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Autofluorescence of the crystalline lens in early and late onset diabetes

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Abstract

Blue/green autofluorescence (excitation around 420 to 430 nm, emission around 520 nm) of the crystalline lens has been studied by an in vivo photographic method in two populations of diabetics and controls. The populations consisted of a geographically based survivor group of 161 mixed early and late onset diabetics (and 133 non-diabetic controls) and a second group of 104 early onset insulin dependent diabetics (and 138 non-diabetic controls), the latter all with non-cataractous lenses. Powerful associations ($p<10^{-6}$) were found between the presence of diabetes and increased lenticular autofluorescence in both populations. Among the mixed diabetics diabetic type was a significant factor after accounting for the effects of age and diabetic duration. In the early onset group (clear lenses) a powerful association existed between autofluorescence and diabetic duration (p=0·000011) after allowing for the effect of age, while in a subgroup of late onset diabetics with clear lenses this effect was modest (p=0·015). In the early onset diabetic group diabetic retinopathy (p=0·0064) was associated with increased lenticular autofluorescence after allowing for the effects of age and diabetic duration. In addition a powerful interaction between diabetic duration and the presence of diabetic retinopathy ($p<10^{-6}$) was found in this subgroup. Among the geographically based population of diabetics, increased nuclear brunescence was powerfully associated ($p<10^{-6}$) with increased autofluorescence after allowing for the effects of age, diabetic duration, and type of diabetes. This association was not found in the non-diabetic population. Non-enzymatic glycosylation of lens proteins should be considered as a possible mechanism of production of the fluorogen with emission around 520 nm.

Diabetes is an accepted risk factor for cataract formation[1-3] and it has been estimated that 12% of cataracts extracted in Britain can be attributed to this risk factor alone.[3] Cataract surgery occupies more than 40% of the surgical workload of ophthalmologists,[4] which implies that mechanisms of cataract formation in diabetes refer to more than 5% of all eye surgery. Study of these mechanisms by simple clinical methods can provide clues to more basic events taking place in the diabetic lens and could be of value in the development and testing of treatment strategies designed to diminish lenticular damage in the clinically and economically important disease combination of diabetes and cataract.

The adult human lens contains molecules that exhibit autofluorescence.[5,6] This phenomenon can be elicited at various wavelengths, some in the visible spectrum (blue/green fluorescence may be observed using the blue light of a standard slit-lamp biomicroscope, excitation around 420 to 430 nm, emission around 520 nm)[7-11] and some in the ultraviolet spectrum (UV/blue, excitation around 360 nm, emission around 440 nm).[6,8,12-15] In vitro 'purple' fluorescence (290/340 nm)[6,12] (probably due to tryptophan) and longer wave fluorescence, red (647/672 nm), near-red (568/633 nm), and orange (568/591 nm) have also been reported.[16] Autofluorescence of the lens increases with age in normal individuals[5,8,9] and is increased in nuclear cataract.[6,8,9,16,17] Blue/green fluorescence is increased in diabetes[7,10,11,18] and this increase has been noted to be duration dependent[11] and related to diabetic control.[18] The nuclear and perinuclear cortical regions of the lens show the most intense blue/green autofluorescence[9,11,19] and an assumption that a single blue/green fluorophor exists is supported on theoretical grounds by Beer–Lambert analysis of in vivo fluorophotometric scans.[20] The fluorogen responsible for the UV/blue fluorescence is closely associated with the yellow pigment of ageing and nuclear brunescent cataract,[17,21-23] and may be derived from tryptophan, possibly photochemically,[8,14] or by non-enzymatic glycosylation of lens proteins.[21,22,24,25] In diabetes fluorescent products of non-enzymatic glycosylation of 'browned' protein have been demonstrated, with excitation and emission maxima around 370 nm and 440 nm respectively.[26,27] The longer wavelength blue/green fluorogen (studied here) may be derived from the shorter wavelength fluorogen.[8,19]

Methods

STUDY POPULATIONS

Two groups of diabetics and controls have been studied.[28] Briefly the survivors of a geographically based population sample of diabetics[29] and controls, group matched for age and sex were recruited. These diabetics will be referred to as the 'mixed' diabetic population, the age distribution of these diabetics and controls being shown in Fig 1. The majority of the mixed diabetics were in fact late onset non-insulin dependent diabetics, and from this mixed group a subgroup of exclusively late onset diabetics was withdrawn for analysis. A second population of early onset diabetics with non-cataractous lenses was also recruited, these individuals being identified in a general diabetic outpatient clinic, a paediatric diabetic outpatient clinic, and an ophthalmological diabetic outpatient clinic. This second

