Chapter 6

Spectral and Directional Reflectance of the Fovea in Diabetes Mellitus

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Submitted

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Abstract:
Aim: To assess the integrity of the photoreceptors in the fovea, and to measure the optical density of the macular pigment and the eye lens in patients with diabetes mellitus, and to compare the results with those of a group of healthy subjects. Methods: The directional and spectral properties of the light reflected from a 1.9 deg field centered on the fovea were measured simultaneously, in a single one second flash, with the Foveal Reflection Analyzer. The directional characteristics, i.e., the optical Stiles-Crawford effect, provided information on the integrity of the foveal photoreceptors. Model analysis of the spectral reflectance yielded optical densities of the macular pigment and the lens. Results: The amplitude of the directional reflectance in diabetic eyes was significantly lower compared to controls \((P < 0.001)\). Surprisingly, the directionality (a measure for the peakedness) was similar in diabetics and controls \((P = 0.3)\). The density of macular pigment was not different from that in controls.
The optical density of the lens increased with age in both groups, but the rate of increase was larger in the diabetics. \( P < 0.05 \). **Conclusion:** The integrity of the photoreceptors in the fovea was altered in diabetics. Possibly, the lens optical density increasing at a higher rate with age reflects changes preceding cataract formation.

### 6.1 Introduction

Diabetes mellitus is one of the largest threats to health in the western world\(^{132}\) and its incidence is expected to rise.\(^{132, 133}\) The main characteristic of the disease is an elevated blood glucose level.\(^{134, 135}\) Many if not all patients develop visual complaints. Major complications are the development of diabetic retinopathy and cataract.\(^{136}\) Diabetes causes vascular changes in the retina. These changes play an important role in the development of the retinopathy, and can be divided in two opposing effects: Capillary dilatation, which causes edema, and capillary occlusion, leading to ischemia.\(^{137}\) The amount of retinal edema is to be monitored closely after first detection. Common methods for assessment of retinal edema are fluorescein angiography, stereo fundus photography, and optical coherence tomography.\(^{137, 138}\) These methods are suitable for mapping the posterior pole and they facilitate general diagnoses.

Detecting retinal edema specifically in the macula, where it is most threatening to visual acuity, asks for dedicated techniques. Additional tests on the structural integrity of the foveal region could be useful for diagnosis and prognosis. DeLint *et al.* compared results on visual pigment density and the optical Stiles–Crawford effect and concluded that both provide information on the integrity of the cone photoreceptors in the fovea.\(^{6}\) Lardenoye *et al.* observed reduced visual pigment density and a decrease in the directionality of the optical Stiles–Crawford effect in eyes with macular edema.\(^{7}\) It must be noted that the etiology of the edema was not always diabetes, but also uveitis. Furthermore, the authors compared patient eyes with and without edema. Direct comparison of the optical Stiles–Crawford effect in diabetic versus healthy eyes is still lacking.

Retinopathy and the associated occurrence of retinal edema as discussed above are well known phenomena. Davies and Morland recently reported a hitherto unknown retinal abnormality in diabetes, *viz.* reduced levels of macular pigment.\(^{139}\) The pigment is a collection of the carotenoids lutein and zeaxanthin,\(^{8}\) and absorbs blue light with a peak absorption at 460 nm.\(^{25}\) It is located along the axons in the
center of the fovea, in front of the receptors, and as such acts as a preretinal filter for blue light.\textsuperscript{29,30} Several authors have suggested that macular pigment reduces the risk for age-related macular degeneration.\textsuperscript{9–11} For the macular pigment being reduced in diabetes, Davies and Moreland discussed three possible causes.\textsuperscript{139} First, the difference could result from a genetic influence, but this was considered unlikely. Second, diabetics might have a lower dietary intake of lutein and zeaxanthin, or a lower absorption in the gut. And third, as preferred by the authors, the reduction could point to a change in either the rate of deposition or removal of lutein and zeaxanthin in or from the retina. In that case, understanding of the deficit might be valuable in a broader context, \textit{i.e.}, in relation to age-related macular degeneration.

