Role of Glycation in Modification of Lens Crystallins in Diabetic and Nondiabetic Senile Cataracts

TIMOTHY J. LYONS, GIULIANA SILVESTRI, JOHN A. DUNN, DANIEL G. DYER, AND JOHN W. BAYNES

To assess the significance of glycation, nonenzymatic browning, and oxidation of lens crystallins in cataract formation in elderly diabetic patients, we measured three distinct products of glycation, browning, and oxidation reactions in cataractous lens crystallins from 29 diabetic patients (mean ± SD age 72.8 ± 8.8 yr) and 24 nondiabetic patients (age 73.5 ± 8.3 yr). Compounds measured included *1*) fructoselysine (FL), the first stable product of glycation; *2*) pentosidine, a fluorescent, carbohydrate-derived protein cross-link between lysine and arginine residues formed during nonenzymatic browning; and *3*) N^{ϵ}-(carboxymethyl)lysine (CML), a product of autoxidation of sugar adducts to protein. In diabetic compared with nondiabetic patients, there were significant increases ($P < 0.001$) in HbA_1 (10.2 ± 3.1 vs. 7.1 ± 0.7%), FL (7.6 ± 5.4 vs. 1.7 ± 1.2 mmol/mol lysine), and pentosidine (6.3 ± 2.8 vs. 3.8 ± 1.9 μmol/mol lysine). The disproportionate elevation of FL compared with HbA_1 suggests a breakdown in the lens barrier to glucose in diabetes, whereas the increase in pentosidine is indicative of accelerated nonenzymatic browning of diabetic lens crystallins. CML levels were similar in the two groups (7.1 ± 2.4 vs. 6.8 ± 3.0 mmol/mol lysine), providing no evidence for increased oxidative stress in the diabetic cataract. Thus, although the modification of lens crystallins by autoxidation reactions was not increased in diabetes, the increase in glycation and nonenzymatic browning suggests that these processes may acclerate the development of cataracts in diabetic patients. *Diabetes* 40:1010–15, 1991

From the Sir George E. Clark Metabolic Unit and Department of Ophthalmology, Royal Victoria Hospital, Belfast, Northern Ireland, United Kingdom; the Division of Endocrinology, Metabolism and Nutrition, Medical University of South Carolina, Charleston; and the Department of Chemistry, and School of Medicine, University of South Carolina, Columbia, South Carolina.

Address correspondence and reprint requests to Timothy J. Lyons, MD, MRCP, Division of Endocrinology, Metabolism and Nutrition, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425.

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Diabetes is considered a significant risk factor for the development of "senile-type" cataracts (1–3). Cotlier (4) has estimated that, on average, cataracts reach maturity 9.6 yr earlier in the presence of diabetes, and in the Framingham study, hyperglycemia itself was identified as a risk factor (although admittedly weak) for the development of cataracts (5). Leske and Sperduto (2) have concluded that, overall, the evidence "tends to support the existence of an association between diabetes and cataracts, which appears to be more evident at younger [50–69 yr] than at older [>70 yr] ages." Several different pathogenetic mechanisms have been proposed to explain the accelerated cataractogenesis of diabetes. These mechanisms include increased glycation and browning of lens crystallins (6–8) and increased osmotic (9) and oxidative (10–12) stress induced by increased sorbitol-pathway activity. However, many of the studies supporting these mechanisms, particularly those conducted in animal models of diabetes, are more relevant to the development of the acute white "snowflake" cataract than the brunescent (browned) "senile-type" diabetic cataract more common in humans.

To assess the role of glycation, nonenzymatic browning, and oxidation reactions in the accelerated development of cataracts in diabetes, we measured the concentration of distinct products of these reactions in nuclear lens crystallins from senile-type cataracts obtained from elderly patients with and without diabetes. The specific products were fructoselysine (FL), pentosidine, and N^{ϵ}-(carboxymethyl)lysine (CML).

FL is the stable ketoamine-linked Amadori adduct formed on glycation of ε-amino groups of lysine residues in protein (Fig. 1) (13). In previous work, we have shown that the FL content of control (nondiabetic noncataractous) lens crystallins increases between the ages of 0 and 5 yr and thereafter remains relatively constant at ~0.15% of lysine residues through 80 yr of age (14,15). This constant level of glycation of lens crystallins is thought to result from a steady-state

