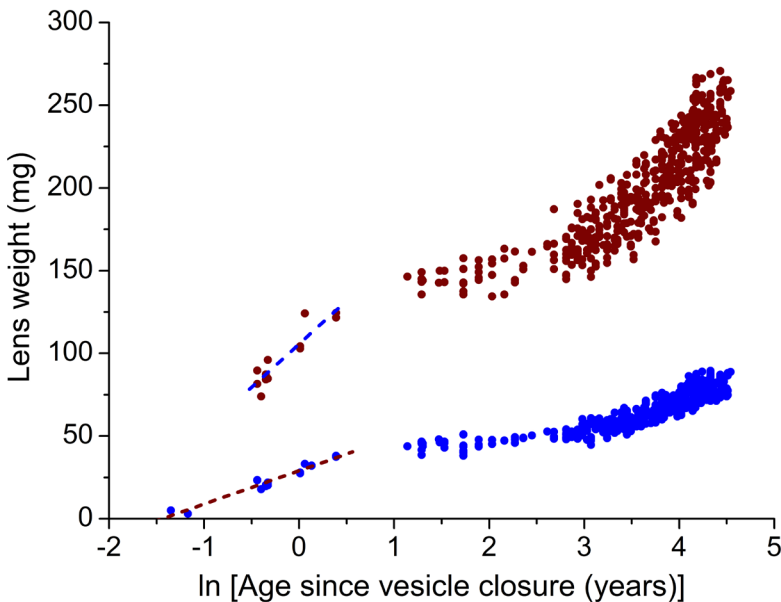


Figure 4. Semilogarithmic analyses of lens Wet (brown symbols) and Dry (blue symbols) weights as a function of age. Regression analysis of all less than 2 year data yielded straight lines with wet weight = $105.65 (\pm 2.32) + 52.36 (\pm 7.0) \times \ln [A_v]$ ($R^2=0.85$; $p<0.0001$; $n=12$) and dry weight = $29.02 (\pm 0.65) + 20.16 (\pm 1.67) \times \ln [A_v]$ ($R^2=0.95$; $p<0.0001$; $n=16$).

that were combined with the logistic equations to produce the following equations for lens growth from vesicle closure and over the whole lifespan:

$$\text{Wet weight} = 1.32 \times A_b + 145.4 (-5.93 \text{ for female lenses}) \times \exp[-0.2/A_v]$$

$$\text{Dry Weight} = 0.5 \times A_b + 44.3 (-1.78 \text{ for female lenses}) \times \exp[-0.2/A_v]$$

DISCUSSION

In this study, we obtained wet and dry weight data from 549 human lenses, covering the age range 0–93 years. This represents, by far, the largest data set obtained in any single

laboratory. Of particular interest is the set of 523 dry weights covering the whole of the postnatal age range. Such data have not been available previously as most previous studies measured only wet weights. Great care was taken to ensure uniformity in the collection and handling of lenses.

All phases of the experimental work were conducted by only one person (AM). This included collecting donor eyes from a single eye bank, removing lenses within a median of 22 h after death, measuring wet weights within 30 min, placing the lenses in fixative immediately afterward, and determining the dry weights once constant weight was