Diabetes also affects the anterior segment of the eye; it is a well known risk factor for cataract.\textsuperscript{140} During the phase preceding the appearance of cataract, which may take years, diabetic lenses are already abnormal in various aspects.\textsuperscript{141} One property, the transmission of light, can be inferred from the ratio of the autofluorescence in the posterior and anterior part of the lens.\textsuperscript{142–145} Zeimer \textit{et al.} and Mosier \textit{et al.} found no significant difference between diabetic eyes and healthy controls.\textsuperscript{142,145} Van Best \textit{et al.} observed a strong decrease in lens transmission of healthy subjects above the age of about 50 years.\textsuperscript{143} In a second paper, for a subgroup with duration of diabetics longer than 10 years, the steep decrease in transmission occurred about 15 years earlier.\textsuperscript{144} While two of the three of the autofluorescence studies did not report significant differences, two psychophysical studies have demonstrated a lower transmission at 420 nm in diabetes compared with age matched subjects.\textsuperscript{139,146} The autofluorescence method yields a transmission at approximately 500 nm. Possibly the transmission changes are limited to lower wavelengths and were not noticed.

In the present paper we tested three hypotheses in a group of diabetic patients: first, that photoreceptor integrity is affected, second, that macular pigment is reduced, and third, that the optical density of the lens is increased compared with age matched subjects. The patients were of both type I and II, diabetes duration was on average 19 years, and almost all patients showed signs of retinal pathology. In most studies, the retina and anterior segment are studied separately. Our study of such differing hypotheses was facilitated by a recently developed instrument: the Foveal Reflection Analyzer (Chapter 3).\textsuperscript{72} This apparatus is capable of simultaneous assessment of the spectral and directional properties of the light reflected from the retina. Model analysis of spectral reflectance provides optical densities of photo stable ocular absorbers such as the lens and macular pigment (Chapter 3).\textsuperscript{2,3,72} The directional characteristics exhibit the optical Stiles–Crawford effect (Chapter 3),\textsuperscript{16–20,72} which contains information on the integrity of foveal cones.\textsuperscript{6,7} The investigated properties of diabetic eyes were obtained in single snap shot measurements.


**Table 6.1**: Clinical data for the 14 diabetic eyes. *Age, gender, type of diabetes, duration of the disease and visual acuity were obtained from the medical record. †The presence of edema, small hard exudates, and ischemia in the measurement region (− absent, + present) was assessed from a fluorescein angiography and red-free and color photographs, obtained immediately after our investigations. ‡Availability or absence of the early phase (EP) recording (+/−). Without an early phase recording, the state of ischemia is uncertain. Three patients’ profiles and spectra are depicted with triangles§, squares¶, and crosses# in Figs. 6.1 and 6.2.

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### 6.2 Methods

#### 6.2.1 Subjects

The research followed the tenets of the Declaration of Helsinki and was approved by the local Medical Ethics Committee. Before testing, all subjects gave written informed consent after the nature and possible consequences of the study were explained. Diabetic patients were recruited randomly from all the patients who had an appointment for a fluorescein angiography consult in our clinic. Exclusion criteria based on the medical record were age above 65 years, correction outside the range −5 D to +3.5 D, cataract, visual acuity less than 0.2, large hard exudates within the
fovea, intra ocular lens implant, having other ocular or systemic diseases, or a history of allergic reactions on fluorescein. In total 14 eyes of 14 subjects were included; an overview of the clinical data is given in Table 6.1. In general, the right eye was selected. In a single case the left eye was measured. The patients’ ages, rounded to the nearest integer, ranged from 23 to 61 years, mean 46 years, with a standard deviation of 11 years. Both type I and II diabetics were included. Duration of the disease ranged from 6 to 28 years, mean 19 years, with a standard deviation of 6 years. Visual acuity as obtained with a letter projection system was taken from the medical record and can only serve as a rough indication. The presence of edema, hard exudates and ischemia in the measurement region was assessed by an ophthalmologist of our clinic from fluorescein angiography recordings, and red-free and color photographs. Table 6.1 also indicates whether an early phase (EP) recording was obtained for the included eye. The assessment of ischemia is hampered without an early phase recording. The control group consisted of 14 eyes of 14 age matched healthy subjects, without a history of eye disease and no complaints on visual acuity. They were aged 23 to 60 years, mean 47 years, with a standard deviation of 11 years.

