Clinical Detection of Precataractous Lens Protein Changes Using Dynamic Light Scattering

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Objective: To use dynamic light scattering to clinically assess early precataractous lens protein changes.

Methods: We performed a cross-sectional study in 380 eyes of 235 patients aged 7 to 86 years with Age-Related Eye Disease Study clinical nuclear lens opacity grades 0 to 3.8. A dynamic light-scattering device was used to assess α-crystallin, a molecular chaperone protein shown to bind other damaged lens proteins, preventing their aggregation. The outcome measure was the α-crystallin index, a measure of unbound α-crystallin in each lens. The association of the α-crystallin index with increasing nuclear opacity and aging was determined.

Results: There was a significant decrease in the α-crystallin index associated with increasing nuclear lens opacity grades ($P<.001$). There were significant losses of α-crystallin even in clinically clear lenses associated with aging ($P<.001$). The standard error of measurement was 3%.

Conclusions: Dynamic light scattering clinically detects α-crystallin protein loss even in clinically clear lenses. α-Crystallin index measurements may be useful in identifying patients at high risk for cataracts and as an outcome variable in clinical lens studies.

Clinical Relevance: The α-crystallin index may be a useful measure of the protective α-crystallin molecular chaperone reserve present in a lens, analogous to creatinine clearance in estimating renal function reserve.

Arch Ophthalmol. 2008;126(12):1687-1693

CLINICAL DETECTION OF precataractous lens damage in clinically clear lenses and identification of patients at high risk for cataracts would be useful for many reasons. This ability would help alert a patient in advance of functional change and allow lifestyle adjustments to reduce factors, such as sun exposure, cigarette smoking, alcohol intake, and poor control of diabetes mellitus, which could increase the risk of developing cataracts. It could also help determine patient eligibility for clinical trials of anticataract drugs because studies[1-12] have suggested that once lens opacities develop, it may be too late to intervene. Exposure to noxious physical agents (such as cigarette smoke, x-radiation, and sunlight[2]) or drug treatments may also result in an increased risk of cataracts: early detection would be important to assess lens damage and avoidance of these agents or treatments. For example, long-term studies of drugs that lower intraocular pressure have suggested a possible increased risk of nuclear opacity.[13] Because the development of overt nuclear cataracts can proceed slowly, a highly sensitive and quantitative means of assessing precataractous changes also would be useful as an outcome variable in clinical trials for assessing treatment effects (protective or toxic) on the lens.

Dynamic light scattering (DLS) was developed more than 30 years ago, and it has been used by numerous researchers to study lens protein changes.[14-22] Recently, a new DLS device developed by Ansari et al[23-29] was tested in animals under a National Aeronautics and Space Administration–National Eye Institute interagency agreement and was shown to detect lens protein changes much earlier than conventional optical methods. These studies were conducted using different animal models of cataract, including cold cataract and radiation-, diabetic-, selenite- and hyperbaric oxygen–induced cataracts.[23-29] Following these findings, a clinical device was developed. The DLS probe was mounted on a movable carriage inside a keratoscope (Keratron; Optikon 2000 SpA, Rome, Italy) with a 3-dimensional aiming system to improve repeatability.[23,24,30-32] Preliminary clinical studies with this DLS device demonstrated its safety and the repeatability of its results.[31] In this article, we describe further clinical studies using this device to assess early lens changes in a large group of healthy and cataractous individuals in a cross-sectional study, and further laboratory studies to clarify the information obtained using DLS.

