Age-Related Nuclear Lens Opacities Are Associated with Reduced Growth before 1 Year of Age

Jennifer R. Evans, Abdul Rauf, Avan Aihie Sayer, Richard P. L. Wormald, and Cyrus Cooper

PURPOSE. The aim of this study was to assess the relationship between fetal and infant growth, as measured by birthweight and weight at 1 year and the development of age-related lens opacities.

METHODS. A total of 1428 men and women who were born in Hertfordshire, United Kingdom, between 1920 and 1930, and for whom records of birthweight and weight at 1 year were available, were traced and invited for examination. Of these, 717 (50%) attended for ophthalmic examination. After dilation with tropicamide 1%, lens opacities were graded using the Lens Opacities Classification System (LOCS) III.

RESULTS. In this population of English men and women aged 64 to 74 years, most opacities were of the nuclear type. There was no association between birthweight and nuclear lens opacities. Weight at 1 year was negatively correlated with nuclear opacity score in adult life (P = 0.001). Subjects in the highest tertile for weight at 1 year (>23 pounds) had an odds ratio of 0.35 (95% confidence interval, 0.17 to 0.74) for having a significant nuclear lens opacity (LOCS score of ≥3) compared with people in the lowest tertile for weight at 1 year (<21 pounds). This association remained after controlling for age, sex, smoking, social class, adult height, and diabetes.

CONCLUSIONS. To our knowledge, this is the first time that such an association has been reported; it needs to be replicated in other populations. It could provide part of the explanation for the observed excess risk of cataract in developing countries. (Invest Ophthalmol Vis Sci. 1998; 39:1740–1744)

Age-related cataract (nuclear opacity) is the most important cause of blindness in the world; nearly 16 million people are estimated to be blind because of cataracts. The normal lens is transparent, largely because its major constituents, lens proteins (crystallins), are densely packed to form a nonordered array with a uniform refractive index. Accumulated damage to these proteins progressively increases light scatter and leads to opacification. Several metabolic pathways have been suggested to explain age-related damage to lens proteins, including oxidation, glycosylation, carboxylation, and radiation-induced cross-linking. In human populations, cataract is associated with lower social class, shorter stature, smoking, diabetes, and increased mortality. Animal and human studies have shown that undernutrition in early life permanently 'programs' the body's structure and metabolism. Impaired early growth is associated with an increased risk of age-related adult diseases (raised blood pressure, cardiovascular disease, and diabetes) and increased mortality. In a cohort of people aged 64 to 74 years living in Hertfordshire, United Kingdom, we examined the association between early growth, as measured by birthweight and weight at 1 year, and age-related lens opacities. These data were presented at the 1996 ARVO Annual Meeting.

METHODS

At the beginning of the 20th century, most births in Hertfordshire took place at home. From 1911 to 1948, all women giving birth were attended by a midwife who recorded birthweight. During the first year of life, newborn infants were visited at home by a health visitor who recorded various parameters of the child's well-being, including the child's weight at 1 year. These data were transferred to ledgers, which have been used in several studies of early growth and adult disease. Men and women born between 1920 and 1930 were traced using the National Health Service Central Registry, and those still living in North Hertfordshire in 1995 were invited to take part in the study. A trained research nurse interviewed them at home. The study was approved by the Local Research Ethics Committee for North Hertfordshire. All subjects were treated in accordance with the tenets of the Declaration of Helsinki; informed consent was obtained from all subjects after explanation of the nature and possible consequences of the study.

At a local health center, each participant was given a full ophthalmic examination, which included clinical grading of lens opacity using the Lens Opacities Classification System (LOCS) III after dilation of the pupil using 1% tropicamide. All clinical observations were made by one ophthalmologist (AR) and were collected in a manner that was masked to exposure data. Height and weight were measured. Social class was classified according to occupation. Smoking status and diabetes were self-reported.

For analysis purposes, the lens opacity score in the worst affected eye was taken as the score for that person. All analyses were done using SAS (SAS Institute, Cary, NC). Crude associations were assessed using the correlation coefficient r; its significance was assessed with a Student's t-test. Because the nuclear opacity score was skewed, its logarithmic transformation was used to calculate mean scores. These mean scores were antilogged for presentation purposes. Logistic regression was used to model the association between birthweight and weight at 1 year and nuclear lens opacities, adjusting for the effect of other risk factors. Nuclear lens opacity score also was modeled as a continuous variable, and similar findings were obtained. Because the interpretation of the data is simpler using categories, these data only are presented.
RESULTS

Between 1920 and 1930, there were 6803 live-born singletons in Hertfordshire. Of these births, 75% had records of birth weight and weight at 1 year. Using the National Health Service Central Registry, 40% were traced, and 1428 people (21%) were found to be living in north Hertfordshire in 1994. These people comprised the study population. Of these, 824 (58%) were interviewed at home. Subsequently, 717 (50%) attended for clinical examination. Responders and nonresponders were divided into tertiles by weight at 1 year. This demonstrates that the population by weight at 1 year. This demonstrates that the effect is not purely confined to people with grade 3 or higher scores but reflects a general shift in the distribution of lens opacity scores.

