

Autofluorescence of the Lens in Diabetic and Healthy Subjects by Fluorophotometry

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Autofluorescence ($\lambda = 530$ nm) of the human lens was determined as a function of age by fluorophotometry in insulin-dependent diabetics and in healthy controls. Statistical analysis revealed a significant linear age dependency for both groups ($7.98 \text{ ng eq} \cdot \text{ml}^{-1} \cdot \text{y}^{-1}$ for diabetic patients, $P < 0.001$ and $6.36 \text{ ng eq} \cdot \text{ml}^{-1} \cdot \text{y}^{-1}$ for healthy controls, $P < 0.001$) and a significant dependency on diabetes duration ($8.78 \text{ ng eq} \cdot \text{ml}^{-1}$ per year of diabetes duration, $P < 0.001$). The increase with age was found from about zero value at early childhood. Invest Ophthalmol Vis Sci 27:791–794, 1986

Fluorescence of the human lens was first described in 1858 by Regnaud¹ and has been subject of many investigations.^{2–6} Fluorescence of several specific lens proteins excited by UV or visible light of short wavelength has been demonstrated.^{7–11} Chronic exposure to ambient UV-radiation may cause photochemical generation and accumulation of various fluorescent proteins.^{8,10,12–14} Tryptophan may act as the initial absorbing chromophore.¹⁵ In accordance with this theory, aging of the lens has been associated with an increase in fluorescence at 420 and 530 nm.^{2,4,6,9} An increase of fluorescent chromophores is associated with increasing absorption of UV and some visible light and a corresponding decrease in transmission that may protect the retina from cumulative photochemical damage.^{16,17}

Diabetes seems to cause increase of autofluorescence of the lens,^{6,18} although a connection was not always found.³ Most data on fluorescence have been obtained by *in vitro* experiments.^{2,5,7,9,10,11,14,19} Only few quantitative *in vivo* methods have been described based on comparison of lens fluorescence with a fluorescence standard,³ on micro densitometric analysis of UV-slit-lamp photographs,^{20,21} or on scanning of the lens with a fluorophotometer.⁶

In this report results of high resolution fluorophotometric scanning of the lens in healthy subjects and diabetic patients are described.

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Materials and Methods

Material

Autofluorescence in the central anterior part of the lens was measured in 58 insulin-dependent diabetic patients and compared with the results in 56 healthy volunteers. The diabetic patients aged 18 to 74 yr (mean 44.8) were recruited from the Diabetic Outpatient Department of the University Hospital of Leiden. The duration of the diabetes ranged from 1–48 yr (mean 13.4). Informed consent was obtained after the nature of the procedure had been explained fully. The volunteers, aged from 8–91 yr (mean 43.1), showed no signs of ocular or general diseases.

Instrumentation

Autofluorescence was measured with a commercially available computer fluorophotometer (Fluorotron Master, Coherent Radiation Inc. (Palo Alto, CA)) fitted with a special lens ("anterior segment adapter") for detailed scanning of lens, anterior chamber, and cornea. Measurements were performed within 1 min without use of mydriatics, contact lens, or fluorescein. Autofluorescence of the lens, excited by a beam of continuous blue light, was scanned along the optical axis in steps of 0.1 mm by moving the internal lens system of the fluorophotometer by a computer-controlled motor. The wavelengths of excitation and fluorescent light were set by noninterchangeable color filters with peak transmission at 490 nm and 530 nm respectively. The measured autofluorescence, expressed in equivalents of fluorescein concentration, was recorded on magnetic disc as a function of distance in the eye (Fig. 1). The fluorescence measured outside the lens capsule can be attributed to the finite measurement volume of the fluorophotometer (black diamond in Fig. 1).