6.2.2 Instrument

The instrument has been described in detail elsewhere (Chapter 3). A number of essentials are summarized below. The key element of the instrument was an imaging spectrograph with its entrance slit conjugate to the pupil plane. Spectral range of the spectrograph was 420–790 nm. The slit of the spectrograph defined a horizontal $0.8 \times 12$ mm exit pupil, placed over the dilated pupil of the subject. The coil of a 30 W halogen lamp was relayed to the pupil plane of the eye, defining a $0.8 \times 1.2$ mm entrance pupil. This small entrance pupil was placed centered with, and below the horizontal bar shaped exit pupil. Their separation was 0.7 mm. The intensity of the measuring light entering the eye was $1.10 \times 10^6$ Troland. A 2.8 deg diameter spot centered on the fovea was illuminated. Ametropia was corrected for with the front lens focus. The subject fixated on a central cross hair. The central 1.9 deg of the illuminated spot was sampled. A chin rest and temple pads connected to a headrest were used to maintain head position. A video channel was available for observation of both retina and pupil.

With the imaging spectrograph, a spectral image of the bar shaped field in the pupil plane was projected on the chip of a cooled CCD camera. For calibration purposes, reference spectral images were obtained from a diffuse reflecting surface painted with Eastman 6080 white paint and a dark black cloth in each session. For each pixel of the CCD, the counts in the measurement and reference spectral images
were converted to an equivalent percentage reflectance $R$, and an estimate of the error in reflectance. Prior to the calculation of reflectance, the images were binned and interpolated to 5 nm spectral and 0.1 mm spatial resolution. The array of reflectance values can be looked upon as the distribution of light within a narrow bar placed over the pupil, versus wavelength.

6.2.3 Protocol

The pupil of one eye in the controls was dilated with one or two drops tropicamide 0.5%. In the patients, for the purpose of the fluorescein angiography assessment following immediately after our investigations, the pupils of both eyes were dilated with tropicamide 0.5% and phenylephrine 5%. Both drops were administered a second time after ten minutes. Subjects were aligned with the instrument and instructed to fixate the cross wire at all times. The entrance beam was focused in the pupil plane by moving the headrest back and forth. Fixation and front lens focus were checked in the retinal image. We then searched for the maximum in the directional reflectance (i.e., the maximum of the Stiles–Crawford effect) using the measuring field. During the search, continuously directional profiles near 540 nm were read from the CCD. At that wavelength the directionality showed up prominently. In the horizontal direction in the pupil plane (along the spectrograph slit) the maximum position was readily observed in a profile plot on a computer display. The maximum in the vertical direction was found by manual search. While moving the headrest vertically, the actual profile was compared by eye to the highest profile till then. The search procedure took approximately 2 minutes. In this period, visual pigments were bleached away by the measuring light at approximately 97%. At the optimal entrance position 5 spectra were obtained. In some of the patients it was impossible to observe a directional component in the reflectance. In that case, the measurements were obtained near the center of the pupil. Integration time was set at 1.0 s. All patients underwent fluorescein angiography as part of their regular treatment immediately after our measurements. Before injection of the fluorescein, red-free and color photographs were obtained.

6.2.4 Data Analysis

The array of reflectance values obtained from the spectrograph can be looked upon as pupil profiles exhibiting the directional reflectance (i.e., the optical Stiles–Crawford effect) versus wavelength (Chapter 3). Only the profile at 540 nm was used. The array can also be interpreted as spectra versus location along the bar shaped exit pupil. We selected a single spectrum, at the location where the 540 nm profile
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had a maximum. In cases where no such maximum was present, the spectrum was obtained at the center of the pupil.

The spectra were fitted with the van de Kraats et al. fundus reflectance model using a least-squares method. Each data point was assigned a weight 1 over its error squared. Spectral data at wavelengths above 750 nm was discarded because the signal to noise ratio in this region was too low (Chapter 3). The fundus reflectance model describes radiation transfer in the eye with a limited number of reflecting, absorbing, and scattering layers. Spectral properties of the absorbers are taken from the literature. Eight parameters were optimized: reflectance from disks in the outer segments of the photoreceptors, at the inner limiting membrane, and at the cornea, the optical densities of melanin, macular pigment, and the aging component of the lens, the thickness of the blood layer, and a parameter accounting for scattering in the choroid. The parameter accounting for reflectance of the cornea was added to the model. Visual pigment density was assumed zero, Stiles–Crawford parameter SC was set at unity. The non-aging component of the optical density of the lens at 420 nm was fixed at 0.31. Values for other fixed parameters and a detailed description of the model are given in the original paper. The optical density of the lens is given at 420 nm and the optical density of macular pigment at 460 nm.