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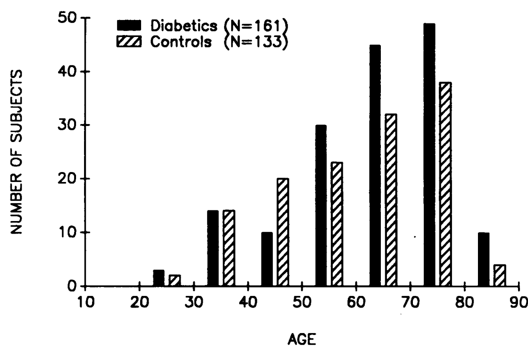

Figure 1 Age distribution of the 'mixed' diabetic population and non-diabetic controls.

population will be referred to as the 'early onset' diabetics. Early onset diabetes was defined as diabetes requiring insulin treatment from diagnosis in patients with an age of onset ≤30 years, and all others were regarded as late onset (non-insulin dependent) diabetics. The non-diabetic control population for the early onset group consisted of individuals with non-cataractous lenses selected from the above control group, supplemented by individuals with clear lenses and no anterior segment disease who were recruited from ophthalmological casualty and outpatient departments. The age distribution of the early onset diabetics and controls is given in Fig 2. (In the case/control comparisons between these diabetics and non-diabetics, statistical compensation for imperfect age matching was achieved by treating age, a potentially confounding variable, as a covariate.) Clear lenses were defined by the Oxford Clinical Cataract Classification and Grading system[30 31] as follows: Nuclear scatter and nuclear brunescence up to and including Grade 2 were allowed, spoke opacities and waterclefts of Grade 1 were allowed, but all lenses with any anterior or posterior subcapsular opacities were excluded. Minor lens changes such as isolated vacuoles, retrodots, and focal dots were permitted. For all participants a minimum of 2 weeks from exposure to fluorescein (intravenous or by drops) was considered reasonable for inclusion in the study.

SUBJECT EXAMINATION

Approval for the study was obtained from the Central Oxford Research Ethics Committee (ref no 1211). Following recruitment, informed verbal consent was obtained from each subject.

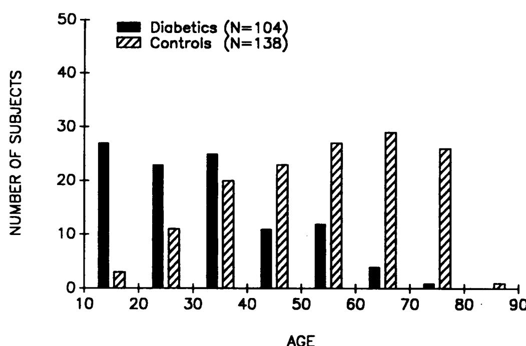

Figure 2 Age distribution of the early onset diabetic population and non-diabetic controls.

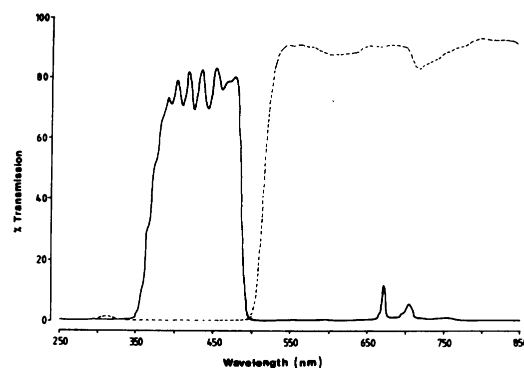

Figure 3 Transmission characteristics of the excitation (__) and barrier (....) filters used for recording lenticular autofluorescence.

All participants were examined and photographed by a single examiner (JS) at the Oxford Eye Hospital. The assessment consisted of the administration of a questionnaire and a full ocular examination (under mydriasis) with special reference to the lens of the eye. Lens changes were quantified by slitlamp examination using the Oxford Clinical Cataract Classification and Grading System,[30 31] and autofluorescence, Scheimpflug[32–34] and retroillumination[35] lens photography were performed at the end of the assessment.[28 36]