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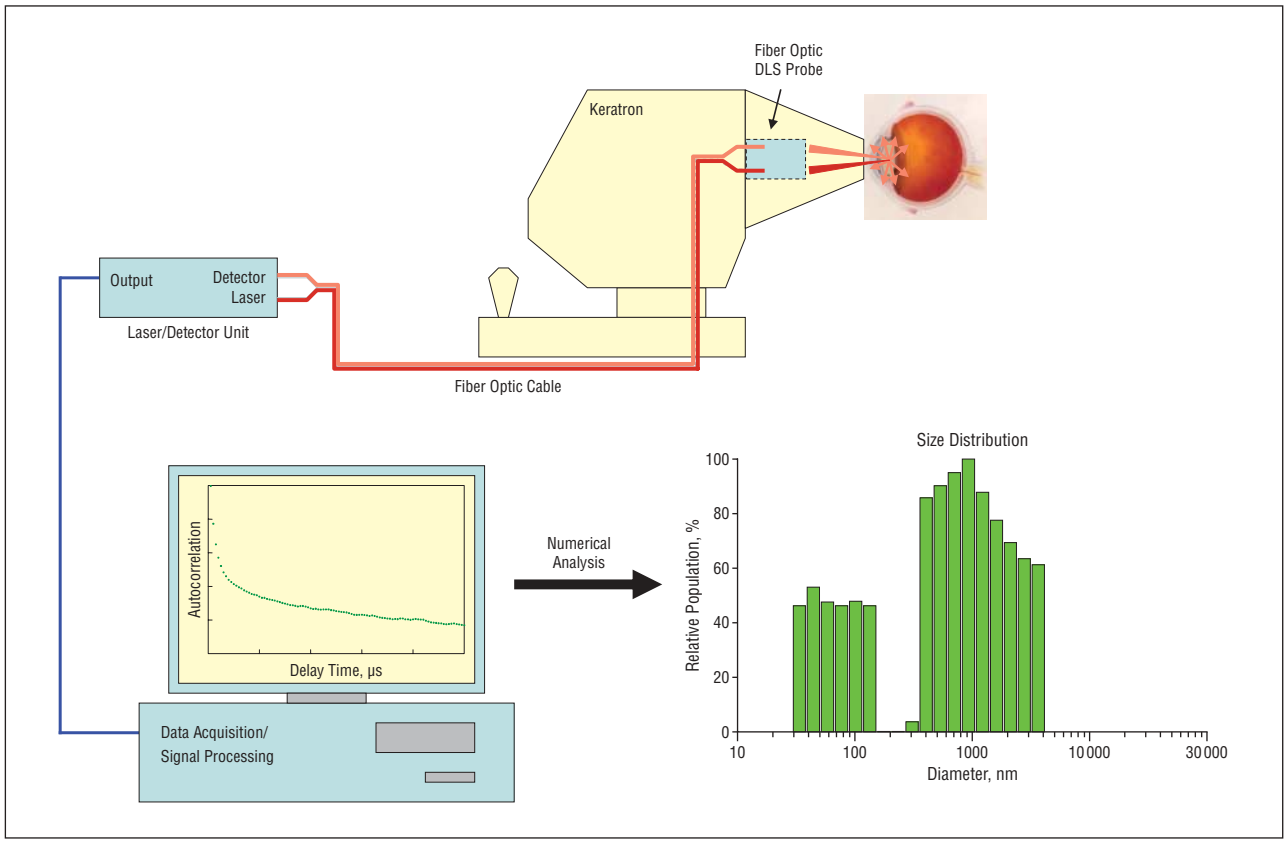

Figure 1. Schematic diagram of how the National Aeronautics and Space Administration–National Eye Institute dynamic light scattering (DLS) clinical device works. The DLS device[33] directs a beam of light to the lens nuclear region (see the text for method of determination), and the light scattered by randomly moving particles in the lens is collected by a photodetector during a 5-second interval. The time autocorrelation function of the measurements is then processed to estimate a profile of intensities and their corresponding particle sizes, resulting in a frequency distribution of particle sizes.

METHODS

CLINICAL DLS SYSTEM

In this new clinical DLS system, a beam of light is directed to a specific area in the lens, and light scattered by randomly moving particles in that area is collected during a 5-second interval (**Figure 1**).[31] The time-autocorrelation function of the measurements is then processed to estimate a profile of intensities. Because the intensity is directly related to particle size, this provides an estimate of the frequency distribution of particle sizes. This profile is typically bimodal (see the example in Figure 1): the first peak represents scattering from particles in the size range of α-crystallin proteins (see the following paragraph), and the second peak represents scattering from high-molecular-weight particles, which are mainly large aggregates of lens protein, cellular organelles, and membrane components.[17,18,20,21,28]

SIGNIFICANCE OF α-CRYSTALLINS

Results of recent studies[28,33-45] have highlighted an important role played by α-crystallins in the lens. α-Crystallins are members of the small heat shock protein family and have been found to act as molecular chaperones that prevent the unfolding and uncontrolled aggregation of damaged lens proteins. α-Crystallins have been shown to be highly efficient in recognizing, and binding to, proteins in the early stages of unfolding. Because the formation of large protein aggregates in the lens causes light scatter and leads to cataracts, the net effect of the chaperone activity of α-crystallin is the maintenance of lens transparency. As a person ages, there is a loss of α-crystallins, which diminishes the capacity of the lens to prevent uncontrolled protein aggregation due to the irreversible binding of damaged proteins and the fact that most of the lens lacks the capacity to synthesize new proteins. Previous biochemical analyses[33,35-38,46-48] of lens extracts have shown that, whereas young, clear lenses have abundant unbound α-crystallin, eyes with nuclear cataracts have little or none. Thus, lenses remain clear as long as there is available α-crystallin binding capacity. When that capacity is overwhelmed, no further chaperone reserve remains to prevent protein aggregation, and lens proteins aggregate in an uncontrolled manner, resulting in light scattering and opacity. Thus, a measure of the α-crystallin remaining in the lens may reflect the level of protective reserve, much as an assessment of creatinine clearance allows us to estimate the renal functional reserve.

