Loss of contrast sensitivity in diabetic patients with LOCS II classified cataracts

Leo T Chylack Jr, Nita Padihye, Patricia M Khu, Caroline Wehner, John Wolfe, Daniel McCarthy, Bernard Rosner, Judith Friend

Abstract

Contrast sensitivity function (CSF) was assessed in a population of diabetics with moderate cataracts to determine if CSF testing provides more information about visual dysfunction than Snellen or Lotmar interferometric visual acuity. With the Lens Opacities Classification Systems Version II (LOCS II) of cataract classification it was possible to grade accurately the type and severity of cataract and nuclear brunescence. The presence of statistically significant relationships between increasing LOCS II classification (worsening cataract) and diminished function, even when the regression model was controlled for Snellen visual acuity, supports the thesis that CSF measurements do provide more information about cataract related visual loss than Snellen acuity alone. Statistically significant (p<0.05) relationships existed between different morphological types of cataract, nuclear colour, and CSF at specific frequencies. The frequencies affected differed with cataract type or nuclear colour, and with distance and near CSF. (Br J Ophthalmol 1993; 77: 7-11)

For decades clinicians have noted the discrepancy between the extent of an age-related cataract and the severity of the visual disability measured using high contrast Snellen acuity charts which may not be sensitive to visual disability characterised by loss of contrast sensitivity function (CSF). To quantify low contrast visual disability, ophthalmologists recently have measured CSF; there are several commercial methods available for this purpose including the Vistech placards (Model No 6500) or the Pelli-Robson charts. It is not clear if CSF testing provides more useful information about visual dysfunction than Snellen acuity alone. Moreover, it is not clear if one type of cataract affects CSF more than another type. Several investigators have reported changes in CSF with specific cataract types. For example, Skalka and Paulsson and Sjöstrand demonstrated loss of contrast sensitivity in posterior subcapsular cataract; Elliott and Gilchrist found that advanced cortical, nuclear, and posterior subcapsular cataract adversely affected CSF. Typically, loss of contrast sensitivity in patients with cataract has been reported to be greater at higher spatial frequencies, though some have reported effects at low frequencies in normal persons and diabetics.

Recently, we and others have published descriptions and validation data for the Lens Opacities Classification Systems LOCS I and II. With these highly reproducible systems, it is now possible to define cataract type and extent very accurately. This, in turn, permits us to define the effects of specific cataract types and degrees on specific visual functions. These validated systems of cataract classification now permit us to control for cataract type and severity in regression analyses of sources of abnormal CSF.

The purposes of this study were: (1) to determine the relationship between the extent and type of cataract and the severity of loss of near and distance contrast sensitivity in a population of diabetic patients whose cataracts have been classified with a valid system of cataract classification; and (2) to determine if Vistech CSF testing charts provide any more information than Snellen acuity alone in quantitate cataract-related visual loss. To do so, we measured cataract severity using the LOCS II system, near and distance CSF at five spatial frequencies with the Vistech VCTS 6500 and 6000 charts, and visual acuity in a population of diabetic patients.

Materials and methods

PATIENT POPULATION

Data were collected from 88 eyes of 49 diabetic volunteers (19 males and 30 females) ranging in age from 32-75 (mean age 62.5). The patients were recruited for a study of the natural history of diabetic cataractogenesis from the Brigham and Women's Hospital, The Massachusetts Eye and Ear Infirmary, and Joslin Diabetes Center, and met the following inclusion criteria: 21-75 years of age; Type I or II diabetes mellitus with stable diabetic control (that is, there was no change in the type of therapy for diabetes other than dosage adjustment). Diabetic control was assessed at baseline and at each visit by determination of Hba,c; the mean Hba,c at baseline was 10.0% (range 6.6-15.9%) which corresponds to fair to poor diabetic control. Other inclusion criteria were: early immature cataract; patient was unlikely to require cataract extraction within 2/7 years; and the patient was willing to give informed consent. There were no pregnant or nursing women; no subjects with cataracts other than senile or diabetic (for example, steroid-induced, traumatic, etc); no eye infections within the past month; no patients with proliferative diabetic retinopathy or history of laser photocoagulation; no persons who had used therapeutic doses of systemic steroids for a period longer than 4 weeks, who took aspirin (>1300 mg/day), or used a topical opthalmic medication chronically; no persons who were on concomitant therapy with an investigational
EXPERIMENTAL PROCEDURES AND EVALUATIONS