PHOTOGRAPHIC RECORDING OF LENTICULAR AUTOFLUORESCENCE

Lenticular autofluorescence was photographed using a slightly modified Zeiss anterior segment camera. The system contains a blue excitation filter (Balzers, interference type), in the illuminating beam, and a yellow barrier filter (Wratten 12) in the light path before the photographic emulsion. The filters were chosen in order to examine the visible range lenticular autofluorescence seen in lenses of diabetic subjects,[7 9–11] the transmission characteristics of the filters being illustrated in Fig 3. The fluorescence recorded has an excitation wavelength of around 420 to 430 nm, and an emission wavelength of around 520 nm. The arrangement of the filters was such that reflected blue light from the eye could not reach the light sensitive film, only light emitted by fluorescence was able to pass through the barrier filter and expose the photographic emulsion. A small amount of 'leaked' red light (between 660 and 720 nm) was able to pass through both filters. This 'contaminating' red light is seen in the image as a small specular corneal reflection of the flash light source (Fig 4). The spectral sensitivity of the black and white film used in this study (Ilford XP1) was low in the region of this contaminating red wavelength.

IMAGE ANALYSIS OF AUTOFLUORESCENCE PHOTOGRAPHS

The intensity of the lenticular autofluorescence was measured by examining digitally a rectangular sample of grey levels from the autofluorescence photographs as shown in Fig 4. The rectangle analysed covered part of the fluorescing lens and part of the background of the photographic image, care being taken to avoid

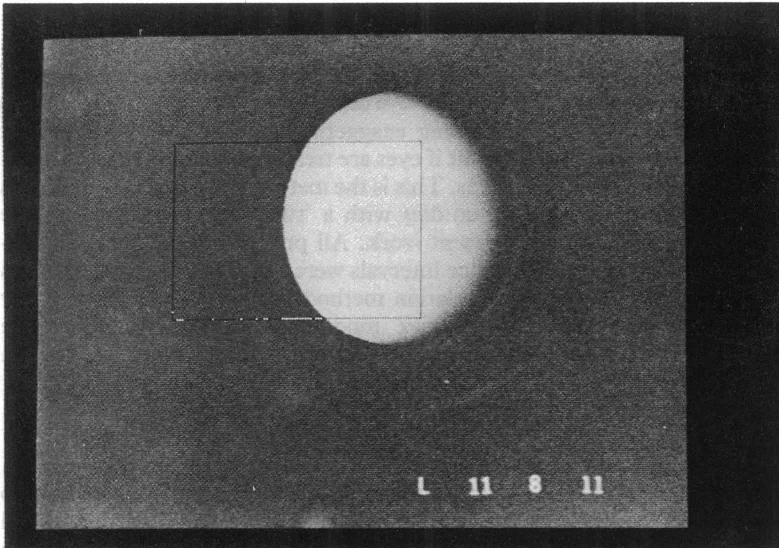

Figure 4 Autofluorescence image as seen on the video screen of the digital image analysis system. The rectangular 'area of interest' is the region used for making the fluorescence measurement. (Reproduced from Eye *with permission of the Editor.)*

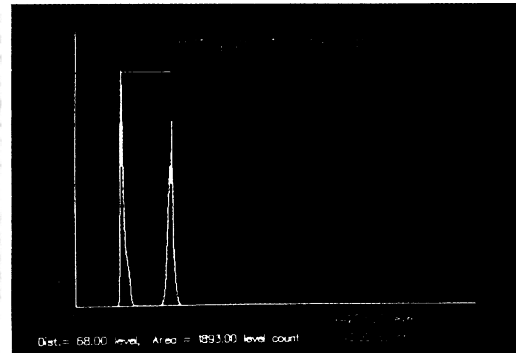

Figure 5 Histogram of the grey levels from the rectangular 'area of interest' indicated in Fig 4. The bimodal distribution represents the fluorescing lens (light peak) and the image background (dark peak). The measure (arbitrary units) is taken as the difference between the two peaks of the (standardised) histogram. (See ref 37 for details of image analysis system.) (Reproduced from Eye *with permission of the Editor.)*