CLINICAL STUDY

We conducted a prospective cross-sectional study of control subjects with clear lenses and patients with varying ages and grades of nuclear lens opacity using the DLS device at the National Eye Institute. This study was approved by the National Eye Institute institutional review board. All the tenets of the Declaration of Helsinki on human subjects were followed, and all the participants gave informed consent. Individuals who had tear film disorders, corneal opacities or disorders, uveitis, or glaucoma were excluded. Those who were thought to be at risk for or who had a history of allergic or adverse reaction to one of the dilating or anesthetic agents used were also excluded. No one was excluded based on sex or race.

All the participants underwent a comprehensive dilated eye examination by one of us (M.B.D.), including Age-Related Eye Disease Study clinical lens nucleus grading and lens photography.[49] Age-Related Eye Disease Study photographs were masked and sent for grading to the University of Wisconsin Reading Center (Madison). The DLS testing was performed by one of us (M.B.D.) using the clinical DLS device, as previously described.[31] The DLS measurements of the lens nucleus were repeated 2 to 3 times. After the first measurement, the participant disengaged from the DLS device; after several minutes, the device was refocused for another measurement. The lens nucleus location was determined by performing an A-scan ultrasound measurement of each eye. The DLS probe depth (as an offset from the corneal vertex) was set to the anterior chamber depth plus half of the lens thickness.

The DLS autocorrelation data, with masked identifiers, were sent to the National Aeronautics and Space Administration John H. Glenn Research Center at Lewis Field for processing and analysis. The autocorrelation curve from each measurement was analyzed using an exponential sampling algorithm[50] provided by Brookhaven Instruments Corporation, New York, New York to produce 18 separate exponential terms corresponding to 18 particle size intervals (Figure 1). The final output of the processing is the set of 18 particle size intervals and the scattering intensity for each particle size interval is scaled relative to the highest intensity. The output resembles a histogram of particle size, divided into low- and high-molecular-weight particles (Figure 1).

Because of the biological significance of α-crystallins as chaperone molecules, we derived the α-crystallin index (ACI) as the DLS main summary variable and the primary outcome variable. The ACI is the percentage of scatter from small particles (the first peak) relative to the scatter from all particles, weighted by their corresponding intensities. The ACI was computed as the sum of intensities of the first 6 particle size intervals (corresponding to the first peak, representing mostly unbound α-crystallin) of the DLS output, divided by the sum of the intensities of all 18 particle size intervals, expressed as a percentage (range, 0%-100%). Other potential indices were also explored extensively. Variables that reflect the higher-molecular-weight proteins (second peak)[17-20] represent the complement of the ACI in the precataractous and early stages of lens opacification. No other variables were more highly associated with age or lens opacity than was the ACI. The ACI also had the biological characteristic of being directly associated with unbound α-crystallin.

We focused on lenses with clinically nonsignificant nuclear opacities and normal visual acuity (nuclear grade 0-1); hence, visual acuity was not a primary outcome variable. We applied linear regression and graphical techniques to examine the associations between ACI values, lens grades, and aging. The repeatability of the ACI value was estimated as the square root of the pooled within-eye variances,[51] divided by the mean value of the ACI.

LABORATORY STUDY

A parallel lens protein study was performed on fetal calf lenses to determine which lens proteins accounted for the first and second peaks of the DLS bimodal scattering distribution. The DLS measurements were obtained on (1) purified fetal calf α-crystallin, (2) a known mixture of β- and γ-crystallins, and (3) a complete mixture of soluble lens proteins. Lenses were homogenized in 0.05M Tris buffer solution, pH 7.4, containing 0.1M potassium chloride and 0.02% sodium azide. The homogenate was centrifuged at 25 000*g* to remove the insoluble material. The α-, β-, and γ-crystallin fractions were then separated by means of gel exclusion chromatography using a Superose 6 column (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Isolated fractions were concentrated using Centriprep YM-10 concentrators (Millipore Corporation, Bedford, Massachusetts) and were kept at 4°C until the DLS analyses were complete.