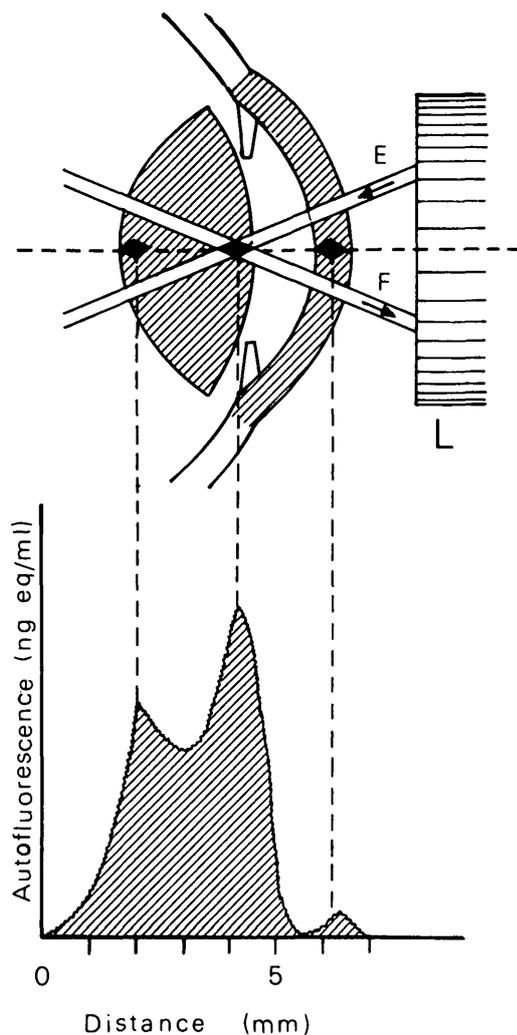


Fig. 1. Upper part: measurement of autofluorescence along the optical axis of the lens. E = excitation beam; F = fluorescence; L = lens system of fluorophotometer. Lower part: relative autofluorescence as a function of distance along the optical axis of the eye.

To avoid loss of excitation and fluorescent light by scatter and absorption within the lens,^{6,22,23} the peak value of axial autofluorescence in the central anterior part of the lens was used as the parameter for lens fluorescence. The spread of the measurements was estimated by ten times measuring autofluorescence of one of both lenses in three volunteers aged 27, 49, and 69 yr respectively. In all three cases the standard deviation was within the 3% limit. Each eye was measured two times and an average value of both eyes was calculated for each subject.

Results

Autofluorescence of Left and Right Eyes

The distribution of the ratios between autofluorescence of left and right lenses is presented in a histogram for both healthy controls and diabetic patients (Fig. 2). The histogram suggests a symmetric distribution for both groups indicating an equal probability for highest

fluorescence in the left as in the right lens. The low percentage of healthy controls at ratios between 0.975 and 1.025 is due to the choice of intervals and is therefore artifactual.

Autofluorescence and Age

Autofluorescence of the central anterior part of the lens as a function of age is presented for healthy persons in Figure 3 and for diabetic patients in Figure 4. The solid line in Figure 3 represents the course of the average lens fluorescence (in ng equivalent fluorescein/ml) of healthy subjects as a function of age. The dotted lines in Figures 3 and 4 account for the 95% prediction intervals for healthy controls (see statistical analysis). Both figures show a linear increase of autofluorescence with age starting in the first 5 yr of life and suggest absence of lens autofluorescence at birth.

Autofluorescence and Diabetes Duration

To assess the effect of diabetes duration on lens autofluorescence in Figure 4, a subdivision was made in three groups of patients with different diabetes duration. This subdivision reveals on an average higher autofluorescence of the lens for patients with longer duration of diabetes.