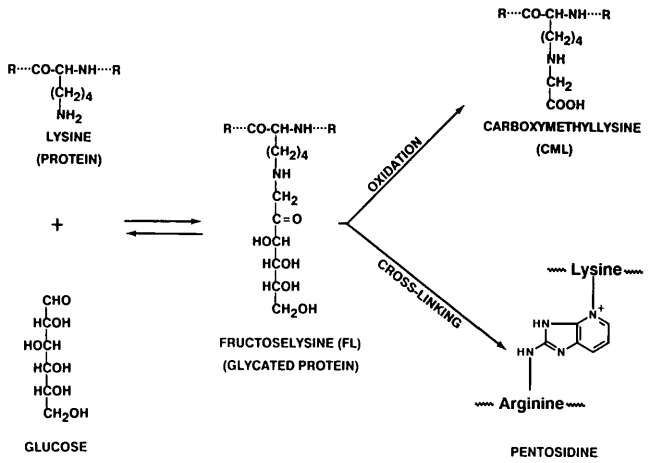

FIG. 1. Maillard reaction pathways for formation of fructoselysine (FL), pentosidine, and N^{ϵ}-(carboxymethyl)lysine (CML) in protein.

relationship between glycation and ambient glucose concentration in the lens (14–16). Thus, FL in lens crystallins should be useful as an intermediate-term indicator of lens glucose concentration.

Pentosidine is a fluorescent cross-link formed between lysine and arginine residues in proteins during the later stages of the Maillard (nonenzymatic browning) reaction (17; Fig. 1). Originally considered to be the product of reactions of pentoses with protein (17,18), there is now evidence that pentosidine can also be formed by reaction of glucose (19,20)* and other hexoses (unpublished observations) with protein. Although pentosidine accounts for <1% of the nondisulfide cross-links in proteins dimerized by glucose in vitro (20), it increases in concert with total fluorescence and cross-linking of proteins during glycation and browning reactions in vitro and with the total fluorescence in human skin collagen with age and in diabetes (17,18). Thus, pentosidine is considered to be a useful biomarker for the overall extent of browning and cross-linking of proteins by glucose in vivo (16–21).

CML has been identified as a major product of metal-catalyzed oxidative degradation of FL in glycated proteins in vitro (22,23; Fig. 1). It is also found in human lens crystallins (14), collagen (16), and urine (23). The CML content of lens crystallins increases linearly with age from trace levels (<0.05% of lysine residues) in the neonate to ~0.7% of the lysine residues at 80 yr of age (14). We have learned recently that, in addition to its origin from FL, CML may also be formed in autoxidative reactions of ascorbate and other sugars with proteins (24). Because CML is a product of autoxidation of sugar adducts to proteins, the CML content of a protein is thought to reflect its cumulative exposure to both glycative and oxidative stress (16,21). This study was undertaken to compare the concentrations of FL, pentosidine, and CML in cataractous nuclear lens crystallins from a group of diabetic and nondiabetic patients of similar age to explore the hypothesized role of glycation and oxidation in the development of cataracts in diabetes.

RESEARCH DESIGN AND METHODS

Nuclear portions of lenses were obtained from 53 patients undergoing routine extracapsular cataract extraction at the Royal Victoria Hospital, Belfast, Northern Ireland. Of these patients, 29 were known to be diabetic: 27 (10 men, 17 women) had non-insulin-dependent diabetes mellitus (NIDDM), and 2 (both women) had insulin-dependent diabetes mellitus (IDDM). Of the 27 NIDDM patients, 17 were treated with diet alone and 10 with diet plus oral hypoglycemic agents. The remaining 24 patients (12 men, 12 women), selected as controls, had no history of diabetes and had normal random plasma glucose values (range 4–7.5 mM). Of these patients, 8 had HbA_1 values >7.2% (determined by agar gel electrophoresis; 25), the upper limit of the normal range of the assay in our laboratory. For statistical analyses, these patients were retained in the nondiabetic group; however, they are identified separately in the presentation of data in tables and figures. In no case did exclusion of these patients from the control group affect the statistical interpretation of the data. Clinical data on the patient groups are given in Table 1. This project was approved by the Ethical and Human Subjects Review Committees of the participating institutions, and informed consent was obtained from all patients.

Lens was prepared as follows. The insoluble nuclear portions of cataractous lenses were collected on a fine screen during cataract removal, then homogenized and dialyzed against deionized water as described previously (14,15). Protein concentration was determined by the biuret method (26), with bovine serum albumin as standard. Unless otherwise stated, reagents were obtained from Sigma (St. Louis, MO) and Aldrich (Milwaukee, WI). The preparation of N^{α}-formyl-N^{ϵ}-fructose-lysine (the standard for the measurement of FL) and CML have been described previously (22). Pentosidine was synthesized from glucose, *N*-acetylarginine and N^{α}-acetyl-[4,5-^{3}H]lysine and was purified to homogeneity by high-performance liquid chromatography (HPLC). The detailed procedure will be described elsewhere.