Cataract classification using the Lens Opacities Classification System II (LOCS II)

LOCS II cataract classifications were derived from Neitz-CTR retrolumination, Topcon Scheimpflug, and Zeiss colour slit-lamp lens photographs taken on diluted eyes according to a standard protocol. The grades used were consensus grades by LTC and DM. Consensus grades were arrived at as follows: each of the two graders graded each photograph independently. Then, the two graders discussed any discrepancy in grading and a grade that was acceptable to both graders was decided upon and used as the grade for that photograph. Cataract (C) opacities were graded from 0–5; posterior subcapsular cataracts (P) were graded from 0–4; nuclear opalescence (nuclear cataract, NO) from 0–4; and nuclear colour (brunescence, NC) from 0–2.

Contrast sensitivity function (CSF)

Distance CSF was tested monocularly at 10 feet with the best distance spectacle correction in place using the Vistech distance contrast sensitivity chart (VCTS 6500) with a constant illuminance of 30–70 foot Lamberts. The test stimuli were sinewave gratings of varying contrast at five spatial frequencies: 1.5 cpd (cycles/degree), 3.0 cpd, 6.0 cpd, 12.0 cpd, and 18.0 cpd. The test proceeded from higher to lower contrast and from lower to higher spatial frequency. At each spatial frequency, the target with the lowest contrast that was correctly identified before any preceding error was recorded as the contrast sensitivity for that frequency. Contrast sensitivity values, derived from the table provided in the Vistech manual, range from 3 to 260 and depend on the frequency. To obtain a normal distribution of contrast sensitivity measurements in the population, the logarithmic transform of 1 plus the CS value was used for statistical analysis. Near CSF was tested similarly with the VCTS 6000 chart at 18 inches. The results of near CSF are analysed with respect to Snellen distance visual acuity (VA). While in principle it would have been desirable to use Jaeger near VA instead of Snellen distance VA, that was not possible since Jaeger scores were not available for all patients.

For statistical analyses, the responses to the five individual spatial frequencies tested were averaged into three groups: low frequency (1.5 and 3.0 cpd), middle frequency (6.0 cpd), and high frequency (12.0 and 18.0 cpd).

Best corrected visual acuity (VA)

1 Snellen visual acuity (Snellen VA). Best corrected Snellen visual acuities were measured with a Projecto-Chart (American Optical) in a 20 foot lane. For statistical analyses, the Snellen acuity was converted to ln(1/Snellens acuity).

2 Lotmar interferometric visual acuity (LI VA).

After measurement of Snellen VA and CSF, pupils were dilated and the Lotmar interference fringe acuitymeter attached to the Haag-Streit slit-lamp was used to assess the macular interference fringe acuity. Lotmar interferometric visual acuity (LI VA) was recorded as a decimal from 0–1 (with 0-1 equivalent to 20/200 Snellen acuity and 1-0 equivalent to 20/20 Snellen acuity). The end point was the smallest fringe for which the patient correctly indicated all four azimuthal directions (vertical, horizontal, and 45* to right and left). For statistical analyses we used ln(1/LI VA).

3 Macular appearance. This was designated either visually normal (0) or abnormal (1) as viewed with the direct ophthalmoscope. It was considered abnormal if retinal thickening or hard exudates were at or within 500 μm of the centre of the macula.

The retinal status was designated normal if there were no extramacular signs of diabetic retinopathy: microaneurysms, retinal haemorrhages, exudates, venous beading, or proliferative retinopathy. If any of the above signs of diabetic retinopathy were seen, the retina was regarded as abnormal. Retinopathy was graded on the following coarse categorical scale: 1=normal; 2=mild; 3=moderate; 4=severe. There were no cases of proliferative retinopathy. The standardised methods of grading diabetic retinopathy were not available when this study began.