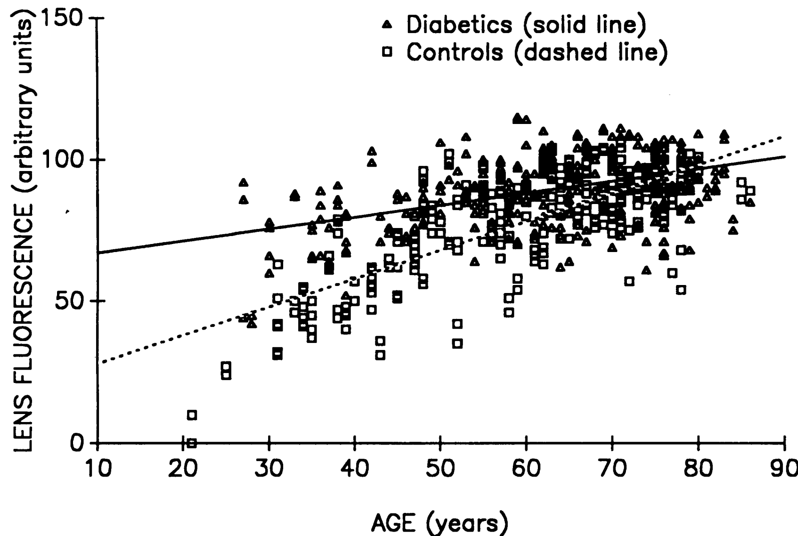

Figure 6 Plot of lenticular autofluorescence (arbitrary units) against age (years) for eyes of 'mixed' diabetics and controls. (See text for details of these populations.) Separate linear regression lines have been shown for diabetics and controls.

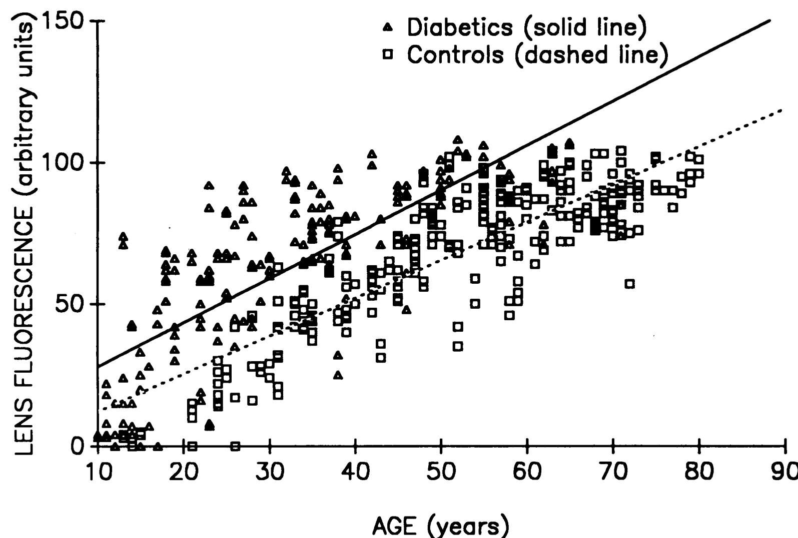

Figure 7 Plot of lenticular autofluorescence (arbitrary units) against age (years) for eyes of early onset diabetics and controls. (See text for details of these populations.) Separate linear regression lines have been shown for diabetics and controls.

the corneal specular reflex. The frequency distribution of the pixels across the grey scale within the rectangular 'area of interest' produced a bimodal distribution (Fig 5) the 'white peak' representing the fluorescing lens, and the 'dark peak' representing the background. The measure used for the intensity of the autofluorescence was the difference between the two peaks of the standardised histogram. The units of fluorescence were determined partly by technical constraints and partly for convenience resulting in a total range of between 0 and 130 arbitrary units. In a dilution experiment the fluorescence units were found to follow a sigmoid curve when plotted aginst the logarithm of fluorescein concentration (R^2=0·99 for third order polynominal regression, n=84 samples measured).[28] The linear portion of the sigmoid curve however lay within the range 10 to 110 units, which is the range covering the vast majority of measurements made in the present work (Figs 6 and 7). The details of the digital image analysis system have been described previously.[37]

REPEATABILITY OF THE AUTOFLUORESCENCE MEASURE

The repeatability of the method for measuring lenticular autofluorescence was assessed by examining repeat images of the same eyes taken at two separate photographic sessions several months apart. A population of differences between initial and subsequent assessments was obtained from analysis of 60 images of 30 eyes (half were left eyes and half were right eyes). The average difference between initial and subsequent measurements was negligible (0·37 fluorescence units). Assuming the 'true' value for a particular lens to be the mean of the two measurements, the standard deviation (SD) of the errors away from the 'true' value describes the accuracy of the method. Assuming a normal distribution (this was approximately true) there is a probability of 0·95 that any individual error away from the 'true' value will lie within the range ±(1·96 SD).[38] The relevant range for errors away from the 'true' value was ±15 fluorescence units. In order to ensure better