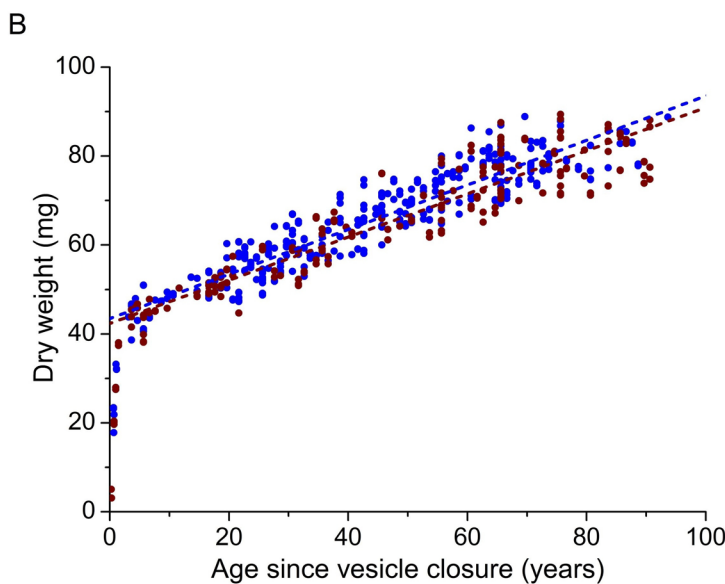
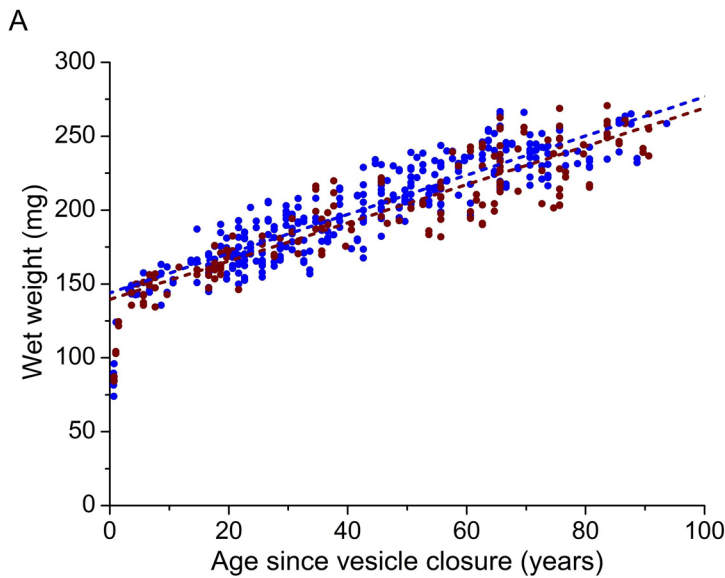


Figure 5. Human lens wet (A) and dry (B) weights as a function of age since vesicle closure for women (brown symbols) and men (blue symbols). Linear regression analyses of the 3- to 93-year-old data yielded the following relationships: female wet weight = $139.51 (\pm 3.14) + 1.30 (\pm 0.06) \times A_b$ ($R^2=0.84$; $p<0.0001$; $n=96$); female dry weight = $42.36 (\pm 1.05) + 0.49 (\pm 0.02) \times A_b$ ($R^2=0.87$; $p<0.0001$; $n=100$); male wet weight = $143.97 (\pm 2.14) + 1.33 (\pm 0.05) \times A_b$ ($R^2=0.82$; $p<0.0001$; $n=199$); male dry weight = $43.44 (\pm 0.66) + 0.50 (\pm 0.01) \times A_b$ ($R^2=0.87$; $p<0.0001$; $n=202$).

achieved. As a result, only 3% had to be discarded because of obvious physical damage. Another 6% were discarded after the first weighing, predominantly because of hydration changes that can occur if the lenses have even minor capsular damage [13,16].

It proved to be difficult to obtain data for young lenses hampering determination of prenatal growth. However, the limited weight data were similar to those in the larger data sets pooled from several studies in different laboratories [1], and curve fitting yielded essentially the same relationships between weight and age as previously. For the older lenses, the conclusions that may be drawn from the present data are also similar to the previous conclusions which, again, were based on much more variable pooled data from different laboratories [1,2]. The standard deviation for wet and dry weights obtained in the present study is around 2.5% of the mean for any age, compared with the 7.0% observed previously for wet weights.

The plots of weight against age and the semilogarithmic and allometric plots are consistent with the biphasic growth model identified previously by Augusteyn [2]: rapid self-limiting prenatal growth and slow postnatal linear growth. Extrapolation of the two phases in the semilog plots suggests that they intercept at around 1.8 years since vesicle closure or 1.2 years since birth. This corresponds to the age at which the growth phase change can first be observed, not the start of linear growth. The signal for the transition from the prenatal to the postnatal growth mode would have occurred earlier than this as cells already committed to the rapid prenatal growth mode need to complete their development to fiber cells and continue to dominate the growth rate until they have matured. It is not known how long this might take. However, decreased γ -crystallin and increased $\beta_s(\gamma_s)$ -crystallin synthesis, characteristic of postnatal fiber cells, have been observed in lenses as early as 8 weeks after birth [17].