Amounts of FL, CML, and lysine in lens crystallins were measured by gas chromatography–mass spectrometry with selected ion monitoring (SIM-GC/MS) as previously described in detail (14). Briefly, the homogenized lens samples were hydrolyzed in 7.8 N HCl for 24 h at 110°C under N_2. After removal of HCl by centrifugal evaporation, amino acids were converted to their *N*-trifluoroacetyl methyl ester derivatives for analysis by SIM-GC/MS. FL and CML were quantified by standard addition (14,27), and with a standard curve, the molar ratios of FL and CML to lysine were calculated based on relative peak areas of major fragment ions in the SIM-GC/MS chromatograms. Day-to-day coefficients of variation for FL/lysine and CML/lysine determinations were 10.7 and 10.5%, respectively.

The pentosidine content of lens crystallins was measured by reversed-phase HPLC, with fluorometric detection (19). Briefly, an aliquot of lens protein homogenate containing 8 mg protein was dissolved in 1 ml H_2O, then mixed with an

*The compound, Maillard fluorescent product 1 (MFP-1) had been identified as a fluorescent cross-link formed during browning reactions between glucose and protein (19,20). Using nuclear magnetic resonance spectroscopy and mass spectrometry, we recently determined that MFP-1 is identical to the compound pentosidine, characterized earlier by Sell and Monnier (17,18). There is some debate about whether pentosidine is formed in vivo from ribose (17,18), glucose (19,20), or other sugars.

TABLE 1
Patient characteristics

	n	Age (yr)	Plasma glucose (mM)	HbA_1 (%)†
Diabetic	29	72.8 ± 8.8 (54–89)	9.0 ± 3.5 (3.2–17.5)*	10.2 ± 3.1 (6.2–16.4)
Nondiabetic	24	73.5 ± 8.3 (58–88)	5.5 ± 1.0 (4.0–7.5)‡	7.1 ± 0.7 (5.6–8.5)
High HbA_1	8	73.4 ± 10.1	5.4 ± 1.0 (4.4–7.4)	7.9 ± 0.4 (7.3–8.5)
Normal HbA_1	16	73.6 ± 7.5	5.5 ± 1.1 (4.0–7.5)	6.6 ± 0.4 (5.6–7.1)

Values are means ± SD with ranges shown in parentheses. Mean ± SD duration of diabetes was 9.1 ± 10.7 yr (range 0–38 yr). $P < 0.001$, diabetic vs. nondiabetic for plasma glucose and HbA_1 (nonparametric Mann-Whitney *U* test); NS for age.
*Fasting plasma glucose measured by glucose oxidase procedure.
†Determined by agar gel electrophoresis (25); normal range 3.6–7.2%.
‡Random plasma glucose measured by glucose oxidase method.

equal volume of 0.1 N NaOH containing 1 M $NaBH_4$. After incubation for 12 h at room temperature, residual borohydride sodium was discharged, and the sample was diluted to 2 mg/ml by the addition of an equal volume of concentrated HCl. The protein was then hydrolyzed under N_2 for 24 h at 110°C. The HCl was removed by centrifugal evaporation and the sample dissolved in 1 ml of 1% heptafluorobutyric acid. An aliquot was removed for amino acid analysis to determine lysine content of the hydrolysate. A second aliquot containing 1.5 mg protein was analyzed by reversed-phase HPLC with a C-18 column (Supelco, 25 cm × 4.6 mm, 5-μm particle size) and a water-acetonitrile solvent system containing 0.1% heptafluorobutyric acid. The solvent gradient was 0 min, 5% CH_3CN; 40 min, 9% CH_3CN; 60 min, 9% CH_3CN; 65 min, 50% CH_3CN. Fluorescence (excitation/emission 328/378 nm) was monitored by quantitation of pentosidine, which eluted at ~57 min in the gradient (Fig. 2). Peak areas were integrated with a Baseline Chromatography Workstation (Dynamic Solutions), and pentosidine quantitation was based on the peak area and radioactivity content of the synthetic pentosidine standard containing [4,5-^{3}H]lysine of known specific radioactivity. The pentosidine concentration was normalized to the lysine content of the protein. All laboratory work on the lens samples was done blind, i.e., with no knowledge of clinical details of individual samples.

Data are summarized throughout as means ±SD. Regression analyses were done by the linear least-squares method. Differences between groups were analyzed nonparametrically by the Mann-Whitney *U* test. Statistical data are summarized in the figure legends.

