Lens fluorescence and accommodative amplitude in pre-presbyopic and presbyopic subjects

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Abstract

Accommodative amplitude (AA; the difference, measured in diopters, between the near and far points of vision) declines steadily with age such that, by midlife, most individuals are unable to focus clearly on near objects and, thus, are said to be presbyopic. Conversely, intrinsic lens fluorescence (LF) increases steadily with age. Previous studies have suggested that AA and LF are negatively correlated, independent of age. Were this to be the case, it might suggest that the biochemical modifications underlying increased tissue fluorescence (for example, glycation of lens proteins) contribute to presbyopia. We used quantitative techniques to re-evaluate the relationship between AA and LF in 161 healthy volunteers aged between 25 and 70. Our data confirmed that AA decreases with age, becoming essentially zero by age 55, and LF increases with age. However, in marked contrast to previous reports, statistical analysis failed to detect any correlation between LF and AA independent of age. Thus, the biochemical processes responsible for increased LF observed in the aged lens are unlikely to contribute directly to presbyopia.

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variously been ascribed to changes in the lens or ancillary accommodative structures (Atchison, 1995; Croft et al., 2001; Strenk et al., 2005). Helmholtz (1855) argued that lens sclerosis, or hardening, might be an important contributing factor. Recent biomechanical studies, in which the stiffness of the lens material was shown to increase markedly during aging, have lent support to this view (Heys et al., 2004; Pau and Kranz, 1991).

One property that increases steadily as AA declines is lens fluorescence (LF; Vannas and Wilska, 1935). LF is spectrally complex and thought to result from the accumulation of various fluorophores in the lens cell cytoplasm. A large fraction of LF may be attributed to the glycation of lens proteins. During glycation, glucose (or structurally-related molecules, such as dehydroascorbate (Slight et al., 1990)) binds to the ε-amino group of lysine residues. Such sugar adducts increase in abundance with age (Garlick et al., 1984) and contribute significantly to fluorophore formation in the lens (Monnier and Cerami, 1981). Once glycated, proteins undergo further reactions, giving rise to poorly-characterized structures called advanced glycation end products (AGEs). AGEs are heterogeneous molecules that cause protein cross-linking, tissue browning and fluorescence. Fluorescent and non-fluorescent AGEs have been identified in aging lenses (Ahmed et al., 2003; Argirov et al., 2005; Biemel et al., 2002; Frye et al., 1998; Kessel et al., 2002; Sell and Monnier, 2004; Zarina et al., 2000).

The relationship between LF and AA was investigated by Hemenger et al. (1990) who found that these parameters were negatively correlated, independent of age. Thus, at a given age, those individuals with the most fluorescent lenses tended to have the least AA. This intriguing finding is consistent with the notion that lens stiffening may result from the glycation and subsequent cross-linking of cytoplasmic components. This is a provocative model because compounds have recently been introduced that purportedly break AGE-crosslinks. One such compound, DMPTB, successfully disrupted high molecular weight aggregates extracted from diabetic lenses (Hollenbach et al., 2003) and, in the aging human vascular system, treatment with a related compound, ALT-711, restored arterial compliance (Kass et al., 2001). If lens hardening is due to glycation, treatment with AGE-crosslink breakers might represent a plausible strategy for restoring tissue elasticity and reversing presbyopia. The hypothesis, however, rests on the initial report that LF and AA are negatively correlated and that conclusion was reached using a small sample size (11 subjects) in which data from left and right eyes were considered to represent independent measurements (Hemenger et al., 1990). Moreover, in the earlier study AA was determined using a subjective, “push up” method. This approach has generally been shown to overestimate true AA (Wold et al., 2003). Because of the potential clinical implications of the findings, we elected to repeat the study of Hemenger et al. (1990) on a larger sample size, using quantitative methods to assess LF and AA.

LF and AA were measured in 161 healthy volunteers (71 males and 90 females) aged 25 to 70. Informed consent was obtained in accordance with protocols reviewed and approved by the Human Studies Committee of Washington University. The study followed the tenets of the Declaration of Helsinki. Participants had no known ocular disease and refractive errors of <3 D.

