Laser Light Scattering Spectroscopy of In Vivo Human Lenses

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Laser light scattering spectroscopy measures the thermal random movement of protein as characterized by the diffusion coefficient. This technique has been used in assessing cataract formation in animals. The changes detected appear to predict the later development of lens opacities. The sensitivity and quantitative aspects of this technique offer advantages over other presently available methods of detecting cataract formation. First studies in humans indicated a significant correlation between the diffusion coefficient and age \( (P < 0.05) \). The age adjusted mean diffusion coefficient for nondiabetics \( (4.60 \pm 0.29; \text{mean} \pm \text{SEM}) \) was significantly higher compared to diabetics without retinopathy \( (3.59 \pm 0.41; \ P = 0.0473) \), diabetics with background or preproliferative retinopathy \( (2.73 \pm 0.27; \ P = 0.0001) \), or to diabetics with preproliferative or proliferative retinopathy receiving laser photocoagulation within 1 year of measurement \( (3.02 \pm 0.37; \ P = 0.0012) \). Diabetics with laser treatment more than 1 year prior to measurement \( (3.96 \pm 0.51) \) did not differ significantly from nondiabetics. Invest Ophthalmol Vis Sci 25:594-598, 1984

A major difficulty in lens research is the lack of a sensitive and quantitative method by which early changes in the state of the lens may be determined in vivo. Techniques such as fluorescence, image luminescence, and densitometric analysis of backscattered light\(^1\)\(^-\)\(^4\) detect lenticular damage only after secondary phenomena may have already obscured the primary cause(s) of cataractogenesis. The early detection of lenticular damage while it may still be reversible is a key prerequisite for devising potential cures for this process. Laser light scattering spectroscopy,\(^5\) a technique that meets the above requirements,\(^6\)\(^-\)\(^7\) measures the thermal random motions of lens proteins, which can be quantitated by the diffusion coefficient. This coefficient directly represents the equilibrium and dynamic properties of the lens cytoplasm.

The first in vivo animal study of cataract formation using laser light scattering spectroscopy has been completed.\(^8\) The results may be summarized as follows:

1. In the normal lens, protein motions were slowed at the center of the lens and became monotonically faster as the radial position shifted towards the periphery. This corroborated previous observations that the protein concentration decreases radially across the lens. (2) In the normal lens, for a fixed radius, the protein motion slowed with age. This was probably a reflection of an increase in molecular size and in protein concentration. (3) Within 1 week after irradiation, protein motion became much faster at every point in the x-ray irradiated lens compared with that in the controls. This phenomena may result from changes in the local hydration of the lenticular protein.\(^9\) It is interesting to note that biomicroscopic abnormalities (observed by JNW) were not seen until 5 weeks after irradiation. These results were confirmed by in vitro laser light scattering spectroscopy on the normal and x-ray irradiated lenses after excision.

In humans, the diabetic patient provides a relatively short-term model of cataract development in an already high risk population, which is unavailable in nondiabetic individuals. This paper describes the application of laser light scattering spectroscopy to a series of normal controls and diabetic patients.\(^10\)

Materials and Methods

The apparatus used consisted of two components: (1) an illumination optical system using a He-Ne laser and (2) a detection system made up of fiber-optic transmission and receiving devices interfaced with a biomicroscope (Optech Research Associates, P.O. Box 1037; Brookline Village, MA). The operating principle

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of the laser light scattering apparatus in brief is as follows. The random motions of lens proteins give rise to concentration fluctuations, and the intensity of scattered light fluctuates accordingly. The light is recorded in the form of a time-correlation function, which relates the scattered light intensity I(t) to that at a certain time later I(t + τ): \( \langle I(t)I(t + \tau) \rangle \) where \( \langle \rangle \) denotes averaging over a time t. The decay time of the correlation function is affected by particle mobility. A fast moving particle exhibits a quickly decaying correlation function; a slower moving particle exhibits a slowly decaying correlation function. In summary, the correlation function decays exponentially and the decay time directly reflects the random motions of lens protein.

Informed consent was obtained from 38 diabetic and 19 age-matched nondiabetic volunteers. All subjects were questioned regarding medical and ocular history. An eye examination, including visual acuity, applanation tonometry, biomicroscopic, and dilated fundus examinations was obtained. Stereo lens photography was performed. Approximately two-thirds of the diabetic patients' lenses were clear or exhibited 1-2+ nuclear sclerosis. The remaining one-third exhibited 1-2+ cortical opacities, 1+ posterior subcapsular changes and/or 2-3+ nuclear sclerosis. Twenty-two nondiabetic lenses were clear, and five showed 1-2+ nuclear sclerosis.