RESULTS

LABORATORY STUDY

To determine whether the first peak in the DLS distribution could be attributed to scatter from unbound α-crystallin, we chose a fetal calf lens model to ensure that the crystallins would be in their nascent state without aging effects. **Figure 2** shows the results of DLS analysis of the soluble proteins from fetal calf lenses and the purified α-crystallin and the mixture of β- and γ-crystallin proteins. The profile of the complete mixture of soluble proteins showed 2 peaks (similar to the human distributions), with the relative proportion of scattering from the first peak being particularly high. The α-crystallin proteins scattered light only in the first DLS peak; the mixture of β- and γ-crystallin proteins also scattered light in the first peak, as would be expected for these relatively small molecules. These in vitro measurements were made at much higher light intensities than are used clinically because the β- and γ-crystallin fraction did not yield a detectable peak at the intensities used clinically (limited to <100 μW for patient safety). For comparison, the DLS profile for a 28-year-old volunteer with a clear lens nucleus (nuclear opacity grade N=0) is shown in Figure 2D.

In a further experiment, we modeled the effects of aging on the crystallins by exposing solutions of total lens soluble proteins to elevated temperatures (50°C-60°C), inducing denaturation of β- and γ-crystallins. We found that this stress caused the first peak to diminish, with a concomitant increase in the second scattering peak, indicating an increase in protein aggregates. This is demonstrated in **Figure 3** by comparison of a representative sample before heating and after 60 minutes at 55°C. Note the shift in both scattering peaks to a higher molecular size and the decrease in area for the first peak and the increase for the second.

CLINICAL STUDY

We examined 235 patients (380 eyes) aged 7 to 86 years. Age-Related Eye Disease Study clinical nuclear lens opacity grades ranged from 0 (clear) to 3.8 (opaque). The **Table** provides the distribution of age, nuclear lens opacity grade, and ACI values for study participants. The ACI values ranged from 0% to 41.4%. **Figure 4** shows a significant decrease in the ACI with increasing nuclear lens opacity grade. For each 1-U increase in nuclear grade, the ACI decreased by 8.9 U ($P<.001$). Results were similar for photographic grades (data not shown).

In a multiple regression model, ACI values significantly decreased with increasing nuclear grade even after adjusting for age. For each 1-U increase in nuclear opacity grade, the ACI decreased by 4.0 U ($P<.001$). The mean value of the ACI in clear lenses from control subjects younger than 22 was 31%, whereas the mean value of the ACI in eyes with nuclear grades of 2 or greater (significant