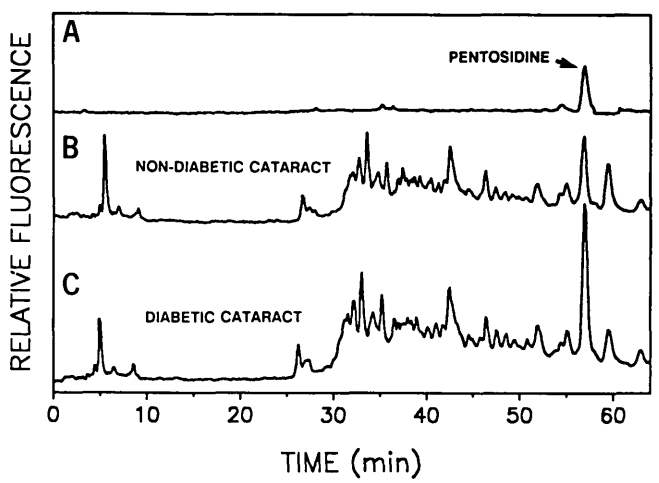

FIG. 2. Identification of pentosidine in lens crystallins. *A*: chromatogram of authentic pentosidine standard (160 fmol, 61 pg pentosidine). *B*: chromatogram of hydrolysate (1.4 mg protein) from nondiabetic cataract (age 88 yr) with average content of pentosidine. *C*: similar chromatogram of hydrolysate from diabetic cataract (age 74 yr) with average (increased) content of pentosidine. These chromatograms are truncated at 65 min, when column was washed with 50% CH_3CN.

RESULTS

In previous studies on normal (noncataractous nondiabetic) lens crystallins obtained postmortem from donors aged 20–80 yr, we observed 1.4 ± 0.4 mmol FL/mol lysine in total lens crystallins (14). We noted that the concentration of FL in lens protein was relatively constant between 20 and 80 yr of age and that the FL content was similar in the soluble (largely cortical) and insoluble (largely nuclear) lens protein fractions (14). The results of this study on the FL content of insoluble nuclear cataractous lens crystallins from nondiabetic patients (1.7 ± 1.2 mmol FL/mol lysine; Fig. 3*A*) were therefore in good agreement with the earlier analyses of total lens crystallins, indicating that glycation of lens crystallins is essentially normal in nondiabetic patients with cataracts (14). As also shown in Fig. 3*A*, the concentration of FL in lens crystallins was more than fourfold higher (7.6 ± 5.4 mmol FL/mol lysine) in diabetic patients. This increase was disproportionately large compared with the less than twofold increases in HbA_1 (Table 1) and lens pentosidine (Fig. 3*B*) in the diabetic patients. Lens FL correlated significantly with fasting plasma glucose in diabetic patients and with HbA_1 in the total and diabetic populations (Fig. 4). However, there was no significant correlation between lens FL and HbA_1 in the nondiabetic group, perhaps because of the limited dispersion of the HbA_1 values, which tended to be in the high normal range in this elderly population. There was also no significant correlation between FL and age in either group or between FL and known duration of diabetes.

The fluorescent carbohydrate-derived protein cross-link pentosidine is readily detected in lens crystallins (Fig. 2). The identity of the pentosidine peak in the lens protein chromatograms was confirmed by its fluorescence spectrum, pH-dependent changes in fluorescence (17), chromatographic mixing experiments, and comparative HPLC analyses of standard and lens samples with different solvent gradients, organic modifiers (trifluoroacetic acid) and col-

umns (phenyl vs. C-18). The concentration of pentosidine was elevated ~65% in diabetic compared with nondiabetic cataracts (6.3 ± 2.8 vs. 3.8 ± 1.9 μmol pentosidine/mol lysine) (Fig. 3*B*). A significant correlation was observed between pentosidine and FL ($r = 0.51$, $P < 0.001$) in the total patient group but not within the diabetic population. There were no significant correlations between pentosidine and plasma glucose, HbA_1, patient age, or known duration of diabetes.

CML concentrations were similar in nondiabetic and diabetic cataracts (Fig. 3*C*). Because CML increases with age in lens crystallins, the data were corrected for possible bias introduced by differences in patient age by expressing the observed CML level as a percentage of that predicted for a normal lens of equivalent age (14). There was no significant difference in these age-standardized CML levels between diabetic and nondiabetic cataractous lenses (112 ± 35 vs. 98 ± 30% of predicted value), and indeed, neither group