Laser light scattering measurements were made at the central nuclear position, the nasal and temporal cortical position, and the anterior and posterior subcapsular regions of one or both eyes. In order to determine the diffusion coefficient, the log of the correlation function was obtained and resulting curve empirically fit to a fifth order polynomial function (5th order cumulant analysis),11 as it provided the best fit to the measured autocorrelation function.

For the purpose of this study, only the first order cumulant (average diffusivity) was used. The higher order cumulants that characterize the shape of the distribution were not considered at this time. The system has been designed to insure patient safety, for example, at the power used to make these measurements (1.5 mw) the maximum permissible exposure time (ANSI Standards) is greater than 5000 sec.12 The measurement duration was 5 sec. A total of 100 eyes in 57 subjects were examined. There were no patient complaints, complications, or side-effects.

The diffusion coefficient of each eye was classified into one of five study groups on the basis of the subject's diabetes status, results of ophthalmoscopic examination, and length of time since laser photocoagulation. The study groups were as follows: nondiabetics (NDM); diabetics without ophthalmoscopically visible retinopathy (DNR); diabetics with background or preproliferative retinopathy (DR); diabetics with preproliferative or proliferative retinopathy who had laser photocoagulation within 1 year of study (DRL1); and diabetics with preproliferative or proliferative retinopathy who had laser photocoagulation greater than 1 year prior to study (DRL2). Some subjects had both eyes in the same study group (n = 28), others had eyes in two different study groups (n = 15), and the remaining subjects had only one eye studied (n = 14). The demographic characteristics of the subjects and the number of eyes in each study group are summarized in Table 1.

The null hypothesis of zero difference between diffusion coefficients for right and left eyes in 8 NDM and 20 diabetics with both eyes in the same study group was tested by Student's paired t-test. The relationship of age and duration of diabetes to the diffusion coefficient was evaluated by Pearson's product moment correlation. Overall analysis of the data was done using three different general linear models. The first was a one-way analysis of variance using study group as the main effect. The second was a one-way analysis of covariance using study group as the main effect and age as a covariate. The third was a three-way analysis of covariance with study group, sex, and eye as main effects and age as a covariate. Post hoc comparison among the means were tested for statistical significance using a least squares means procedure. Analyses were performed by Statistical Analysis System (SAS) programs13,14 on an IBM 4341 computer at the Harvard School of Public Health Computing Facility.

### Table 1. Demographic data and summary of results by study group

<table>
<thead>
<tr>
<th>Study group</th>
<th>Number of subjects</th>
<th>Age (yrs)</th>
<th>Duration of diabetes</th>
<th>Number of eyes studied</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SEM</td>
<td>Range</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>NDM</td>
<td>19 (7M, 12F)</td>
<td>36 ± 3</td>
<td>21–67</td>
<td>12 ± 4</td>
</tr>
<tr>
<td>DNR</td>
<td>7 (4M, 3F)</td>
<td>38 ± 8</td>
<td>17–66</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>DR</td>
<td>24 (12M, 12F)</td>
<td>39 ± 3</td>
<td>22–68</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>DRL1</td>
<td>15 (9M, 6F)</td>
<td>37 ± 4</td>
<td>22–68</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>DRL2</td>
<td>7 (2M, 5F)</td>
<td>39 ± 6</td>
<td>23–65</td>
<td></td>
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</tbody>
</table>

* Number of subjects with both eyes in the same study group is in parentheses.
Results

A correlation function versus time was generated for each point measured. Visual comparison of these multi-exponential decays demonstrates differences between the diabetic and nondiabetic data (Fig. 1A, B). Using a fifth-order cumulant analysis, a diffusion coefficient was generated for each correlation function (Fig. 2A, B).

A comparison of diffusion coefficients from the right and left eye, respectively, in the eight nondiabetic controls with both eyes tested, also was made. The mean difference (± standard error of the mean) was −0.21 ± 0.37 (range −1.30−1.51), which was not significantly different from zero ($P < 0.60$). A highly significant correlation coefficient was obtained between the right eye diffusion coefficient and left eye diffusion coefficient in the same subject ($r = 0.941; P < 0.001$). This indicates that in the nondiabetics measured, both eyes responded to the laser light scattering method similarly.