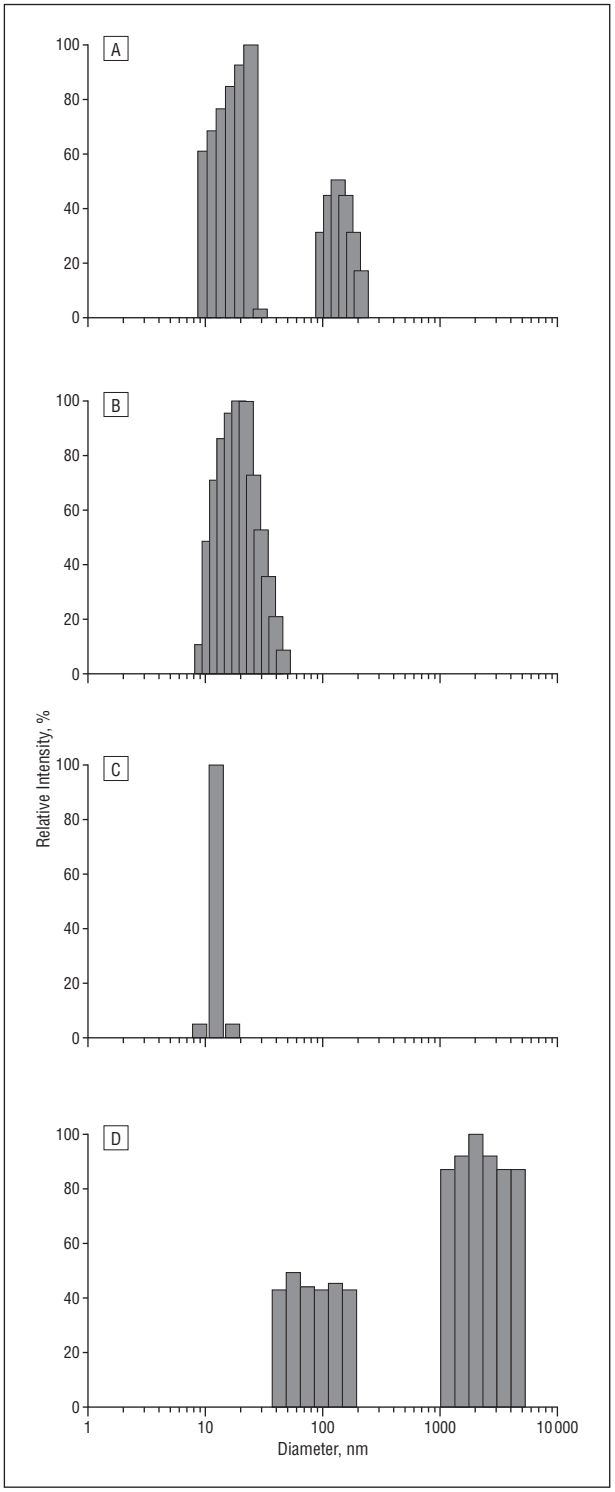

Figure 2. Results of dynamic light scattering (DLS) analysis of the soluble proteins from fetal calf lenses. A, The bimodal DLS distribution for the total soluble protein isolated from a fetal calf lens. B, The DLS profile for α-crystallin isolated from the same soluble lens protein preparation. Note that there is only a single scattering peak, which coincides with the first peak in A. C, The DLS profile for the purified β- and γ-crystallins is also a single peak at the leading edge of the first peak in A. This is to be expected because these proteins are much smaller than α-crystallin. D, For comparison, the DLS profile is shown for the clear lens of a 28-year-old individual (nuclear opacity grade N=0). The 2 peaks in the profile indicate higher particle sizes as is the case for all species tested. This is in part due to the increased age of the tissue but also reflects interactions among the protein species in the very protein-dense milieu of the intact lens and the fact that the second peak also includes scattering from cellular constituents, such as membranes and organelles that are removed in the soluble protein preparations.

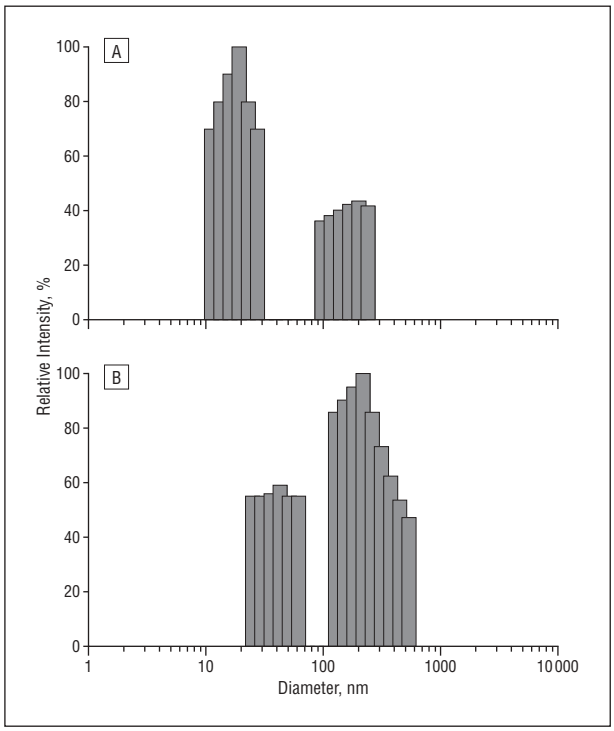

Figure 3. The effect of heating on the dynamic light scattering profile of fetal calf soluble protein. A, The initial profile obtained before the sample was heated to 55°C. B, The profile of the same sample after 60 minutes at 55°C. Note the shift in scattering intensity from the first to the second peak and the increased particle size seen in both peaks after heating.

nuclear opacity) was 2%. Two-thirds (67%) of persons who were 70 years or older with a clinical nuclear grade of 2 or more had an ACI of 0%. This finding also suggests that ACI measurements may be most useful and meaningful when used for lenses with nuclear grades of less than 2.

In participants with clinically clear nuclear regions (nuclear grade 0-1), there was a significant decrease in the ACI associated with an increase in age ($P<.001$) (**Figure 5**). The mean amount of unbound α-crystallin is highest (ACI=31%) in those younger than 22 and declines at each successive decade, approaching 0% for persons older than 75 years. Thus, even in clinically clear lenses, there are demonstrable losses of α-crystallin associated with aging.