DATA ANALYSIS

A GLMIC, a multivariate, generalised least squares regression model with intraclass correlation was used to analyse the data. For example, in this model, the effects of a specific type of cataract (for example, cortical) on low frequency CSF can be defined by controlling for Snellen visual acuity, age, and sex:

\[ y_i = \alpha + \beta_1 x_{i1} + \beta_2 x_{i2} + \beta_3 x_{i3} + \beta_4 x_{i4} + \beta_5 x_{i5} + \beta_6 x_{i6} + e_i \]

\[ i \text{ denotes patient} \]
\[ j = 1 \text{ if right eye (OD) and 2 if left eye (OS)} \]

where

\[ y_i = \text{averaged contrast sensitivity score at low frequency for eye } j \text{ of patient } i \]
\[ x_{i1} = \text{LOCS II cortical grade} \]
\[ x_{i2} = \ln(1/\text{Snellen}) \text{ (best corrected) VA} \]
\[ x_{i3} = \text{retinal status } (1 = \text{normal}; 2 = \text{abnormal}) \]
\[ x_{i4} = \text{macular status } (0 = \text{normal}; 1 = \text{abnormal}) \]
\[ x_{i5} = \text{age in years} \]
\[ x_{i6} = \text{sex } (1 = m; 0 = F) \]
\[ e_i = \text{error term assumed to be normal with mean } 0 \text{ and variance } \sigma^2. \]

The correlation between \( e_i \) and \( e_j \) is 0 which represents the intraclass correlation between CSF for the right and left eyes after controlling for the covariates. This is similar to multiple linear regression except that the error terms are assumed to be correlated, and one estimates both the regression coefficient \( \beta \) and the correlation parameter \( \rho \) in the same model using maximum likelihood methods. Similar models were run for the other cataract types (P, NO, and NC) and for
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The Spearman correlation coefficients between cataract types and nuclear colour are given, with the p value in parentheses, for each possible relationship. The relationship is considered statistically significant and the p value presented if p<0.05. If p>0.05, the relationship is considered NS (not significant).

**Table 2** Significance of Spearman correlation coefficients (r) between cataract type and nuclear colour

<table>
<thead>
<tr>
<th>Cataract</th>
<th>Posterior subcapsular</th>
<th>Nuclear opacity</th>
<th>Nuclear colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical</td>
<td>1.00</td>
<td>0.150 (NS)</td>
<td>0.287 (NS)</td>
</tr>
<tr>
<td>Posterior subcapsular</td>
<td>na</td>
<td>1.00</td>
<td>0.230 (NS)</td>
</tr>
<tr>
<td>Nuclear opacity</td>
<td>na</td>
<td>na</td>
<td>1.00</td>
</tr>
<tr>
<td>Nuclear colour</td>
<td>na</td>
<td>na</td>
<td>1.00</td>
</tr>
</tbody>
</table>

The results are expressed as regression coefficients (beta, b) indicating the direction and magnitude of the effects and p values indicating the statistical significance of specific regression coefficients. Spearman rank correlation coefficients were calculated to determine relations between grades of severity of different cataract types.

**Results**

**CATARACT FREQUENCY**

The distribution of grades of cataracts in the population is given in Table 1 for C, P, NO, and NC.

**CORRELATION BETWEEN CATARACT TYPES**

There are significant relationships between grades of nuclear opacity and nuclear colour, but not between grades of other cataract types or nuclear colour (Table 2).

**VISUAL ACUITY AND CATARACT OR NUCLEAR COLOUR**

Table 3 illustrates the relationships between the Snellen and interferometric visual acuities and the grades of cataract or nuclear colour defined using LOCS II. All types of cataract and nuclear colour had adverse effects on Snellen visual acuity in this population.

Nuclear opalescence, posterior subcapsular cataract, and nuclear colour, but not cortical cataract, had adverse effects on LI VA.

**DISTANCE AND NEAR CSF AND CATARACT OR NUCLEAR COLOUR**

When the relationship between distance and near CSF and LOCS II cataract or nuclear colour is studied, there is a maximum contrast sensitivity at 3.0 cpd and a minimum at 18.0 cpd with cataract resulting in a drop in CS. (Figures 1A–1D demonstrate this for distance CS. Near CS was similar; the data are not presented.) The statistical significance of the relationships is presented below and in Tables 4 and 5.