Table 1 Summary of p values for associations between lenticular autofluorescence and patient characteristics in the community based, mixed (early and late onset) diabetic population and non-diabetic controls. A subgroup analysis of exclusively non-cataractous lenses has also been presented*

	All lenses	*Clear lenses*
Diabetics and controls (early and late onset)		
Age	$<10^{-6}$	$<10^{-6}$
Sex	0·038	0·0042
Sta	$<10^{-6}$	$<10^{-6}$
Age.sta	$<10^{-6}$	$<10^{-6}$
R for model	0·71 (*n*=294)	0·75 (*n*=224)
Diabetics (early and late onset)		
Age	$<10^{-6}$	$<10^{-6}$
Sex	NS	NS
Dur	0·013	0·0054
Type	0·0028	0·0017
Dur.type	0·023	NS
Ret	NS	NS
Dur.ret	NS	0·043
R for model	0·53 (*n*=161)	0·63 (*n*=114)
Controls		
Age	$<10^{-6}$	$<10^{-6}$
Sex	NS	NS
R for model	0·75 (*n*=133)	0·77 (*n*=110)

*Probabilities calculated in a multivariate analysis by the intraclass correlation method of Rosner (see refs 39 and 40). (Model terms were fitted in the order in which they appear in the table.) Sta=diabetic or non-diabetic status; Dur=diabetic duration; Type=early or late onset type of diabetes; Ret=any diabetic retinopathy; Age.sta=interaction term between age and diabetic/non-diabetic status; Dur.type=interaction term between diabetic duration and type of diabetes; Dur.ret= interaction term between diabetic duration and retinopathy; *R*=generalised correlation coefficient; *n*=number of individuals in subgroup.

standardisation of the repeatability measure, the errors can be expressed as a percentage of the total dynamic range of the test within a given population of measurements (the coefficient of repeatability). By this measure there was a probability of 0·95 that any individual error would lie within the range ±13%.

STATISTICAL METHODS

A series of statistical model fitting exercises was employed. The method consisted of a multivariate extension of Rosner's intraclass correlation method.[39 40] This method takes account of the inter-eye correlation within individuals, thus avoiding exaggerated significance levels which result if eyes are treated as independent observations. This is the method of choice in the analysis of studies with a 'two eye' design such as the present work. All probability values and confidence intervals were calculated by this intraclass correlation method using the generalised linear interactive modelling (GLIM) system of the Numerical Algorithms Group (Wilkinson House, Jordan Hill Road, Oxford OX2 8DR).

Table 2 Summary of p values which illustrate differences in autofluorescence associations between late onset and early onset diabetic populations and controls. (See text for descriptions of these populations.) To facilitate comparability between the early and late onset groups, a separate analysis of exclusively clear lenses has been presented for the late onset group*

	Late onset		*Early onset*
	All lenses	*Clear lenses*	*clear lenses*
Diabetics and controls			
Age	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$
Sex	NS	0·031	NS
Sta	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$
Age.sta	$<10^{-6}$	$<10^{-6}$	NS
R for the model	0·72 (*n*=267)	0·77 (*n*=199)	0·82 (*n*=242)
Diabetics only			
Age	0·010	0·014	$<10^{-6}$
Sex	NS	NS	0·0032
Dur	0·042	0·015	0·000011
Ret	NS	0·027	0·0064
Dur.ret	NS	NS	$<10^{-6}$
R for the model	0·32 (*n*=136)	0·43 (*n*=91)	0·88 (*n*=104)

*Probabilities calculated in a multivariate analysis by the intraclass correlation method of Rosner (see refs 39 and 40). (Model terms were fitted in the order in which they appear in the table.) Sta=diabetic or non-diabetic status; Dur=diabetic duration; Ret=any diabetic retinopathy; Age.sta=interaction term between age and diabetic/non-diabetic status; Dur.ret=interaction term between diabetic duration and retinopathy; *R*=generalised correlation coefficient; *n*=number of individuals in subgroup.

Results

Among the mixed diabetic population and group matched controls there were 161 diabetics and 133 non-diabetics with at least one eye suitable for inclusion in the study. There were likewise 104 early onset diabetics and 138 controls (all clear lenses) in the second population. These main groups were divided into a number of subgroups, the numbers in each subgroup being indicated in the relevant summary tables (Tables 1, 2, and 4).