Figure 6 shows the distribution of DLS measurements in a patient with grade 3 cataracts. In this lens, there is complete disappearance of the first peak, representing unbound α-crystallin (ACI=0%), and a single large particle size peak, representing high-molecular-weight particles (mainly large lens protein aggregates, cellular organelles, and membrane components[17,18,20,21,28]).

Figure 7 shows that in the group of older persons aged 60 to 70, a significant decrease in the ACI is associated with increasing nuclear lens opacity grade. For each 1-U increase in nuclear opacity grade, the ACI decreased by 4.8 U ($P<.001$). This decrease in the ACI remained significant even after adjusting for age within the 60- to 70-year interval: for each 1-U increase in nuclear opacity grade, the ACI decreased by 3.9 U ($P<.001$). Thus, the ACI may be useful for identifying older persons who still have clear lenses who are at higher risk for cataract development.

Table. Characteristics of the 235 Study Participants

Characteristic	Participants or Eyes, No. (%)	Value, Mean (SD) [Range]
Age, y[a]		52.7 (19.6) [7.5-86.0]
7-25	38 (16.2)	
26-35	11 (4.7)	
36-45	22 (9.4)	
46-55	36 (15.3)	
56-65	55 (23.4)	
66-75	50 (21.3)	
≥76	23 (9.8)	
Sex[a]		NA
Female	140 (59.6)	
Male	95 (40.4)	
Clinical nuclear lens opacity grade[b]		0.68 (0.73) [0.0-3.8]
0.0	66 (17.4)	
0.1-0.2	80 (21.0)	
0.3-0.5	86 (22.6)	
0.6-0.8	20 (5.3)	
0.9-1.4	63 (16.6)	
1.5-1.9	26 (6.8)	
≥2.0	39 (10.3)	
Photographic nuclear lens opacity grade[c]		2.62 (1.36) [0.9-6.0]
0.9-1.9	97 (40.6)	
2.0-2.9	56 (23.4)	
3.0-3.9	36 (15.1)	
4.0-4.9	37 (15.5)	
≥5.0	13 (5.4)	
α-Crystallin index, %[b]		15.77 (10.55) [0-41.41]
0.0	46 (12.1)	
>0.0 to <10.0	74 (19.5)	
≥10 to <20	136 (35.8)	
≥20 to <30	80 (21.0)	
≥30	44 (11.6)	

Abbreviation: NA, not applicable.
[a] For 235 participants.
[b] For 380 eyes.
[c] For 239 eyes.

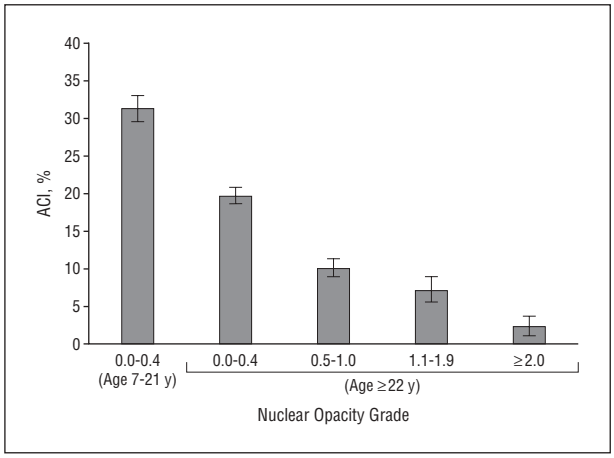

Figure 4. Association between mean α-crystallin index (ACI) values and Age-Related Eye Disease Study nuclear lens opacity grade showing a significant inverse relationship ($P<.001$). The first bar on the left represents ACI values for young, healthy individuals (aged 7-21 years, n=48 eyes). The remaining bars represent individuals 22 years or older (n=332 eyes). Error bars represent 95% confidence intervals.

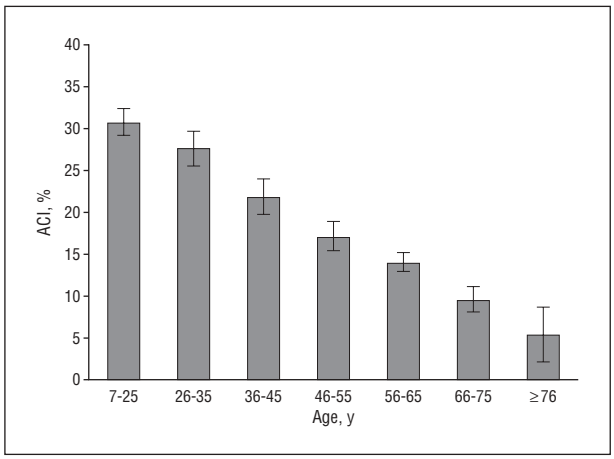

Figure 5. Association between mean α-crystallin index (ACI) values and age for all eyes with no cataract (nuclear lens opacity grade ≤1.0) showing that the dynamic light scattering device detects early protein changes even while the lenses remain clear by clinical evaluation ($P<.001$). Error bars represent 95% confidence intervals.