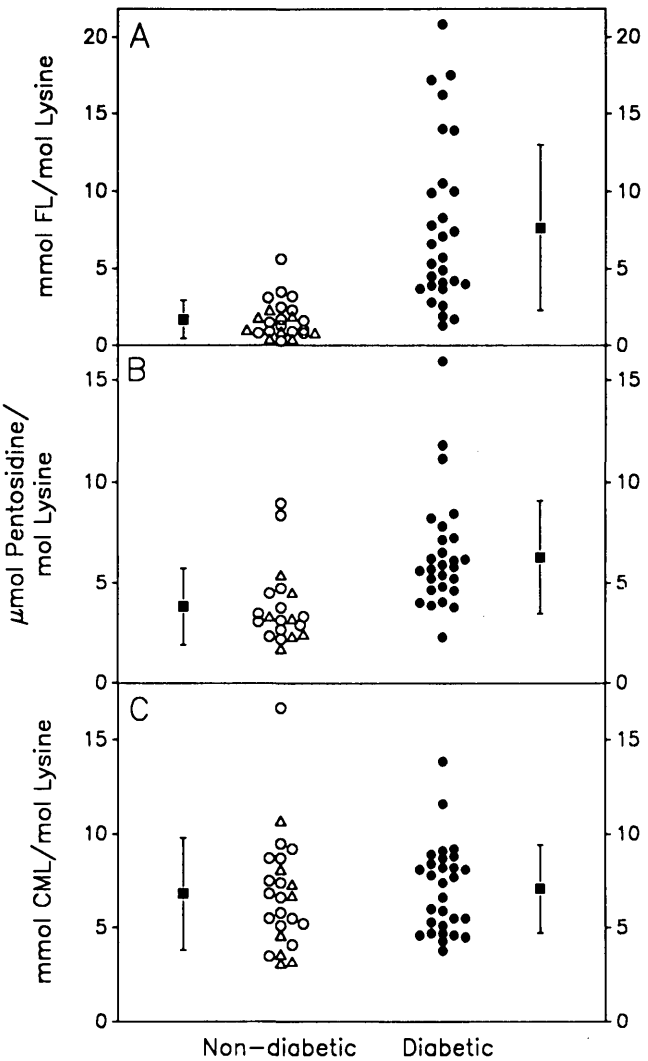

FIG. 3. Levels of fructoselysine (FL; *A*), pentosidine (*B*), and N^{ϵ}-(carboxymethyl)lysine (CML; C) in cataractous lens crystallins from nondiabetic patients with normal (○) and high (△) HbA_1 levels, and from diabetic patients (●). Means ±SD (■) for each group are as follows: FL (mmol FL/mol lysine): nondiabetic 1.7 ± 1.2, diabetic 7.6 ± 5.4, $P < 0.001$; pentosidine (μmol pentosidine/mol lysine): nondiabetic 3.8 ± 1.9, diabetic 6.3 ± 2.8, $P < 0.001$; CML (mmol CML/mol lysine): nondiabetic 6.8 ± 3.0, diabetic 7.1 ± 2.4, $P = 0.5$.

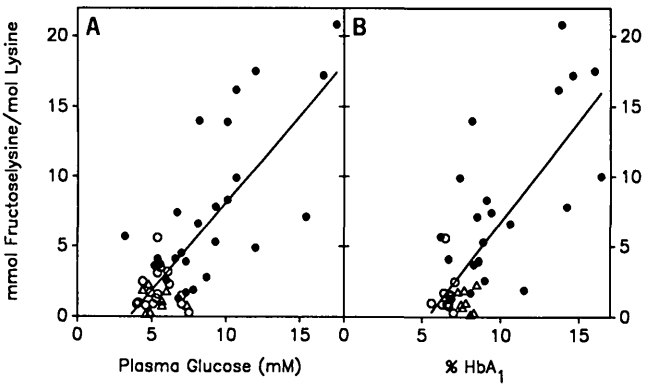

FIG. 4. Relationship of fructoselysine (FL) in lens crystallins to plasma glucose and HbA_1. *Lines*, least-square fits to data points. ○, Nondiabetic with normal HbA_1 level; △, nondiabetic with high HbA_1 levels; ●, diabetic. Plasma glucose values are random in nondiabetic group and fasting in diabetic group. Significant correlations: overall lens FL vs. plasma glucose: $r = 0.78$, $P < 0.001$; diabetic patients only: $r = 0.71$, $P < 0.001$; overall lens FL vs. HbA_1: $r = 0.76$, $P < 0.001$; diabetic patients only: $r = 0.66$, $P < 0.001$.

differed significantly in CML content from whole normal lenses of similar age (14). Lens CML did not correlate with plasma glucose, HbA_1, lens FL, or pentosidine or with known duration of diabetes.

There were no significant differences in plasma glucose, HbA_1, FL, pentosidine, or CML levels between diabetic patients taking oral hypoglycemic agents and those controlled by diet alone.

DISCUSSION

Increased glycation of lens crystallins in diabetes. The concentration of FL in nondiabetic cataractous nuclear lens proteins was similar to levels of FL measured in whole lens proteins from nondiabetic noncataractous donors of similar age (14,15). These results are consistent with earlier studies showing that there are only slight differences in glycation of lens nuclear and cortical proteins (28) and that glycation of lens proteins is constant with age (14,15). In agreement with earlier studies (28–30), we found that FL was increased in diabetic compared with nondiabetic lens proteins (Fig. 3). The extent of this increase in FL (>4-fold) was unexpected, being significantly greater than the increases in plasma glucose (1.7-fold) or HbA_1 (1.5-fold) (Table 1). However, in a more limited study, Garlick et al. (30) observed an ~4-fold increase in glycation of total lens crystallins (not classified with respect to cataracts) in diabetes. The disproportionate increase in FL compared with plasma glucose and HbA_1, which we observed, suggests that ocular permeability barriers to glucose may be compromised in the diabetic patients.