MIXED DIABETIC POPULATION AND CONTROLS

The first analysis (summarised in Table 1) examined the mixed diabetic population and controls. Age was clearly a very powerful determinant of lenticular fluorescence, with sex playing a relatively minor role. The variable effect of sex (greater fluorescence in males) was much reduced when the order of the terms of the model was altered such that sex was fitted after diabetic/non-diabetic status. The effect of diabetic status, after adjusting for the effects of age and sex was very powerful, and there was in addition a powerful interaction between age and diabetic status. These relationships are well demonstrated in Fig 6, where the regression line of the diabetics is on average above that of the controls (main effect) and the slope of the diabetic regression line is much less steep (interaction effect), with younger diabetics having a relatively greater excess of fluorescence than older diabetics. From the analysis of the diabetic subgroup it is apparent that after adjusting for the effect of age (sex non-significant) there were significant effects of diabetic duration and type of diabetes (early or late onset), with a moderate interaction effect between type of diabetes and diabetic duration. Among the controls, age was the only important determinant of lenticular autofluorescence. A subgroup analysis on clear lenses only gave similar results, with the duration by type interaction no longer reaching the 0·05 probability level, and a significant duration by retinopathy interaction term appearing.

LATE ONSET DIABETIC POPULATION AND CONTROLS

From the mixed diabetic population a subgroup consisting of the late onset only diabetics (and controls) was analysed. A summary of this analysis appears in Table 2. Comparing late

onset diabetics with controls demonstrated powerful effects of age and diabetic status, similar to those noted above for the mixed diabetic group and controls. Within the late onset only subgroups of diabetics (Table 2, left and middle columns), the age effect was far less important than that for the mixed (Table 1) and early onset (Table 2, right column) groups, and the models for late onset diabetics fitted the data less well (see R values, Tables 1 and 2). After adjusting for the relatively modest effect of age in the late onset groups, diabetic duration and retinopathy (in the clear lenses subgroup) were significant effects. There was no important interaction effect between diabetic retinopathy and duration. The powerful effect of age observed above in the combined late onset and controls groups was therefore due mainly to the effect of age amongst the controls (as documented directly in Table 1).

EARLY ONSET DIABETIC POPULATION AND CONTROLS

Analysis of the early onset only diabetics and controls (clear lenses) (Table 2, right column) demonstrated that age and diabetic/non-diabetic status were powerful determinants of lenticular autofluorescence. The interaction term between age and diabetic status however did not play a dominant role. These relationships are illustrated in Fig 7, where it is seen that the regression line of the diabetic subgroup lies well above that of the controls and further that the lines are approximately parallel (no interaction effect). The analyses on clear lenses for the early and late onset diabetic subgroups (Table 2, middle and right columns) illustrated important differences between these groups. The early onset diabetics demonstrated a powerful age effect, with sex, diabetic duration and diabetic retinopathy also having significant effects. The observed effect of sex disappeared ($p>0·05$) when this term was fitted after diabetic duration, which suggests that the apparent effect of sex may have been due to imperfect matching of diabetic duration between males and females (that is duration acting as a confounding variable). The effects of duration and retinopathy were substantially more powerful in the early onset group than in the late onset group, and in addition a powerful duration by retinopathy interaction term was confined to the early onset group. The model for the early onset group fitted the data well (clear lenses: $R=0·88$ for early onset; $R=0·43$ for late onset diabetics).

Table 3 Summary of regression slopes and effect sizes with 95% confidence intervals for diabetics (late onset and early onset) and controls. Late onset and early onset diabetic groups are contrasted, the analysis referring exclusively to non-cataractous lenses*

	Late onset	*Early onset*
Diabetics and controls		
Linear regression in groups for age and diabetic/non-diabetic status (main effects analysis)		
Age slope (effect per year)	0·9271±0·1443	1·092±0·1221
Diabetes effect (mean)	9·076±3·503	15·87±4·230
Subgroup analysis		
A. Simple linear regression for age		
Control subgroup		
Age slope	1·319±0·1253	
Diabetic subgroup		
Age slope	0·2413±0·2132	1·568±0·2483
B. Multiple linear regression for age and diabetic duration (diabetic only)		
(1) Allowing age effect (slope) to vary within model		
Age slope	0·1635±0·2440	0·8274±0·4092
Duration slope	0·4565±0·3430	1·193±0·5463
(2) Age effect (slope) fixed within model to that of relevant diabetic group		
Age slope	0·2413 (fixed)	1·568 (fixed)
Duration slope	0·4224±0·3308	0·3733±0·3236
(3) Age effect (slope) fixed within model to that of non-diabetic controls		
Age slope	1·319 (fixed)	
Duration slope	−0·05113±0·4908	0·6488±0·3136

*Probabilities calculated in univeriate/multivariate analyses by the intraclass correlation method of Rosner (see refs 39 and 40). Slope=effect of regression term (age or diabetic duration) per year.