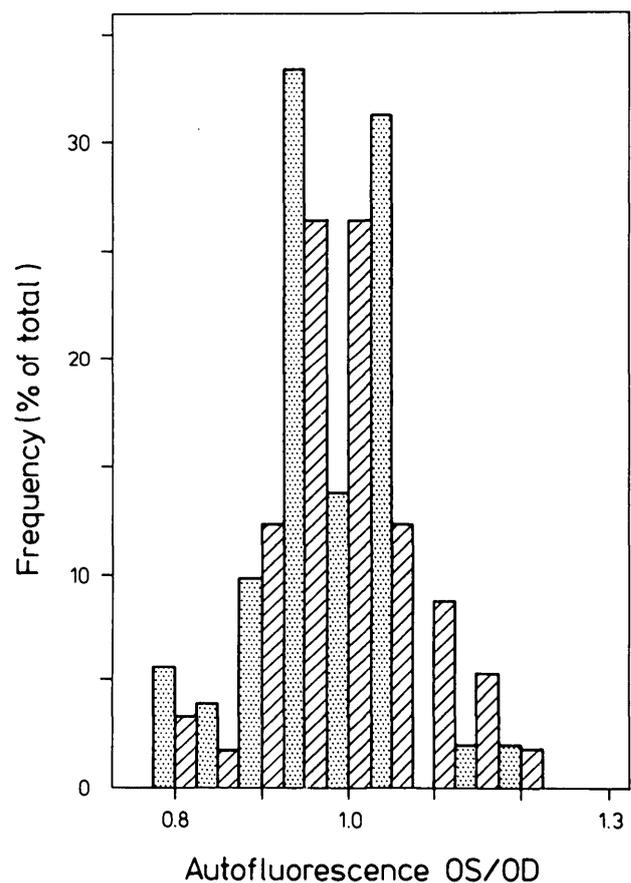


Fig. 2. Left/right autofluorescence ratio histogram. Shaded bars: healthy controls. Hatched bars: diabetic patients.

Statistical Analysis of Results

As the fluorescence values of the two eyes belonging to one person were highly correlated for each subject (see Fig. 2), these two values were averaged to one fluorescence value F_{AV} .

In Figure 3 it can be seen that the standard deviation of these F_{AV} -values of the healthy controls increased approximately proportional with age; eg, the variability near 60 yr is twice as large as that near 30 yr. Therefore a weighted linear regression analysis²⁴ was applied to the data of the healthy controls with weights chosen such that this proportional increase was taken into account. This weighted regression analysis revealed that the expected F_{AV} -value (in ng eq/ml) of a healthy control can be related to his age A (in yr) by the equation

$$F_{AV} = -17.7 + 6.36 A$$

This relationship is presented in Figure 3 by a solid line. The slope of this line differed highly significantly from zero (6.36, SE = 0.34, $P < 0.001$) whereas the intercept just failed to do so (-17.7, SE = 8.9, $P = 0.053$). In addition 95% prediction intervals were computed for normal subjects and are represented in Figure 3 by dotted lines. These dotted lines can be interpreted as indicators of age dependent normal range. Furthermore, the correlation coefficient between F_{AV} and age was found to be 0.90.

For diabetic patients the relationship of F_{AV} with age A and duration of diabetes D in years was investigated by multiple regression analysis with the same weighting system as used for the healthy controls. The analysis revealed the following equation

$$F_{AV} = -69.3 + 7.98 A + 8.78 D$$

The intercept differed from zero (-69.3, SE = 29.1, $P = 0.021$) and the coefficient for age and that for duration of diabetes differed highly significantly from zero (7.98, SE = 0.81, $P < 0.001$ and 8.78, SE = 1.26, $P < 0.001$). This means that F_{AV} increases both with age and duration of diabetes. This is illustrated in Figure 4 by using different symbols for three groups according to duration of diabetes. The solid line and dotted prediction lines are taken from figure 3 as reference. The coefficient for age-dependency for diabetic patients and that for healthy controls were not significantly different (7.98 and 6.36 respectively, $P = 0.067$).

Discussion

Autofluorescence of the lens at $\lambda = 530$ nm was about 50% higher in diabetic patients than in healthy controls of the same age. Autofluorescence in healthy controls shows a linear increase with age starting in early childhood. These results correlate well with previously published data, although such a linear increase was not always obvious,²⁰ and a start in early childhood