Potential confounders were divided into two categories:

1. Variables that are known to be associated with age-related lens opacities and early growth but that are very unlikely to reflect the effect of reduced growth on adult lens opacities. These included sex, social class, and smoking. For completeness, age also was included in this list because it is the strongest determinant of age-related lens opacities, although it was unassociated with weight at 1 year. As expected, nuclear opacity increased with increasing age, female sex, smoking, and lower social class (Table 2). After controlling for these factors, there remained a strong negative association between weight at 1 year and nuclear opacity. People in the highest tertile for weight at 1 year had an odds ratio of 0.35 (95% confidence interval [CI], 0.17–0.74) for nuclear lens opacity compared with people in the lowest tertile for weight at 1 year. This suggests that people in the lowest tertile for weight at 1 year have an increased risk of developing age-related lens opacities at 64 to 74 years of age that is nearly three times that of people in the highest tertile.

Figure 1 shows the distribution of lens opacities in the population by weight at 1 year. This demonstrates that the effect is not purely confined to people with grade 3 or higher scores but reflects a general shift in the distribution of lens opacity scores.

Table 1 shows the association between nuclear opacity and weight at 1 year in men and women. The participants were divided into tertiles by weight at 1 year. The mean nuclear opacity score decreases as weight at 1 year increases. The prevalence of significant nuclear lens opacity (LOCS score ≥ 3) decreases as weight at 1 year increases. In women, the prevalence decreases from 17% in the lowest tertile to 5% in the highest tertile. This effect was highly statistically significant, with a P-value of 0.005. In males, the effect was less strong and not statistically significant, decreasing from a prevalence of 11% to 5% (P = 0.170). After controlling for gender, people in the highest tertile had an odds ratio of 0.35 (95% confidence interval [CI], 0.18–0.76) compared with people in the lowest tertile for weight at 1 year. This suggests that people in the lowest tertile for weight at 1 year have an increased risk of developing age-related lens opacities at 64 to 74 years of age that is nearly three times that of people in the highest tertile.

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2. Variables that are known to be associated with age-related lens opacities and early growth but for which it
is less clear whether they represent genuine confounders. For example, diabetes is associated with early growth and adult lens opacities but might lie on the causal pathway between the two. Adult height reflects exposures throughout life and may be a marker for early growth or a confounder, that is, it may represent nutrition in adult life. There was a nonsignificant increased risk of nuclear opacities associated with diabetes and a decreasing risk with increasing height (Table 2). The association between nuclear lens opacities and weight at 1 year was unchanged after controlling for either diabetes or adult height. Body mass index is associated with early weight and, in other studies, has been shown to be associated with nuclear opacity. In our study, body mass index was associated with weight at 1 year (r = 0.109, P = 0.004) but not with nuclear opacity (r = 0.018, P = 0.639). Including body mass index in the model instead of height had no effect on the association between weight at 1 year and nuclear opacity. Because height and body mass index are strongly co-correlated and, therefore, cannot be modeled together, data for height only are presented.

Table 2 shows the results of a model controlling for all of the potential confounders. The association between weight at 1 year and age-related nuclear lens opacities remains. People in the highest tertile for weight at 1 year had an odds ratio of 0.49 (95% CI, 0.22-1.07) compared with people in the lowest tertile.
TABLE 3. Relationship between Nuclear Lens Opacities and Weight at 1 Year after Controlling for Confounders