Table 4 Summary of p values for associations between lenticular autofluorescence and cataract subtypes in the community based mixed (early and late onset) diabetic population and controls*

	BR	*WS*	*SC*	*PC*
Diabetics and controls (n=284)	$<10^{-6}$	0·35	0·19	0·78
Mixed diabetics (n=154)	$<10^{-6}$	0·31	0·02	0·53
Controls (n=130)	0·14	0·71	0·76	0·90

*Probabilities calculated in a multivariate analysis by the intraclass correlation method of Rosner (see refs 39 and 40). The associations between cataract subtypes and lenticular autofluorescence are indicated as probability values (p values). BR=nuclear brunescence; WS=white nuclear light scatter (clinical grading); SC=central nuclear light scatter (image analysis of Scheimpflug photographs); PC=percentage cortical/subcapsular cataract (image analysis of retroillumination photographs). Confounding variables were included in the model prior to fitting the cataract feature for each group as follows: Diabetics and Controls: Age, diabetic/non-diabetic status, and an interaction term between age and diabetic/non-diabetic status. Mixed diabetics: Age, diabetic duration, type of diabetes (early or late onset), and an interaction term between duration and type of diabetes. Controls: Age.

MAGNITUDE OF EFFECT FOR AGE, DIABETES, AND DIABETIC DURATION

The magnitude of some of the effects noted above are summarised in Table 3. This analysis was confined to an examination of clear lenses only which facilitates comparability between effects seen in the late onset and early onset diabetic groups. In the combined analysis of diabetics and controls (Table 3, upper section) the age slopes for the late onset and early onset groups (each combined with controls) were similar, but the overall effect of diabetes was on average a little greater in the early onset diabetics (that is main effects analysis). In the subgroup analysis the regression slope for age by simple linear regression (Table 3, item A) for the control group lay between the age slopes for the late and early onset diabetic subgroups. The magnitude of the age effect in the early onset subgroup was more than six times that of the late onset subgroup. In the multiple regression analysis where age and duration were allowed to vary independently (Table 3, item B1), the age effect per year in the early onset diabetics was five times that of the late onset group, and the effect per year of diabetic duration more than 2·5 times greater in the early onset group than in the late onset group. Because age and diabetic duration were correlated variables, the fitting of diabetic duration to this type of multiple regression model resulted in a distortion of the estimate for age. In order to avoid this two further analyses were performed in which the age effect was fixed in the model prior to the inclusion of the diabetic

duration term. In the first of these last two analyses the age effect was fixed to that of the diabetic group under consideration (late or early onset), and in the second of these the age effect was fixed to that of the non-diabetic controls. With the age effect fixed to that of the relevant diabetic group (Table 3, item B2) the magnitude of the additional effect of duration after accounting for the effect of age was similar in the early and late onset diabetics. With the age effect fixed to that of the non-diabetic control group (Table 3, item B3), there was no longer any effect of diabetic duration in the late onset group. In the early onset group however a substantial duration effect remained, amounting to approximately an additional 50% per year of the 'normal' ageing effect.

ASSOCIATIONS BETWEEN CATARACT SUBTYPES AND LENTICULAR AUTOFLUORESCENCE

Associations between cataract type and lenticular fluorescence were investigated in the community based mixed diabetic population and controls. The results of these analyses are summarised in Table 4. Confounding variables were included in the models prior to fitting the cataract type under examination. In the combined analysis of diabetics and controls, nuclear brunescence (pigmentation) (BR) was powerfully associated with lenticular fluorescence. Subgroup analysis demonstrated that this association was confined to the diabetics only and further that central nuclear light scatter (SC) as measured by image analysis of Scheimpflug photographs, was also associated with fluorescence within the diabetic subgroup. The association with nuclear scatter does not appear where the measurements have been made by a simple grading method (WS) which may reflect the fact that the grading method is much less sensitive to minor changes than Scheimpflug photography combined with digital image analysis.[30 31 37] No association was found with 'percentage cataract' (PC) as measured by digital image analysis of retroillumination photographs of the lens. The 'percentage cataract' represents a composite statement about the amount of cortical and subcapsular cataract present in a lens.[37]