Diabetics with both eyes in the same study group ($n = 20$) showed more variability than NDM with a mean difference between diffusion coefficients of 0.43 ± 0.34 (range −2.07−4.51). However, this difference also did not differ significantly from zero ($P < 0.30$). The correlation between the left eye and right eye diffusion coefficients was also high ($r = 0.813; P < 0.001$) for this group of diabetic subjects.

The diffusion coefficient was negatively correlated with age ($P < 0.05$ or less) in all study groups (Table 2) but was significantly, inversely correlated with duration of diabetes only in the DR study group ($P < 0.05$).

Because of the significant inverse correlation between age and diffusion coefficient, data were analyzed adjusting for age even though the mean ages in the different groups were not significantly different (Table 1). The results of the diffusion coefficient analyses are summarized in Table 3.

In the first analysis, considering group alone (Table 3A), $r^2$, which measures how much variation in the dependent variable (diffusion coefficient) can be accounted for by the model, was 15.3%. Although this indicates a less than optimal fit for the model, the overall differences among study group means were significant ($P = 0.003$). Also, though the mean diffusion coefficients for the DR and DRL1 study groups were significantly lower, the means for the DNR and DRL2 groups did not differ significantly from NDM controls.

The analysis with age adjustment resulted in an $r^2$ of 57.5%, which indicated that age is a major contributor to variation in the diffusion coefficient, since the addition of age as a covariate accounted for an additional 42.2% of the variability above that obtained from the previous model. The overall differences among study group means were significant ($P = 0.0001$). Post hoc comparison of study group means indicated that those for DNR, DR, and DRL1 were significantly lower, but that the mean diffusion coefficient for DRL2 did not differ significantly from NDM controls (Table 3B).

The third analysis, considering group, age, sex, and eye resulted in an $r^2$ of 59.2%, indicating that the in-

Table 2. Correlation ($r$) of age and duration of diabetes with diffusion coefficient by study group

<table>
<thead>
<tr>
<th>Study group</th>
<th>Number of eyes studied</th>
<th>Diffusion coefficient vs age</th>
<th>Diffusion coefficient vs duration of diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDM</td>
<td>27</td>
<td>−0.72*</td>
<td></td>
</tr>
<tr>
<td>DNR</td>
<td>14</td>
<td>−0.81*</td>
<td>−0.33</td>
</tr>
<tr>
<td>DR</td>
<td>33</td>
<td>−0.79*</td>
<td>−0.39†</td>
</tr>
<tr>
<td>DRL1</td>
<td>17</td>
<td>−0.60*</td>
<td>−0.02</td>
</tr>
<tr>
<td>DRL2</td>
<td>9</td>
<td>−0.71†</td>
<td>−0.41</td>
</tr>
</tbody>
</table>

Significance levels: * $P < 0.001$; † $P < 0.05$. 

Figure 1. A, Correlation functions measured from lens nucleus. (A) 32-year-old nondiabetic; (B) 32-year-old, 6-year history of diabetes, normal fundus exam; (C) 50-year-old nondiabetic; (D) 49-year-old, 33-year history of diabetes, normal fundus exam. B, Correlation functions measured from lens nucleus. (A) 25-year-old nondiabetic; (B) right eye measurement from 25-year-old, 12-year history of diabetes with laser treatment 1.5 years prior to measurement; (C) left eye measurement from same patient, no prior laser treatments.
Fig. 2. A, Diffusion coefficient versus patient age. (O) diabetics with no retinopathy, background, or preproliferative retinopathy and no prior laser treatments; (X) nondiabetics, dotted line represents the lower limit of nondiabetic data. B, Diffusion coefficient versus patient age for diabetics with preproliferative retinopathy having undergone unilateral laser treatment prior to measurement. (●) treated eye; (O) untreated eye; (X) nondiabetics and dotted line superimposed from Figure 2A.

Introduction of sex and eye to the model only accounted for an additional 1.7% of the variability in diffusion coefficients. Partitioning the variance into separate sources indicated that study group and age effects were highly significant \( (P = 0.0001) \), the sex component approached significance \( (P = 0.061) \), and the eye component of variance was not significant \( (P = 0.7448) \).