**STATISTICAL ANALYSES OF CSF AND CATARACT OR NUCLEAR COLOUR ASSOCIATIONS**

GLMIC analysis of the association of cataract with averaged contrast sensitivity from three frequency levels: low (1.5 and 3.0 cpd), middle (6.0 cpd) and high (12.0 and 18.0 cpd) are presented in Tables 4 and 5. Two statistical models were run. One controlled for age, sex, retina status, macular status, and LI VA to detect changes in CSF owing to cataract, and one controlled for age, sex, retina status, macular status, and Snellen VA to test whether CSF provides information beyond that available with Snellen VA testing.

**Statistical model controlled for age, sex, retinal/macular function, and LI VA (Table 4)**

Cortical cataract is associated with decreased distance CSF at all frequencies and decreased near CSF at middle and high frequencies (p<0.05).

Posterior subcapsular cataract is associated with decreased distance and near CSF at all spatial frequencies (p<0.05).

Nuclear opacity is associated with decreased distance and near CSF at all frequencies (p<0.05).

Nuclear colour is associated with loss of distance CSF at high frequencies (p=0.039) and with decreased near CSF at all frequencies ranges (p<0.05).

**Statistical model controlled for age, sex, retinal/macular function, and Snellen VA (Table 5)**

When the model is controlled for Snellen VA, age, sex, and retinal/macular status, increasingly extensive cortical opacification is associated with worsening distance CSF at middle and high spatial frequencies (p<0.05). The near CSF data indicate that worsening cortical cataract has a statistically significant effect only at the middle spatial frequency (p<0.05).
There are no statistically significant (p ≤ 0.05) relationships between the severity of posterior subcapsular cataract and worsening distance or near CSF at any frequency.

There is an association between increasing nuclear opalescence and worsening distance CSF at low frequencies (p ≤ 0.05). There are no statistically significant associations between increasing NO and worsening near CSF.

Nuclear colour is statistically significantly associated with a loss of distance CSF at high frequencies (p ≤ 0.05) and there is a loss of near CSF at low, middle, and high frequencies which is statistically significant at low and middle frequencies (p ≤ 0.05).

Contrast sensitivity data were analysed as the logarithmic transform of a plus contrast value. As a result, the regression coefficients in Tables 4 and 5 are not directly applicable to predicting a linear change in CS with respect to LOCS II change. However, they can be used to predict a change over a specified range. For example, the mean CS at 6 cpd for NO = 0 is 34.9 (see Figure 1). The regression coefficient for NO at 6 cpd is –0.39 (see Table 4). For a unit increase in NO (that is, from 0 to 1), we would predict a decrease of 0.39 in the natural log of 1 plus the CS value; that is to say:

\[
\ln(1 + 34.9) - 0.39 = \ln(35.9) - 0.39 = 3.58 - 0.39 = 3.19
\]

Thus, the contrast sensitivity at NO = 1 is the antilog of 3.19 minus 1, which is 23.3.

### Table 4 Regression analysis of near and distant contrast sensitivity and LOCS II cataract classification controlled for Lotmar interferometric visual acuity