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>n</th>
<th>Unadjusted Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>P Value†</th>
<th>Adjusted Odds Ratio‡</th>
<th>95% Confidence Interval</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td>1.14</td>
<td>1.02–1.27</td>
<td>0.021</td>
<td>1.20</td>
<td>1.06–1.35</td>
<td>0.004</td>
</tr>
<tr>
<td>Female sex</td>
<td>299</td>
<td>1.42</td>
<td>0.85–2.37</td>
<td>0.178</td>
<td>1.17</td>
<td>0.52–2.60</td>
<td>0.704</td>
</tr>
<tr>
<td>Ever smoked</td>
<td>243</td>
<td>1.47</td>
<td>0.83–2.60</td>
<td>0.183</td>
<td>1.78</td>
<td>0.95–3.38</td>
<td>0.081</td>
</tr>
<tr>
<td>Social class§</td>
<td>324</td>
<td>1.19</td>
<td>0.65–2.18</td>
<td>0.578</td>
<td>1.03</td>
<td>0.54–1.96</td>
<td>0.922</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>154</td>
<td>1.28</td>
<td>0.63–2.60</td>
<td>0.493</td>
<td>0.72</td>
<td>0.32–1.63</td>
<td>0.435</td>
</tr>
<tr>
<td>Diabetes</td>
<td>57</td>
<td>1.57</td>
<td>0.68–3.66</td>
<td>0.292</td>
<td>1.56</td>
<td>0.65–3.75</td>
<td>0.319</td>
</tr>
<tr>
<td>Weight at 1 year (pounds)</td>
<td>57</td>
<td>0.82</td>
<td>0.73–0.91</td>
<td>0.0004</td>
<td>0.87</td>
<td>0.76–0.98</td>
<td>0.025</td>
</tr>
</tbody>
</table>

Results are from logistic regression models.
* Reference categories: Male sex; Never smoked; Social class I/II; No diabetes.
† Wald chi-square.
‡ Each factor is adjusted for all others in the table.
§ Social class was classified from occupation. I, professional; II, intermediate; IIIN, skilled nonmanual worker; HIM, skilled manual worker; IV, partly skilled; and V, unskilled.

weight at 1 year had twice the risk of developing age-related nuclear opacity at ages 64 to 74 years compared with people in the highest tertile.

**DISCUSSION**

We must consider whether potential biases in this study might explain the findings. First, misclassification of lens opacities is unlikely to explain the observations because the observer was masked to the data on early life. Nondifferential misclassification would bias the odds ratios toward one. Second, although this cohort is a selected group of the original births in Hertfordshire, the associations within the examined population are likely to be valid. For selection bias to explain the findings, people with a relatively high weight at 1 year and high lens opacities or with low weight at 1 year and low lens opacities would have to have been selected from the cohort. This seems improbable. Third, we adjusted for potential confounding variables, such as social class. Obviously, we cannot exclude confounders that we have not considered or for whom we do not have data. However, we have data for the most likely confounding variables.

It is thought that the different types of lens opacities have different etiologies. This may be why we did not see an association between early life and posterior subcapsular or cortical opacities. Alternatively, we cannot exclude the possibility that, because these lens opacities were less common in our study population, our study lacked the power to examine them.

Oxidative stress is thought to be a potential factor in the development of age-related cataracts in human populations. Free-radical scavengers, such as enzymes with antioxidant properties (superoxide dismutase, catalase, and glutathione peroxidase) and antioxidant vitamins (carotenoids, ascorbic acid, and α-tocopherol), are likely to be protective. Presently, there is no known relationship between growth in utero and in infancy and protection against oxidative insults in adult life. Carbamylation of lens proteins occurs in the presence of cyanate, which is a feature of severe diarrhea and dehydration. Again, there is little evidence that reduced growth in early life is associated with an increased risk of severe dehydrational episodes later on in life. We had no information on severe dehydrational episodes between birth and 1 year of age; however, 16 people from the cohort had experienced some form of diarrhea between birth and 1 year of age, which was unrelated to the later risk of developing nuclear opacity. Low birthweight and poor growth in infancy are associated with impaired glucose tolerance and diabetes in adults. It is hypothesized that poor nutrition at critical times in the growth of the fetus and child affects the development and function of the beta cells of the islets of Langerhans and of muscle tissue, leading to insulin resistance. It has been suggested that reactions with glucose may be important in the age-related damage to proteins that occurs in the lens. It may be that our observations reflect impaired glucose-insulin metabolism. Although we had no direct measure of impaired glucose tolerance in our study population, reported diabetes was associated with a nonsignificant increased risk of nuclear lens opacity. Clearly, if diabetes is on the pathway between impaired early growth and adult lens opacities, it is inappropriate to control for it in the analyses.

Impaired early growth has been shown to be associated with other markers of aging in the ear, muscle, and skin. The effects of early undernutrition particularly may affect those tissues containing a large proportion of long-lived molecules such as lens crystallins, collagen, and elastic fibers. Here, in the relative absence of regeneration, molecular repair processes may be most critical. It may be that early undernutrition may impair the development of repair systems. The lack of association with birthweight may reflect the critical point in time at which the physiologic mechanisms in the lens are vulnerable to programming.

In conclusion, we report the observation that reduced early growth is associated with an increased risk of age-related lens opacities. Cataracts are the most important cause of blindness in the world and, moreover, have an earlier onset and higher incidence in tropical, less industrialized countries. This is the first time that such an association has been reported. It
Injection of Chemoattractants into Normal Cornea: A Model of Inflammation after Alkali Injury

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PURPOSE. The objective of this study was to establish and characterize the invasion of polymorphonuclear leukocytes (PMNs) into a normal cornea after intrastromal injection of the tripeptide chemoattractants generated from alkali-degraded corneas.