LF was measured on undilated eyes using a non-invasive ocular fluorophotometer (Fluorotron, OcuMetrics, Mountain View, CA, USA) fitted with an anterior chamber adapter lens. Fluorescence was excited at 441–480 nm, and emission was collected at 531–634 nm. The signal was calibrated against a solution of fluorescein (pH 7.4) and fluorescence units were, therefore, expressed as nanograms of fluorescein equivalents per milliliter (ng f-eq/ml). Measurements were made along the optic axis in 0.125 mm steps. Two scans were performed per eye.
As is evident from the two examples shown in Fig. 1, LF increased markedly with age, as did the apparent disparity between the anterior and posterior perinuclear fluorescence peaks. Studies on isolated lenses or sectioned material (Jacobs and Krohn, 1981) have shown that the fluorescence distribution in the lens is, in fact, symmetrical (or, even, that the posterior region of the lens is slightly more fluorescent than the anterior) and that the reduced fluorescence observed in the lens posterior \textit{in vivo} is due to absorption/scattering of the incident and emitted light by intervening lens tissue (Larsen and Lund-Andersen, 1991; Zeimer and Noth, 1984). Some investigators have quantified this effect and applied appropriate correction factors (Larsen and Lund-Andersen, 1991) but, in this study, we took the uncorrected anterior lens peak as the measure of lens fluorescence.

Fluorescence data from the left and right eyes of 161 participants of various ages are shown in Figure 2. At any age, there was significant variation in LF between individuals but, as expected, the measurements obtained from the left and right eyes of a participant were highly correlated (Fig. 2A), as indicated by the Bland-Altman analysis (Bland and Altman, 1986). Overall, LF increased with age in a near linear fashion (Fig. 2B).

AA was measured using an infrared auto-refractometer with near target (WR-5100K, Grand Seiko Co., Ltd., Tokyo). Participants were first asked to focus on a distant target (located at 6 meters) and refractive error was determined. They were then asked to focus on a near target as it was moved incrementally towards them. Generally the refractive power of the eye increased as the target approached the eye and the subject accommodated to keep it in focus. As the target passed the near point, no further increase in refraction was measured (Fig. 3A). AA was taken as the difference (in D) between the refractive power of the eye at the near point and at 6 meters. Due to inherent design features of the auto-refractometer used in this study, we were not able to demonstrably exhaust (and, therefore, measure accurately) the accommodative range of the youngest subjects (<28 years of age) and, for this reason, those individuals were excluded from subsequent analysis of AA.

Unsurprisingly, measurements of AA made on the two eyes were highly correlated (Fig. 3B). In agreement with the data of Donders (Donders, 1864), Duane (Duane, 1912) and many others, measurements of AA indicated a progressive decrease in amplitude with age (Fig. 3C), although the data were somewhat offset from the classic data set of Duane (indicated by the dashed line in Fig. 3C). Furthermore, unlike Duane’s data (which indicated that approximately 1D of AA persists into old age) the present data suggested that the eye is essentially unable to accommodate by age fifty five.

The goal of our statistical analyses was to assess the correlation between LF and AA while controlling for age. As both LF and AA are continuous measures, we used a regression approach with a random effects model (Singer, 1998) to take into consideration the correlation (R > 0.97 for both LF and AA) between the left and right eyes. The coefficients from this analysis allowed us to estimate the effect of LF on AA after adjusting for age. The relative importance of age and fluorescence was estimated by calculating the partial correlations of each variable (i.e., the proportion of the variance in accommodation explained by the variable). All statistical analyses were conducted using SAS 9.0 (SAS Institute, Cary N. C., 2002). In analysis of participants aged 29 to 55 (270 eyes in 135 people) we found a strong association between age and accommodation (coefficient = -0.25, p<0.0001), and a weak association between fluorescence and accommodation (coefficient = -0.0004, p=0.72). This indicated that after adjustment for age, there was no detectable relationship between accommodation and fluorescence in our sample.