Dry weight accumulation exhibits positive allometry relative to wet weight (exponent, 1.38); that is, dry weight accumulation is faster during the prenatal growth phase when growth is rapid. This continues in the linear growth phase, but the exponent is lower at 1.08. The ratio of linear accumulation rates ($0.49 / 1.30 = 0.377$ mg dry weight/mg wet weight) is higher than the ratio of dry weight/wet weight that would be in newly formed fiber cells (0.20–0.25 at 100 μ m from the capsule) or in the epithelial cells [18]. This indicates water is being lost from preexisting cells, and compaction continues throughout life. It has been demonstrated previously [18] that the dry weight/wet weight ratio, as judged from the refractive index gradient [19], increases with age from the center of the lens outward, eventually reaching a plateau of 0.385

throughout the nucleus in the fifth decade. Formation of this nuclear plateau appears to coincide with the development of presbyopia.

Our data indicate that male lenses are heavier than female lenses at the same age, by around 6 mg for wet weight over the whole age range studied. This result is consistent with the previous report by Harding et al. [6] that “men have heavier lenses than women” by 7.9 mg. This reduces to 6.4 mg when an outlier is omitted from the Harding et al. data. Although lens wet weights have been measured in several other laboratories, gender has generally not been recorded. No difference was observed by Augusteyn [2] for data from 138 male and 64 female lenses pooled from several sources. A lack of gender difference appeared to be supported by the report that lens weights are the same in males and females in 70 species [20].

Given the current large data set for human lenses, the lower variations and the strong statistical indications, as well as the previous observations, it would now appear that sexual dimorphism affects lens weights. Apart from the agreements for wet weight, this conclusion was also reached from the present dry weight data which indicate a difference of nearly 2 mg throughout postnatal life. The lack of statistical significance in previous analyses of pooled lens wet weights may reflect variations in lens collection, handling, and assessment, which are known to affect wet weights [13].

It would seem that the difference is generated during the prenatal growth mode so that the final asymptotic weight is around 4% greater for men than for women. Thereafter, the post-natal lens growth rate is the same for men and women. The lens weight difference is the same as the 4% difference in bodyweight at birth [21]. Adult male and female bodyweights also increase at the same rate [22]. These similarities suggest that regulation of growth in lens weight and bodyweight may be through similar mechanisms. Sexual dimorphism has been observed in other biometric parameters at birth, including head diameter, limb length, and body length [21]. However, thus far, no differences have been detected in ocular parameters. Multiple regression analysis of the relationship between previously published data for lens [14] and globe [23] dimensions, age and gender, did not detect any statistically significant gender dependence.

The small difference in weights is unlikely to have any substantial effect on gross lens properties. It can be calculated that female lens volume is 4% lower than male lens volume, decreasing to 2% with increasing age. The calculated dimensions are less than 1% lower, on average. Such differences would be difficult to detect with currently available methods. It would appear that the difference may be localized to the nucleus because it is generated during the prenatal growth

phase. In that case, it would amount to around 5% of the nuclear volume throughout life. It seems unlikely that such a small volume difference would affect the function of the nucleus in accommodation.

The wet and dry weights presented here can be used to calculate the volume and density of the lens, using the partial specific volume (ρ) of 0.73 for lens proteins determined by Thomson and Augusteyn [24]. These parameters are particularly important for models that predict radiation doses to the lens in procedures such as computer aided tomography scans of the head or orbit [25], especially in the young.

The data presented in this communication support most previous conclusions regarding biphasic human lens growth. However, the data are more comprehensive and much less variable than previous data, providing greater confidence in the validity of the conclusions. It is hoped that they may be of value in studies aimed at understanding age-related changes, such as formation of the refractive index gradient, stiffening of the lens, and cataract development.

ACKNOWLEDGMENTS

This work was supported, in part, by NIH Research Grants R01EY021834 and P30EY14801 (Center Grant), Hyderabad Eye Research Foundation and the Australian Federal Government CRC Scheme through the Vision Cooperative Research Centre. We are indebted to the Ramayamma International Eye Bank, Hyderabad, India for the supply of eyes.