Discussion

This study confirms the importance of age and diabetic status in determining the visible blue/green (excitation around 420 to 430 nm, emission around 520 nm) autofluorescence of the human lens.[7-11 18] Diabetic duration has been confirmed as an important determinant of blue/green autofluorescence.[11 18] The present work provides new information regarding the effect on lenticular autofluorescence of diabetes and diabetic duration in separate groups of early onset and late onset diabetics and controls. The 'clear lens' analyses presented highlight differences in the impact of diabetic duration on autofluorescence in early and late onset diabetes. In previous studies employing multiple linear regression no analysis has been presented in which the age effect of non-diabetics was imposed on a diabetic group to assess the independent effect of diabetic duration over and above the 'normal' ageing effect.[11 18] Here various methods of estimating the additional effect of diabetic duration after accounting for the effect of age have been presented and these illustrate the effect per year of diabetic duration and demonstrate differences between the early onset and late onset diabetics. The existence of diabetic retinopathy has been found to be of striking importance in early onset diabetics, while the effect of sex was variable. The reasons for the differences between the early and late onset diabetics are not clear and require explanation. Information on metabolic control was not available in this study, so it is not possible to comment upon the published finding that poor metabolic control is associated with increased blue/green autofluorescence.[18]

A powerful association between nuclear brunescence (pigmentation) and autofluorescence has been demonstrated, this effect being confined to the diabetic subgroup. Previous in vitro work has demonstrated increased lenticular fluorescence in the 520 nm emission region in brown nuclear cataracts but not in cortical cataracts, suggesting an association between the existence of the brunescent pigment and the blue/green fluorogen.[8 17] The present in vivo study confirms this association and, importantly, demonstrates that this effect was dominant among the diabetics in the population studied. A weaker association between autofluorescence and increased central nuclear light scatter as measured by image analysis of Scheimpflug photographs has also been identified in the diabetic subgroup.

Non-enzymatic glycosylation of lens proteins is known to occur both in diabetics and in normal individuals. The process increases with age and is increased in diabetes.[24 27 41] Glycosylation occurs at free amino groups with the formation of stable products ('browning'), and may be of importance in the normal processes of protein ageing.[21 22 42] Conformational changes following glycosylation result in unfolding of crystallins, allowing the formation of light scattering macromolecular complexes by oxidation of exposed thiol groups.[25 43 44] In cataracts from human diabetics specific changes of lens proteins,[45] and excess glycosylation products have been found.[41 46] 'Browned' glycosylation products of polypeptides have been shown to exhibit fluorescence at around 370 nm excitation and 440 nm emission.[26 27] Brown nuclear cataracts exhibit fluorescence at these wavelengths[6 8 12-15] but lenses are also known to fluoresce at other wavelengths,[7-11] with the most intense fluorescence in brunescent cataractous lenses in the 520 nm emission range[8 17] (as studied here). Previous workers have stressed the link between the pigment of nuclear brunescence and the UV/blue fluorogen (emission 440 nm).[6 12 13 21-23 26] Photochemical changes to tryptophan within lens proteins have been suggested as a possible mechanism of production of UV/blue 'fluorescent chromophores'.[8 47] UV/blue fluorescence of non-enzymatic glycosylation products of 'browned' proteins have however been demonstrated suggesting that protein derived 'fluorescent chromophors' in the lens nucleus are products of non-enzymatic glycosylation.[21 22 26]

Non-enzymatic fructation of proteins with the production of protein bound UV/blue fluorescence has been demonstrated, the generation of the fluorescent products from fructation being 10 times faster than from glycosylation.[48] Polyol degradation products could account for increased fructose in young diabetic lenses, although the activity of aldose reductase in lenses of adult diabetics is extremely low.[17]

The longer wavelength blue/green fluorogen may be derived from the shorter wavelength UV/blue fluorogen,[8 19] in which case brunescence and the two fluorogens could share a common mechanism of production. It is possible that the pigmented species in nuclear cataract are related to the fluorogen responsible for the blue/green fluorescence seen in diabetic lenses. In the present work, after accounting for confounding effects, there is a powerful association between lenticular fluorescence and brunescence (and to a lesser extent nuclear light scatter) in the diabetic subgroup, with fluorescence and brunescence each increasing with age and diabetic duration. Clearly other mechanisms could be active but non-enzymatic glycation must remain a strong contender as a possible mechanism of production of the fluorogen with emission around 520 nm, which itself may be linked to the pigment of nuclear brunescence.

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