The glucose concentration in the aqueous humor is normally about half that in plasma (31), and in the interior of the lens, it may be as low as 1 mM (32). Consistent with the reported lower glucose concentration in the lens compared with plasma, we have observed that, in nondiabetic subjects, glycation of lens crystallins is significantly lower than that of skin collagen (1.4 vs. 5 mmol FL/mol lysine in lens protein and skin collagen, respectively; 14,16). Because of the procedure used in extracapsular cataract extractions, we were unable to measure the actual glucose concentrations within

our cataractous lenses. However, several years ago, Pirie (33) reported that lens glucose concentrations were undetectable or present only in trace quantities in nondiabetic patients but readily detectable in diabetic patients. Among patients with measurable lens glucose, Pirie described a 3.4-fold increase in diabetic compared with nondiabetic cataracts. Such large changes in lens glucose content might be explained by a breakdown of the blood-aqueous and perhaps the blood-retinal barriers. Supporting this possibility, DiMattio et al. (34) observed that permeation of glucose from the plasma to the aqueous and vitreous compartments is abnormally high in diabetic rats. Also, damage to the ocular vasculature, with increased permeability documented by both vitreous and aqueous humor fluorophotometry (35,36), has been identified as an early abnormality in diabetes. This breakdown of the permeability barriers to glucose in diabetes could amplify the effects of hyperglycemia on the lens. The fact that, in the nondiabetic cataracts, FL levels were similar to those in normal lenses (14) suggests that the mechanisms maintaining the glucose concentration gradients remain intact in nondiabetic cataract patients. Damage to the tissues and mechanisms maintaining this gradient, allowing glucose to leak into the lens, therefore appears to be a consequence of the diabetic state and not of senile alterations in the lens.

Increased nonenzymatic browning of lens crystallins in diabetes. Earlier studies have documented an overall increase in fluorescence in lens proteins in diabetes (6,7) and an increase in a specific nonenzymatic browning product, L_1 (28). In this study, we found that levels of pentosidine were also significantly increased in cataractous lens crystallins from diabetic patients, consistent with the increase in glycation. The 65% difference between the pentosidine content of diabetic and nondiabetic lenses is not as great as the 4.5-fold increase in FL. However, unlike FL, pentosidine is a late-stage product of the Maillard reaction, and its accumulation would depend on patient age and duration of diabetes, severity of hyperglycemia, and perhaps other factors. Detailed knowledge concerning the exact duration of diabetes and a reliable assessment of long-term glycemic control are often not available for patients with NIDDM because glucose intolerance may precede diagnosis by many years. Although pentosidine is clearly increased in cataractous lenses from diabetic compared with nondiabetic subjects, its concentration is nevertheless only at trace level (1000 times lower than FL) and is inadequate to explain the overall increase in cross-linking and decrease in solubility of the proteins. However, studies on glycation of protein in vitro indicate that pentosidine increases in concert with total fluorescence and cross-linking in protein, even though it represents <1% of the total glucose-derived nondisulfide cross-links in proteins dimerized by glucose (16). For this reason, pentosidine should be considered a biomarker rather than a stoichiometric measure of nonenzymatic browning of lens crystallins. Thus, although present only at trace levels, the increased levels of pentosidine in diabetic cataracts are consistent with a significant role for nonenzymatic browning in the early development of cataracts in diabetes.

Oxidative stresss in diabetic lens. Oxidative stress has been implicated in development of cataracts (37) and the accelerated cataractogenesis of human diabetes (9–12). Dietary antioxidants are known to prevent or delay the development of acute sugar cataracts in experimental animals (11,38). Also, Simonelli et al. (39) observed a 3-fold increase in levels of the lipid peroxidation product malondialdehyde (measured as thiobarbituric acid–reactive substances) in cataractous compared with noncataractous lenses, with a further 2-fold increase in diabetic compared with nondiabetic senile cataracts. In contrast, in our study, despite the marked increase in FL, there was no corresponding increase in the oxidation product CML in lens crystallins from the diabetic cataracts. Levels of CML in both diabetic and nondiabetic cataractous lenses were, in fact, similar to those found earlier in lens crystallins from age-matched, whole, normal (nondiabetic noncataractous) lenses (14). The failure to see an increase in CML, despite the 4.5-fold increase in FL, suggests that carboxymethylation of lysine residues via autoxidative reactions of ascorbate (24), rather than oxidation of FL, may be a primary source of CML in the lens. This question is being addressed in studies on ascorbate supplementation in experimental animals. Regardless of the source of CML in human lens crystallins, our results suggest that oxidative damage to proteins is not increased in the diabetic lens. This raises questions about the status of oxidative damage to proteins in the diabetic senile-type cataract and suggests that glycation and browning reactions, and other factors derived from the collapse of ocular permeability barriers, may be more important. The measurement of other chemical indicators of oxidative stress in the cataractous lens, such as products of oxidation of aromatic amino acids (dityrosine, *o*-tyrosine, kynurenines, and *o*-aminobenzoic acid), is planned in order to address this question more decisively.