REFERENCES

- Augusteyn RC. On the growth and internal structure of the human lens. *Exp Eye Res* 2010; 90:643-54. [PMID: 20171212].
- Augusteyn RC. Growth of the human eye lens. *Mol Vis* 2007; 13:252-7. [PMID: 17356512].
- Bours J, Fodisch HJ. Human fetal lens: wet and dry weight with increasing gestational age. *Ophthalmic Res* 1986; 18:363-8. [PMID: 3601352].
- Clapp CA. A communication upon the weight of infant's lenses and their solids. *Arch Ophthalmol* 1913; 42:618-24. .
- Glasser A, Campbell MC. Biometric, optical and physical changes in the isolated human crystalline lens with age in relation to presbyopia. *Vision Res* 1999; 39:1991-2015. [PMID: 10343784].
- Harding JJ, Rixon KC, Marriott FH. Men have heavier lenses than women of the same age. *Exp Eye Res* 1977; 25:651- [PMID: 590390].
- Van Heyningen R. The human lens. 3. Some observations on the post-mortem lens. *Exp Eye Res* 1972; 13:155-60. [PMID: 5013586].
- Nordmann J, Fink H, Hockwin O. Growth curve of the human lens (author's transl). *Albrecht Von Graefes Arch Klin Exp Ophthalmol* 1974; 191:165-75. [PMID: 4547037].
- Scammon RE, Hesdorffer MB. Growth in mass and volume of the human lens in postnatal life. *Arch Ophthalmol* 1937; 17:104-12. .
- Smith P. Diseases of crystalline lens and capsule. 1. On the growth of the crystalline lens. *Trans Ophthalmol Soc U K* 1883; 3:79-99. .
- Maynard FP. Observations on the Weight, Volume, and Ash of Human Lenses. *Br J Ophthalmol* 1920; 4:78-82. [PMID: 18167968].
- Satoh K. Age-related changes in the structural proteins of human lens. *Exp Eye Res* 1972; 14:53-7. [PMID: 5039847].
- Augusteyn RC, Rosen AM, Borja D, Ziebarth NM, Parel JM. Biometry of primate lenses during immersion in preservation media. *Mol Vis* 2006; 12:740-7. [PMID: 16865087].
- Mohamed A, Sangwan VS, Augusteyn RC. Growth of the human lens in the Indian adult population: Preliminary observations. *Indian J Ophthalmol* 2012; 60:511-5. [PMID: 23202388].
- Duke-Elder SS, Wybar KC. The Anatomy of the Visual System. *System of Ophthalmology*. Vol II. St. Louis: C. V. Mosby Company; 1961.
- Augusteyn RC, Cake MA. Post-mortem water uptake by sheep lenses left in situ. *Mol Vis* 2005; 11:749-51. [PMID: 16179906].
- Thomson JA, Augusteyn RC. Ontogeny of human lens crystallins. *Exp Eye Res* 1985; 40:393-410. [PMID: 4065234].
- Augusteyn RC, Jones CE, Pope JM. Age-related development of a refractive index plateau in the human lens: evidence for a distinct nucleus. *Clin Exp Optom* 2008; 91:296-301. [PMID: 18201223].
- Jones CE, Atchison DA, Meder R, Pope JM. Refractive index distribution and optical properties of the isolated human lens measured using magnetic resonance imaging (MRI). *Vision Res* 2005; 45:2352-66. [PMID: 15979462].
- Augusteyn RC. Growth of the eye lens: I. Weight accumulation in multiple species. *Mol Vis* 2014; 20:410-26. [PMID: 24715758].
- Guihard-Costa AM. Gender differences in human prenatal growth. In: Bharati P, Pal M, editors. *Gender disparity; Manifestations, causes and implications*. New Delhi: Anmol Publications; 2005. p. 53–69.
- Devine BJ. Gentamicin therapy. *Drug Intell Clin Pharm* 1974; 8:650-5. .
- Augusteyn RC, Nankivil D, Mohamed A, Maceo B, Pierre F, Parel JM. Human ocular biometry. *Exp Eye Res* 2012; 102:70-5. [PMID: 22819768].
- Thomson JA, Augusteyn RC. On the structure of alpha-crystallin: the minimum molecular weight. *Curr Eye Res* 1988; 7:563-9. [PMID: 3402244].

25. Huang Y, Zhuo W, Gao Y, Liu H. Monte Carlo simulation of tube current modulation. *Phys Med* 2018; 48:72-5. [PMID: 29728232].

eye lens dose reduction from CT scan using organ based [29728232].

Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 31 December 2018. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.