Donders (1864) was the first to clearly define presbyopia. He also noted that because loss of AA is progressive, it is not possible to specify the onset of presbyopia. Nevertheless, he
provided a working definition: the eye is presbyopic when small type can no longer be clearly discerned at a distance of “8 Parisian inches” (about 22 cm). Almost one hundred and fifty years have elapsed since the publication of Donders’ work and, in the interim, numerous studies on accommodative decline have been published (Bruckner et al., 1986; Mordi and Ciuffreda, 1998; Turner, 1958), most notably those of Duane (1912). Although the published studies are in general agreement (that accommodative amplitude does, indeed, decline with age) there is a surprising degree of variability in the results. This is also evident from the results shown in Fig. 3C of the present study, in which our data are compared with those of Duane (1912). The current measurements are systematically offset from the earlier data set. The apparent discrepancy with the data of Duane can be attributed to the fact that, unlike our auto-refractor measurements, the push-up technique used in Duane’s original study is influenced by ocular depth of focus effects (Hamasaki et al., 1956). Other studies using objective measurements of AA have also concluded that AA falls to zero in old age (Koretz et al., 1989).

As with measurements of AA, it has long been recognized that LF varies in an age-dependent manner (Jacobs and Krohn, 1981; Occhipinti et al., 1986; Van Best et al., 1998; Zeimer and Noth, 1984). As early as 1935, Vannas and Wilska observed that lens fluorescence increases with age and that the lenses of diabetic patients are particularly fluorescent (Vannas and Wilska, 1935). Interestingly, fluorescence is not uniformly distributed within the lens. Studies on sectioned material indicate that fluorescence is generally elevated within the lens nucleus, the oldest region of the tissue, although the peri-nuclear region (located 1–2 mm beneath the lens surface) is the most fluorescent of all (Jacobs and Krohn, 1981). Our cross-sectional data showed an average increase in LF of 6.0 ng fl-eq/ml/year, similar to a value of 6.36 obtained previously on a somewhat smaller study group (56 subjects) using similar instrumentation (Bleeker et al., 1986).

From the data shown in Figs. 2 and 3, and numerous published studies, it is apparent that both AA and LF depend strongly on age. Here, however, we sought to determine whether these parameters were correlated with each other independent of age. In contrast to an earlier study (Hemenger et al., 1990), our analysis did not identify a statistically significant relationship between LF and AA independent of age. The reason for this discrepancy probably lies in the number of subjects enrolled in the two studies. In the earlier work, only 11 subjects were analyzed and data from left and right eyes were considered to be independent. As acknowledged by the authors at the time, this is a questionable assumption (a point underscored by measurements in the present study demonstrating that LF and AA measurements are highly correlated between eyes). Our analysis showed some evidence of a trend towards a negative correlation between the variables that might conceivably reach statistical significance in a much larger study. However, it appears that an age-independent relationship between LF and AA, if it exists at all, must be very weak. In summary, we measured LF and AA in both eyes of 161 volunteers. Our data confirmed an age-dependent decrease in AA and an age-dependent increase in LF. Having adjusted for the effects of age, however, we were unable to demonstrate a statistically significant correlation between AA and LF.

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References


Duane A. Normal values of the accommodation at all ages. Journal of the American Medical Association 1912;59:1010–1012.


Figure 1.
Axial fluorescence profiles through the right lens of a 51- or 31-year-old subject. Fluorescence maxima are observed in the perinuclear region of each lens (arrow heads). In the 31-year-old lens, the fluorescence intensity in the anterior (filled arrowhead) and posterior (open arrowhead) perinuclear region are approximately equal. In the 51-year-old lens, the posterior perinuclear fluorescence peak is attenuated with respect to the anterior peak, due to absorbance and scattering by intervening lens tissue. In all cases, the anterior perinuclear value (filled arrowheads) was taken as the measure of lens fluorescence (LF). For the 51-year-old lens, the location of the anterior and posterior lens surfaces are indicated by arrows. The axial position is given in steps, where each step = 0.125 mm.
Figure 2.
Relationship between LF and age. A. Bland-Altman plot showing the correlation between LF values measured in the left and right eye of each subject. B. LF increases approximately linearly with age (the slope of the dashed linear regression line is 6.0 ng fl-eq/ml/year).
Figure 3.
Relationship between AA and age. A. Triplicate measurements of AA in the right eye of a 31-year old volunteer. The refractive power of the eye (dashed lines) increases in response to the accommodative stimulus but lags behind the ideal response (solid line). The plateau in the accommodative response (arrow) defines the near point. B. Bland-Altman plot showing that AA measurements made on the left and right eye of each subject are highly correlated. C. AA declines with age. Note the absence of accommodative response in subjects older than 55. Replotted data from Duane (Duane, 1912) are included for comparison (dashed line).