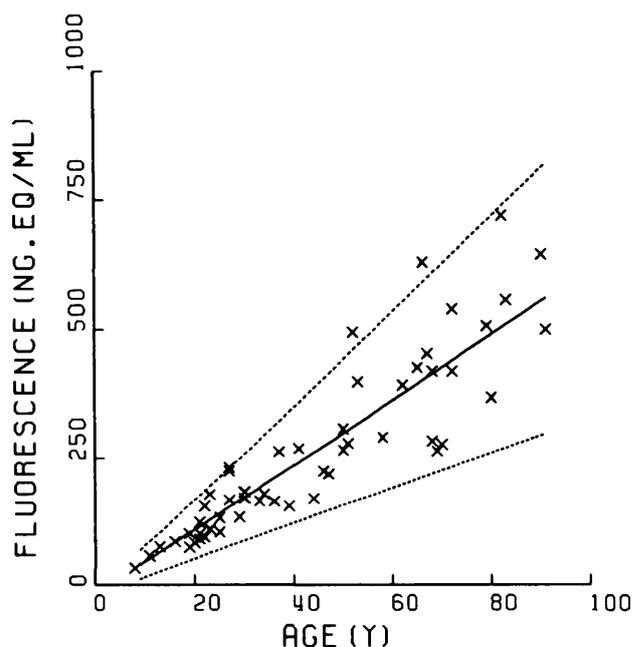


Fig. 3. Autofluorescence of the lens as a function of age in healthy subjects. Each point corresponds to the mean value for both lenses. Solid line: average fluorescence obtained by weighted regression analysis. Dotted lines: 95% prediction intervals.

from about zero value was not observed previously for this part of the autofluorescence spectrum.^{6,20,21} These differences may be attributed in part to the high spatial resolution of about 0.5 mm in our measurements, which was estimated by the use of an eye model. This resolution was achieved by using a special lens ("anterior segment adapter") enabling restriction of mea-

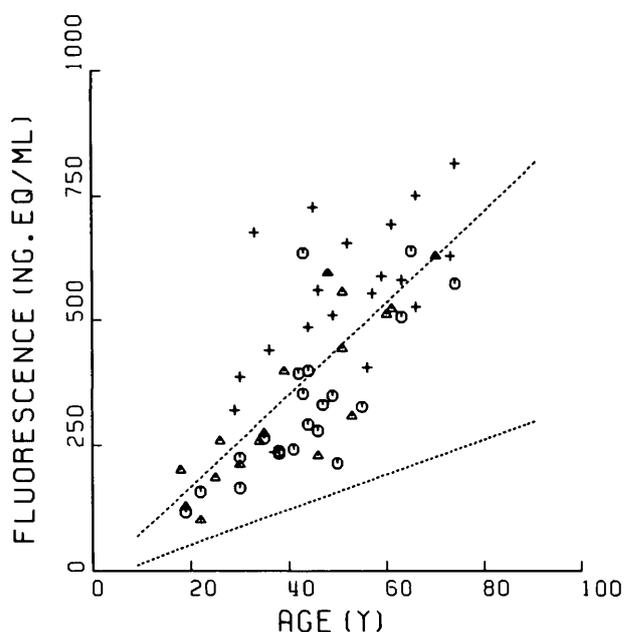


Fig. 4. Autofluorescence of the lens as a function of age in diabetic patients. A subdivision was made to the duration of diabetes: O = less than 6 yr; Δ = 6-15 yr; + = more than 15 yr. Dotted lines: 95% prediction intervals for healthy controls.

surements to the anterior lens cortex thus eliminating light losses due to absorption and scatter within the lens. Furthermore, the high dynamic range (7–91 yr) of our subjects may also have contributed to an improved characterization of the curves. It is also possible that local differences in UV exposure can play a role in the differences between reports.

The increase in lens fluorescence from early childhood seems to confirm theories dealing with UV-light-induced photochemical generation of fluorescent proteins,^{5,13} a process likely to start directly after birth.

The increase in lens fluorescence with age is higher in the diabetic group than in the healthy control group. Multiple regression analysis of the data of diabetic patients shows a significant age-dependency together with a significant diabetes-dependency. As was expected, the age dependency did not significantly differ from that of the healthy controls. The extra increase in fluorescence as a result of 1 yr diabetes is about as large as the increase of 1 yr aging in normals (8.78 ng eq · ml⁻¹ and 6.36 ng eq · ml⁻¹ respectively). An increased lens fluorescence in diabetic patients had also been suggested by others.^{6,25}

It should be noted that this study is cross sectional and shows the relationship between autofluorescence, age, and diabetes duration at a given point in time for persons of different ages. A longitudinal study of the pattern of changes in autofluorescence of diabetic patients is at this moment carried out.

The described technique for measuring autofluorescence of the lens is fast, simple, reproducible, and is no burden to the patient. This report shows that autofluorescence measurement quantitates a change in the lens that is not yet well understood but holds promise since it varies with a disease such as diabetes.

Key words: lens, fluorescence, aging, diabetes, fluorophotometry

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