Conclusions. The diabetic senile-type cataract, although morphologically similar (at least by the time of cataract extraction), is chemically distinct from its nondiabetic counterpart based on the increase in glycation and pentosidine content. A breakdown in the lens-glucose barrier followed by increased glycation and nonenzymatic browning may contribute to the initiation and acceleration of cataractogenesis in diabetes. We found no evidence of increased oxidative modification of cataractous lens crystallins based on measurements of CML concentration. Further studies, particularly on the concentration of other oxidation products in cataractous lens crystallins may clarify the role of protein oxidation in the accelerated development of senile cataracts in diabetes.

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REFERENCES

1. Van Heyningen R, Harding JJ: Risk factors for cataract: diabetes, myopia and sex. *Colloq INSERM* 147:381–85, 1986
2. Alberti KGMM, Hockaday TDR: In *The Oxford Textbook of Medicine*. Wetherall DJ, Ledingham JGG, Warrell DA, Eds. Oxford, Oxford Univ. Presss, 1987, p. 9.83–9.84
3. Leske MC, Sperduto RD: The epidemiology of senile cataracts: a review. *Am J Epidemiol* 118:152–65, 1983

4. Cotlier E: Senile cataracts: evidence for acceleration by diabetes and deceleration by salicylate. *Can J Ophthalmol* 16:113–18, 1981
5. Ederer F, Hiller R, Taylor H: Senile changes and diabetes in two population studies. *Am J Ophthalmol* 9:381–95, 1981
6. Stevens VJ, Rouzer CA, Monnier VM, Cerami A: Diabetic cataract formation: potential role of glycosylation of lens crystallins. *Proc Natl Acad Sci USA* 75:2918–22, 1978
7. Monnier VM, Cerami A: Non-enzymatic browning in vivo: possible process for aging of long-lived proteins. *Science* 211:491–93, 1981
8. Brownlee M, Vlassara H, Cerami A: Non-enzymatic glycosylation and the pathogenesis of diabetic complications. *Ann Intern Med* 101:527–37, 1984
9. Kador PF, Akagi Y, Kinoshita JH: The effect of aldose reductase and its inhibition on sugar cataract formation. *Metabolism* 35 (Suppl. 1):15–19, 1986
10. Monnier VM, Stevens VJ, Cerami A: Non-enzymatic glycosylation, sulfhydryl oxidation, and aggregation of lens proteins in experimental sugar cataracts. *J Exp Med* 150:1098–107, 1979
11. Ross WM, Creighton MO, Trevithick JR, Stewart-DeHaan PJ, Sanwal M: Modelling cortical cataractogenesis. VI. Induction by glucose in vitro or in diabetic rats: prevention and reversal by glutathione. *Exp Eye Res* 37:559–73, 1983
12. Cheng H-M, González RG: The effect of high glucose and oxidative stress on lens metabolism, aldose reductase, and senile cataractogenesis. *Metabolism* 35 (Suppl. 1):10–14, 1986
13. Baynes JW, Watkins NG, Fisher CI, Hull CJ, Patrick JS, Ahmed MU, Dunn JA, Thorpe SR: The Amadori product on protein: structure and reactions. In *The Maillard Reaction in Aging, Diabetes and Nutrition*. Baynes JW, Monnier VM, Eds. New York, Liss, 1989, p. 43–67.
14. Dunn JA, Patrick JS, Thorpe SR, Baynes JW: Oxidation of glycated proteins: age-dependent accumulation of N^{ϵ}-(carboxymethyl)lysine in lens proteins. *Biochemistry* 28:9464–68, 1989
15. Patrick JS, Thorpe SR, Baynes JW: Non-enzymatic glycosylation of protein does not increase with age in normal human lenses. *J Gerontol Biol Sci* 45:B18–23, 1990
16. Dyer DG, Blackledge JA, Katz BM, Hull CJ, Adkisson HD, Thorpe SR, Lyons TJ, Baynes JW: The Maillard reaction in vivo. *J Nutr Sci*. In press
17. Sell DR, Monnier VM: Structure elucidation of a senescence cross-link from human extracellular matrix: implication of pentoses in the aging process. *J Biol Chem* 264:21597–602, 1989
18. Sell DR, Monnier VM: End-stage renal disease and diabetes catalyze the formation of a pentose-derived crosslink from aging human collagen. *J Clin Invest* 84:380–84, 1990