<table>
<thead>
<tr>
<th>LOCS II type of cataract or nuclear colour</th>
<th>Spatial frequency</th>
<th>Low (1.5 and 3.0 cpd)</th>
<th>Middle (6.0 cpd)</th>
<th>High (12.0 and 18.0 cpd)</th>
<th>Beta</th>
<th>p</th>
<th>Beta</th>
<th>p</th>
<th>Beta</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Distance CSF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortical</td>
<td></td>
<td>-0.182</td>
<td>-0.01398</td>
<td>-0.273</td>
<td>0.0011</td>
<td>-0.221</td>
<td>0.0044</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior subcapsular</td>
<td></td>
<td>-0.264</td>
<td>-0.00417</td>
<td>-0.199</td>
<td>0.0481</td>
<td>-0.191</td>
<td>0.0343</td>
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<tr>
<td>Nuclear opalescence</td>
<td></td>
<td>-0.426</td>
<td>-0.0003</td>
<td>-0.390</td>
<td>0.0052</td>
<td>-0.316</td>
<td>0.0143</td>
<td></td>
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<td></td>
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<tr>
<td>Nuclear colour</td>
<td></td>
<td>-0.182</td>
<td>0.178</td>
<td>-0.224</td>
<td>0.157</td>
<td>-0.291</td>
<td>0.0590</td>
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<tr>
<td><strong>Near CSF</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortical</td>
<td></td>
<td>-0.114</td>
<td>0.099</td>
<td>-0.245</td>
<td>0.0053</td>
<td>-0.154</td>
<td>0.0492</td>
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</tr>
<tr>
<td>Posterior subcapsular</td>
<td></td>
<td>-0.300</td>
<td>-0.0001</td>
<td>-0.285</td>
<td>0.0052</td>
<td>-0.241</td>
<td>0.0600</td>
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<td></td>
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<tr>
<td>Nuclear opalescence</td>
<td></td>
<td>-0.363</td>
<td>0.0005</td>
<td>-0.468</td>
<td>0.0009</td>
<td>-0.350</td>
<td>0.0061</td>
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</tr>
<tr>
<td>Nuclear colour</td>
<td></td>
<td>-0.289</td>
<td>0.0125</td>
<td>-0.491</td>
<td>0.0013</td>
<td>-0.215</td>
<td>0.0021</td>
<td></td>
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*The regression coefficient (beta) and statistical significance (p) for the relationships between contrast sensitivity and severity of cataract or nuclear colour averaged for low (1.5 and 3.0 cpd), middle (6.0 cpd), and high (12.0 and 18.0 cpd) spatial frequencies are presented. In addition to Lotmar interferometric visual acuity, results are controlled for age, sex, retinal status, and macular status.*

### Table 5 Regression analysis of near and distant contrast sensitivity and LOCS II cataract classification controlled for Snellen visual acuity

<table>
<thead>
<tr>
<th>LOCS II type of cataract or nuclear colour</th>
<th>Spatial frequency</th>
<th>Low (1.5 and 3.0 cpd)</th>
<th>Middle (6.0 cpd)</th>
<th>High (12.0 and 18.0 cpd)</th>
<th>Beta</th>
<th>p</th>
<th>Beta</th>
<th>p</th>
<th>Beta</th>
<th>p</th>
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</thead>
<tbody>
<tr>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Cortical</td>
<td></td>
<td>-0.089</td>
<td>0.107</td>
<td>-0.169</td>
<td>0.0075</td>
<td>-0.166</td>
<td>0.0251</td>
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</tr>
<tr>
<td>Posterior subcapsular</td>
<td></td>
<td>-0.016</td>
<td>0.831</td>
<td>0.116</td>
<td>0.187</td>
<td>-0.051</td>
<td>0.598</td>
<td></td>
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</tr>
<tr>
<td>Nuclear opalescence</td>
<td></td>
<td>0.203</td>
<td>0.003</td>
<td>0.144</td>
<td>0.099</td>
<td>-0.026</td>
<td>0.103</td>
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<tr>
<td>Nuclear colour</td>
<td></td>
<td>-0.134</td>
<td>0.172</td>
<td>0.168</td>
<td>0.149</td>
<td>-0.287</td>
<td>0.025</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Near CSF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortical</td>
<td></td>
<td>-0.025</td>
<td>0.606</td>
<td>-0.142</td>
<td>0.0405</td>
<td>-0.098</td>
<td>0.188</td>
<td></td>
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<tr>
<td>Posterior subcapsular</td>
<td></td>
<td>-0.035</td>
<td>0.579</td>
<td>-0.252</td>
<td>0.781</td>
<td>-0.103</td>
<td>0.276</td>
<td></td>
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<tr>
<td>Nuclear opalescence</td>
<td></td>
<td>-0.136</td>
<td>0.999</td>
<td>0.082</td>
<td>0.241</td>
<td>0.056</td>
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<td></td>
<td></td>
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<tr>
<td>Nuclear colour</td>
<td></td>
<td>-0.209</td>
<td>0.0089</td>
<td>-0.421</td>
<td>0.0002</td>
<td>-0.225</td>
<td>0.078</td>
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</tr>
</tbody>
</table>