Post hoc comparison of study group means (Table 3C) indicated results similar to those obtained in analysis B, but with slightly higher significance levels. Comparison of mean diffusion coefficients by sex indicated that females were somewhat lower than males \( (3.90 \pm 0.24 \text{ vs } 3.30 \pm 0.22, P = 0.061; \text{ mean } \pm \text{ SEM}) \). Comparison of mean diffusion coefficients by eye in-
that were similar to older nondiabetics. This finding what, if any, impact fluctuations in blood glucose levels towards those of the nondiabetics. While this does not indicate that right and left eyes were nearly identical (3.65 ± 0.22 vs 3.55 ± 0.23, = 0.7448; mean ± SEM).

**Discussion**

The observed results demonstrated changes in laser light scattering that increase with age and diabetes. These changes in the correlation function in human lenses were similar in direction to those seen in irradiated rabbit lenses before the development of visibly detectable lens opacities.7 We feel that these findings represent a preclinical stage of lens damage, and that the laser light scattering technique is a reproducible and quantifiable method of detecting these early stages.

Young diabetics appeared to have measurements that were similar to older nondiabetics. This finding is consistent with the popular idea that diabetic physiologic age is older than chronologic age. It is not known what, if any, impact fluctuations in blood glucose levels had on these measurements. It is possible that acute or subacute change in glucose could account for the overlap seen between the diabetic and nondiabetic diffusion coefficients. Our finding that laser light scattering in eyes measured more than 1 year postlaser were not different from nondiabetics cannot be explained at this time. It is possible that laser energy affects lens protein configuration directly or through an indirect retinal mechanism that gradually returns the measurement towards those of the nondiabetics. While this does not necessarily mean that these lenses are more “normal,” it does, at least, raise the possibility that initial differences in the correlation function are still reversible. This is very important if this technique is to be used in clinical studies of drugs or other treatments aimed at preventing, delaying, or reversing cataractogenesis.

Our findings in diabetics may represent lens changes due to hyperglycemia. Changes in the lens proteins in different areas of the lens, as determined by laser light scattering spectroscopy, could possibly map the metabolic history of the patient and provide an index of long-term glycemic control. Present investigations include alternate methods of analyzing the correlation function and the effects of short and long-term blood glucose levels on laser light scattering measurements.

Table 3. Summary of analysis results for diffusion coefficient using the total group (n = 100 eyes)

<table>
<thead>
<tr>
<th>Study group</th>
<th>Number of subjects</th>
<th>Number of eyes studied</th>
<th>Mean ± SEM</th>
<th>Signif. level (P)</th>
<th>Mean ± SEM</th>
<th>Signif. level (P)</th>
<th>Mean ± SEM</th>
<th>Signif. level (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDM</td>
<td>19</td>
<td>27</td>
<td>4.76 ± 0.41</td>
<td>1.52-9.52</td>
<td>0.1109</td>
<td>0.0073</td>
<td>0.0012</td>
<td>0.0012</td>
</tr>
<tr>
<td>DNR</td>
<td>7</td>
<td>14</td>
<td>3.62 ± 0.57</td>
<td>0.25-10.70</td>
<td>0.3643</td>
<td>0.2764</td>
<td>0.2965</td>
<td>0.0012</td>
</tr>
<tr>
<td>DR</td>
<td>24</td>
<td>33</td>
<td>2.51 ± 0.37</td>
<td>0.34-5.90</td>
<td>0.0001</td>
<td>0.0012</td>
<td>0.0001</td>
<td>0.0012</td>
</tr>
<tr>
<td>DRL1</td>
<td>15</td>
<td>17</td>
<td>3.15 ± 0.52</td>
<td>0.47-8.30</td>
<td>0.0179</td>
<td>0.0012</td>
<td>0.0001</td>
<td>0.0004</td>
</tr>
<tr>
<td>DRL2</td>
<td>7</td>
<td>9</td>
<td>4.00 ± 0.72</td>
<td>0.65-7.87</td>
<td>0.3643</td>
<td>0.2764</td>
<td>0.2965</td>
<td>0.0012</td>
</tr>
</tbody>
</table>

In conclusion, it appears that laser light scattering spectroscopy is a technique that warrants further use in the study of cataractogenesis and diabetes.

**Key words:** laser, spectroscopy, diffusion coefficient, diabetes, lens

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**References**