For measurement of DLS system error, because there were 2 or 3 independent ACI measurements per eye, a variance components model[51] was fit to the data to estimate the within-eye variance, the square root of which is the standard error of measurement. This estimate was 3%, regardless of the size of the mean ACI.

Figure 8 shows baseline and 11-month follow-up ACI measurements for a 42-year-old patient who had rapid development of presenile nuclear cataracts, providing an example of the potential of this system for use in longitudinal clinical studies of nuclear cataracts.

COMMENT

These data show that the clinical DLS device can detect early precataractous protein changes in the living lens nucleus even as the lens remains clinically clear on slit-lamp examination (grades 0-1). Support for the claim that DLS detects α-crystallin is provided by the biochemical studies, which demonstrate that the low-molecular-weight component (the first peak in the DLS measurement profile, ie, the ACI) represents unbound α-crystallin (Figure 2).[19,21,26-29] Based on these clinical and laboratory results, we propose the ACI as a clinical measure of unbound crystallin that indirectly reflects the molecular chaperone reserve of the lens (much as creatinine clearance indirectly reflects the renal functional reserve of the kidney) and can be used as a predictor of future development or progression of cataracts. We believe that a simple DLS clinical assessment can be used in patients to reliably estimate the amount of α-crystallin available as molecular chaperones in the lens nucleus (α-crystallin reserve) and that it will be useful as a screening tool and as an outcome variable.

In this study, we found a strong association between clinical DLS measurements, represented by the ACI, and nuclear lens opacity grading and aging. The association between ACI and nuclear lens grade remains even after adjusting for age. However, these data provide evidence that ACI changes may precede the appearance of clinically observable cataracts because the ACI was 0% for clini-

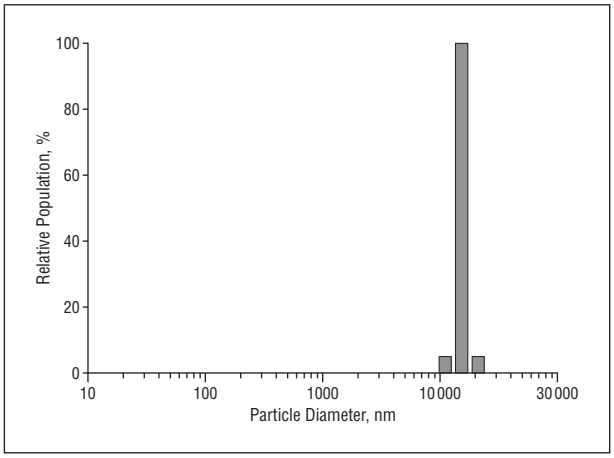

Figure 6. The profile of dynamic light scattering measurements in an eye with advanced cataract (nuclear lens opacity grade N=3) shows unimodal distribution of lens protein sizes, with an absent first peak (corresponding to an undetectable unbound α-crystallin fraction).

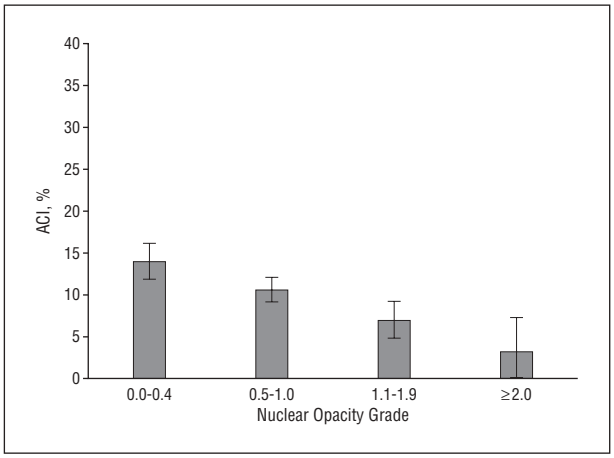

Figure 7. Association between mean α-crystallin index (ACI) values and Age-Related Eye Disease Study nuclear lens opacity grade for eyes from individuals 60 to 70 years of age showing that the dynamic light scattering device can detect lens opacity grade differences independent of age in a group of elderly individuals ($P<.001$). Error bars represent 95% confidence intervals.