METHODS. The following samples were injected into the midstroma of normal rabbit corneas: ultrafiltered tripeptide chemoattractants (N-acetyl-proline-glycine-proline and N-methyl-proline-glycine-proline) generated from alkali-degraded corneas, synthetic N-acetyl-PGP, positive control (leukotriene B4 [LTB4]), or negative control (Hanks’ balanced salt solution [HBSS]). Timed responses of PMN infiltration were established for effective concentrations of LTB4 or the ultrafiltered chemoattractants.

RESULTS. All intrastromal injections resulted in the immediate development of an edematous disc that was 10 mm in diameter. The lesion essentially had cleared in the HBSS-injected eyes by 8 hours, and histologic sections revealed minimal numbers of PMNs in the cornea or limbal tissue. The injection of LTB4 or the ultrafiltered tripeptide chemoattractants induced peak numbers of PMNs within the stroma at 8 hours, subsiding by 16 hours.

Seventy units of ultrafiltered chemoattractants yielded a strong PMN response, similar to $1 \times 10^{-5} \text{ M LTB}_4$. The highest concentration of ultrafiltered chemoattractants (350 U) produced a severe PMN response that was characterized by a solid sheet of neutrophils surrounding the injection site. The injection of synthetic N-acetyl-PGP (2 $\times 10^{-6} \text{ M}$) produced a marked PMN response.

CONCLUSIONS. PMN invasion of the normal cornea after the injection of the ultrafiltered tripeptide chemoattractants or the synthetic N-acetyl-PGP mimicked early PMN infiltration in the alkali-injured eye, confirming the importance of this chemoattractant as an inflammatory mediator. (Invest Ophthalmol Vis Sci. 1998;39:1744-1750)

ALKALI-INJURY TO THE CORNEA IS CHARACTERIZED BY A MARKED INFILTRATION OF POLYMORPHONUCLEAR LEUKOCYTES (PMNS), WHICH LEADS TO CORNEAL ULCEARTATION AND PERFORATION.1,2 THE DIRECT RELEASE OF NEUTROPHIL CHEMOATTRACTANTS FROM HYDROLYZED CORNEAL PROTEINS IS LIKELY TO BE A CRITICAL STEP IN TRIGGERING THE ACUTE INFLAMMATORY RESPONSE TO THE ALKALI-INJURY. PREVIOUS IN VITRO STUDIES HAVE SHOWN THAT ALKALI-DEGRADATION OF VAILABLE OR NONVAILABLE CORNEAL TISSUE DIRECTLY GENERATES TWO CHEMOATTRACTANTS,3 N-ACETYL-PGP AND N-METHYL-PGP, FROM ALL LAYERS OF THE CORNEA.4 THE CHEMOTACTIC ACTIVITY OF THESE COMPOUNDS WAS FIRMLY ESTABLISHED IN VITRO BY THE POLARIZATION AND COLLAGEN GEL-VISUAL CHEMOTACTIC ASSAYS.3

THE PGP CHEMOATTRACTANT SEQUENCE IS FOUND IN A RELATIVELY SMALL NUMBER OF COMMONLY OCCURRING PROTEINS, WHICH ARE REPRESENTED IN THE EXTRACELLULAR AND CELLULAR CORNEAL TISSUE. THE PROTEINS CONTAINING THIS AMINO ACID SEQUENCE, WHICH CAN BE IDENTIFIED THROUGH NATIONAL PROTEIN DATABASES, ARE COLLAGEN, PROTEOGLYCANS, FIBRONECTIN, LAMININ, INTRACELLULAR ADHESION MOLECULE 1, INTEGRIN, AND NA+ K+ ATPASE.5 ONE OR MORE OF THESE POTENTIAL SOURCES OF THE TRIPPEPTIDE CHEMOATTRACTANTS, N-ACETYL-PGP AND N-METHYL-PGP, ARE LOCATED IN ALL LAYERS OF THE CORNEA. THE SYNTHETIC VERSIONS OF THESE CHEMOATTRACTANTS HAVE DEMONSTRATED BIOLGIC ACTIVITY, IN VITRO, SIMILAR TO THE PURIFIED CHEMOATTRACTANTS.5

THE PURPOSE OF THIS STUDY WAS TO DEMONSTRATE THAT THE ULTRAFILTERED AND SYNTHETIC TRIPPEPTIDE CHEMOATTRACTANTS, WHICH

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References