*The regression coefficient (beta) and statistical significance (p) for the relationships between contrast sensitivity and severity of cataract or nuclear colour averaged for low (1.5 and 3.0 cpd), middle (6.0 cpd), and high (12.0 and 18.0 cpd) spatial frequencies are presented. In addition to Snellen visual acuity, results are controlled for age, sex, retinal status, and macular status.*

### Discussion

In this population there is a correlation between different types of cataract and poorer Snellen or Lotmar visual acuities. Although the correlation with Snellen acuity is expected, the correlation with Lotmar interferometric visual acuity was less expected, since this test has been advocated as a measure of retinal acuity which is independent of medial opacities.

In this study we have attempted to isolate the effects of lens opacities on CSF from effects owing to retinal problems and to determine whether measurement of CSF adds any information beyond that obtained with high contrast visual acuity tests such as Snellen visual acuity. To that end we have looked for residual adverse effects of cataract on CSF after controlling the data for LI VA and Snellen VA and found that there are residual effects. In general, increasing severity of LOCS II-classified cataracts and nuclear colour adversely affects both distance and near CSF. However, not all of these adverse effects are statistically or clinically significant and the results after controlling for Snellen VA differ from those after controlling for LI VA. This suggests that CSF provides more information about vision than Snellen acuity alone, but that this additional information varies with different cataract types and degrees.

Using Spearman correlation coefficients we demonstrated that nuclear opalescence is significantly correlated with nuclear colour (Table 2, p ≤ 0.001) in this population. Thus, the presence of nuclear opalescence will affect our results with nuclear colour. This makes it difficult to isolate specific effects of nuclear colour or nuclear opalescence on CSF in this small population.

However, the finding that worsening nuclear colour does appear adversely to affect CSF is potentially important and suggests nuclear colour may be considered an important contributor to loss of contrast sensitivity. This implies that clinical trials aimed at testing the efficacy of potential anticataract drugs must measure and control for nuclear colour when analysing their results. Failure to do this might lead to a failure to detect a beneficial effect on lens opacification (one that was obscured by increasing nuclear colour) or conversely to conclude incorrectly that the beneficial effect of a drug is due to its ability to decrease opacification (when in fact the effect may have been to reduce colour). However, to confirm these results and to separate the effects of nuclear colour and nuclear opalescence completely, it will be necessary to use a more sensitive method of lens colour analysis than LOCS II classification and one which can separate colour effects from nuclear opalescence effects. Fast spectral scanning colorimetry, a computerised method which provides continuous measures of lens colour parameters (for example, purity, dominant wavelength) independent of nuclear opalescence, will be used for these studies.

Howes et al studied contrast sensitivity in diabetics with different extents of cataract, but they did not use a validated, standardised cataract classification system to grade the type and severity of cataract. Rather, they graded the cataracts as mild (if there was no obstruction to the red fundal glow) and moderate (if there was...
obstruction to the view of the retina with the fundus camera). Mild cataract was associated with loss at the low and medium spatial frequencies while moderate cataract suppressed sensitivity at all frequencies. They did not try to correlate nuclear colour with contrast sensitivity.

Two other studies have investigated the link between the type and extent of cataract, graded using a validated system of classification, and loss of contrast sensitivity at specific spatial frequencies. In both studies, frequency-specific changes were noted. Briefly, Chylack et al, using the Vistech 6500 CSF method and LOCS II in a population of non-diabetic patients with early cataracts showed decreased CSF only at high frequency (12-0 to 18-0 cpd) for nuclear opalescence with no apparent effect for early posterior subcapsular or cortical cataract or nuclear colour. Elliott and Gilchrist1 found a CSF loss at 2, 4, and 10-6 cpd for cortical and nuclear cataract and at 1, 2, 4, and 10-6 cpd for posterior subcapsular cataract defined using the Oxford system.28 However, since in both those studies the populations were non-diabetics, the results may not be comparable with those reported here.

We are unable to generalise our results to all diabetics, because we did not derive this patient cohort from the general population, and the cohort is highly selected by the inclusion and exclusion criteria.

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