19. Baynes JW, Dunn JA, Dyer DG, Knecht KJ, Ahmed MU, Thorpe SR: Role of glycation in development of pathophysiology in diabetes and aging. In *Glycated Proteins in Diabetes Mellitus*. Ryall RG, Ed. Adelaide, Australia, Adelaide Univ. Press, 1990, p. 219–36
20. Dunn JA, Dyer DG, Knecht KJ, Thorpe SR, McCance DR, Bailie K, Silvestri G, Lyons TJ, Baynes JW: Accumulation of Maillard reaction products in tissue proteins. In *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*. Finot PA, Aeschbacher RF, Hurrell RF, Liardon R, Eds. Basel, Birkhauser-Verlag, 1990, p. 425–30
21. Lyons TJ, Thorpe SR, Baynes JW: Glycation and oxidation of proteins in aging and diabetes. In *Glucose Metabolism, Diabetes and the Vascular Wall*. Ruderman N, Brownlee M, Williamson JR, Eds. Oxford Univ. Press, New York. In press
22. Ahmed MU, Thorpe SR, Baynes JW: Identification of (carboxymethyl)lysine as a degradation product of fructoselysine in glycated protein. *J Biol Chem* 261:4889–94, 1986
23. Ahmed MU, Dunn JA, Walla MD, Thorpe SR, Baynes JW: Oxidative degradation of glucose adducts to protein: formation of 3-(N^{ϵ}-lysino)-lactic acid from model compounds and glycated proteins. *J Biol Chem* 263:8816–21, 1988
24. Dunn JA, Ahmed MU, Murtiashaw MH, Richardson JM, Walla MD, Thorpe SR, Baynes JW: Reaction of ascorbate with lysine and protein under autoxidizing conditions: formation of N^{ϵ}-(carboxymethyl)-lysine by reaction between lysine and products of autoxidation of ascorbate. *Biochemistry* 29:10764–70, 1990
25. Menard L, Dempsey ME, Blankstein LA, Aleyassine H, Wacks M, Soeldner JS: Quantitative determination of glycosylated hemoglobin A_1 by agar gel electrophoresis. *Clin Chem* 26:1598–602, 1980
26. Layne E: Spectrophotometric and turbidimetric methods for measuring proteins. *Methods Enzymol* 3:450–51, 1957
27. Kennedy JH: *Analytical Chemistry*. New York, Harcourt Brace Jovanovich, 1984, p. 392–95
28. Oimomi M, Maeda Y, Hata F, Kitamura Y, Matsumoto S, Baba S, Iga T, Yamamoto M: Glycation of cataractous lens in non-diabetic senile subjects and in diabetic patients. *Exp Eye Res* 46:415–20, 1988
29. Kasai K, Nakamura T, Kase N, Hiraoka T, Suzuki R, Kogure F, Shimoda S-I: Increased glycosylation of proteins from cataractous lenses in diabetes. *Diabetologia* 25:36–38, 1983
30. Garlick RL, Mazer JS, Chylack LT, Tung WH, Bunn HF: Non-enzymatic glycation in human lens crystallin. *J Clin Invest* 74:1742–49, 1984
31. de Berardinis E, Tieri O, Polzella A, Iuglio N: The chemical composition of the human aqueous humour in normal and pathological conditions. *Exp Eye Res* 4:179–86, 1965
32. Cotlier E: The lens. In *Adler's Physiology of the Eye, Clinical Applications*. 7th ed. Moses RA, Ed. St. Louis, MO, Mosby, 1987, p. 277–303.
33. Pirie A: Epidemiological and biochemical studies of cataract and diabetes. *Invest Ophthalmol* 4:629–37, 1965
34. DiMattio J, Altszuler N, Ellis S, Zadunaisky JA: Glucose transport across ocular barriers of the streptozotocin-diabetic rat. *Diabetes* 30:903–906, 1981
35. Cunha-Vaz J, Faria de Abreu JR, Campos AJ, Figo GM: Early breakdown of the blood-retinal barrier in diabetes. *Br J Ophthalmol* 59:649–56, 1975
36. Waltman SR, Oestrich C, Krupin T, Hanish S, Ratzan S, Santiago J, Kilo C: Quantitative vitreous fluorophotometry: a sensitive technique for measuring early breakdown of the blood-retinal barrier in young diabetic patients. *Diabetes* 27:85–87, 1978
37. Augusteyn RC: Protein modification in cataract: possible mechanisms. In *Mechanisms of Cataract Formation in the Human Lens*. Duncan G, Ed. New York, Academic, 1981, p. 71–115
38. Srivastava SK, Ansari NH: Prevention of sugar-induced cataractogenesis in rats by butylated hydroxytoluene. *Diabetes* 37:1505–508, 1988
39. Simonelli F, Nesti A, Pensa M, Romano L, Savastano S, Rinaldi E, Auricchio G: Lipid peroxidation and human cataractogenesis in diabetes and severe myopia. *Exp Eye Res* 49:181–87, 1989