cal cataract stages greater than grade 2. Thus, DLS should be most useful in studies of precataractous lens changes when the lens is still clear as detected by slitlamp examination (grade <2). It should be useful in detecting individuals at high risk for cataracts while the lens remains clear. Beyond a nuclear cataract grade of 2, conventional optical imaging techniques, such as slitlamp clinical and photographic grading and Scheimpflug and retroillumination photography remain the best ways to assess progression.[22]

Previous studies[1-12] have suggested that once lens opacities appear, it may be too late to prevent or stop the progression of cataracts. This may indicate that persons with clinically clear lenses who are at high risk for cataracts will be ideal candidates for future clinical cataract studies. To test anticataract drugs, the ACI should be useful in patient selection and as a "proof of principle" outcome variable. The finding that DLS measurements have good repeatability confirms a previous study[31] and indicates that the ACI could be used as a reliable, sensitive outcome variable for future longitudinal clinical studies of the lens.

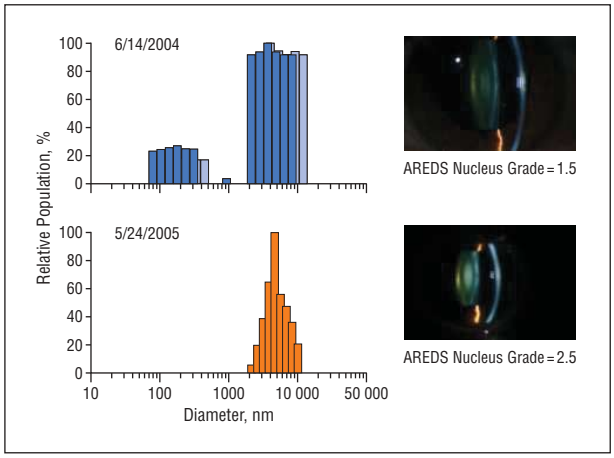

Figure 8. Follow-up data from a 42-year-old patient with early presenile nuclear cataract showing loss of the unbound α-crystallin protein fraction (first peak) when a cataract developed 11 months later. Note that the dynamic light scattering profile on June 14, 2004, represents 2 measurements superimposed on each other to show the repeatability of the system as discussed in the text. AREDS indicates Age-Related Eye Disease Study.

Loss of α-crystallin has recently been found to be associated with presbyopia.[52] The ACI may therefore also be useful in studying, in middle-aged patients, presbyopia and other bothersome visual symptoms that are undetectable by slitlamp and other optical examinations at the molecular level.

The DLS measurements also may be helpful in a variety of clinical situations. For example, in patients with glaucoma, those who have had cornea transplantation, or those who have had retinal surgery and display an ACI of 0% in their lenses, a combined cataract procedure may be preferred. When deciding between laser in situ keratomileusis and lens exchange surgery, if the patient's ACI is low or close to 0%, lens exchange may be preferred because cataract surgery may be needed in the near future anyway. For our National Aeronautics and Space Administration collaborators, ACI measurements may help assess the effects of cosmic radiation in outer space, may aid in developing methods to prevent lens damage from radiation, and may help select individuals at lowest risk for long missions.

In summary, this study demonstrates the usefulness of DLS measurements in assessing early precataractous lens changes. The ACI may be useful in future studies as a screening tool to detect individuals at high risk for cataracts and as a reproducible, sensitive outcome variable in clinical trials or epidemiologic studies. We present data supporting the applicability of the ACI as a measure of α-crystallin chaperone reserve in the lens nucleus, reflecting the risk of cataracts in an individual.

Submitted for Publication: February 26, 2008; final revision received June 9, 2008; accepted June 28, 2008.
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Author Contributions: Dr Datiles had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Financial Disclosure: Drs Ansari and Suh are the owners of patent No. 5973779.
Funding/Support: This study was supported by a National Aeronautics and Space Administration–National Eye Institute interagency agreement.
Previous Presentation: This study was presented in part at the 2007 ARVO Annual Meeting; May 9, 2007; Fort Lauderdale, Florida.
Additional Contributions: Emily Chew, MD, allowed us to enroll some of her patients into this study; Rita Hiller, MS, Linda Goodman, COT, and Cheryl Perry, BSN, served as clinic coordinators for the study; James King provided technical help; and Joram Piatigorsky, PhD, provided helpful discussions.

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