In healthy subjects, the reflectance versus location in the pupil plane can be described as a bell-shaped peak on top of a constant background (Chapter 3). Usually, two sharp drops at about 4 mm nasal and temporal are present, corresponding to the edge of the dilated iris. With pathology, the bell-shaped peak may disappear, leaving a block shaped profile. For both patients and controls, the distribution of reflectance versus location in the pupil plane was fitted with:

\[ R(x) = B \left( \exp\left[-r_1(x-x_L)\right] + \exp\left[-\exp[r_2(x-x_R)]\right] \right) + A 10^{-\rho(x-x_c)^2}, \quad (6.1) \]

where \( R \) is the percentage reflectance, \( x \) is the location in the pupil plane in mm, \( B \) is the non-directional background reflectance, \( A \) is the amplitude of the directional reflectance, \( \rho \) is a measure for the peakedness, \( x_c \) is the center position, \( x_L \) and \( x_R \) are the location of the left and right edge, and \( r_1 \) and \( r_2 \) are a measure for the steepness of the drop at the edges of the profile. The fitted data included a few points at level zero left and right to the pupil edges. As can be inferred from the equation, the left and right edge are modeled in terms of a growth curve from zero to \( B/2 \). All profiles were fitted with Eq. (6.1) using a least-squares method, with all data points assigned equal weights.

The observed amplitudes \( A \) and \( B \) are reduced due to absorption in the lens. The non-aging component of lens absorption is zero at 540 nm, and can be neglected. The same is true for macular pigment. A correction for light losses due to the aging
Figure 6.1: Percentage reflectance at 540 nm versus location in the pupil plane for three diabetic patients (triangles, squares, and crosses) and one healthy subject (circles). Nasal (N) and temporal (T) side are indicated. The solid lines represent model fits to the data with Eq. (6.1). The drop in amplitude of the directional reflectance in two of the patients (squares and crosses) indicates that the integrity of the photoreceptors was altered.

The aging component of the lens absorption was obtained from a model fit to the accompanying spectra. The corrected amplitude is given by:

$$A' = A10^{f_{\text{D}_{\text{lens}}-a}},$$

where $A$ is the amplitude of the directional reflectance as found in the original fit, $D_{\text{lens}}-a$ is the aging component of the lens density, and $f = 0.247$, the ratio of lens aging density at 540 nm to 420 nm.\textsuperscript{64} The same relation holds for $B'$ and $B$. Directionality and the maximum position are far less altered by absorption in the ocular media, although in theory the shape of the profile might be altered, even at 540 nm.\textsuperscript{41, 73} We neglected these minor differences.

### 6.3 Results

Four examples of a pupil profile exhibiting the directional reflectance from the fovea, i.e., the optical Stiles–Crawford effect, are shown in Fig. 6.1. The symbols depict the amount of reflected light versus location along a horizontal bar in the pupil. The edges of the dilated pupil can be recognized as the sharp drops at about 4 mm nasal
Figure 6.2: Spectral reflectance for the same subjects as in Fig. 6.1, three diabetic patients (triangles, squares, and crosses) and one healthy subject (circles). The solid curves are fits to the data with van de Kraats et al.’s model. Vertical lines (not discernable in most cases) indicate measurement errors. A low reflectance at the shortest wavelengths is indicative of a high optical density of the lens. For two patients (squares and crosses) reflectance was reduced between 540 and 580 nm, in accordance with their lowered directional reflectance at 540 nm (c.f., Fig. 6.1). The absorption edge of macular pigment near 520 nm was clearly present in all the spectra.

and temporal. The solid curves are model fits with Eq. (6.1). The circles represent data obtained from a healthy male control, aged 42 years. Reflectance showed a prominent maximum 1.4 mm nasal of the pupil center. The triangles represent a male type I diabetic subject, aged 42 years. Duration of his diabetic condition was 21 years and visual acuity was 1.0. Fluorescein angiography and fundus photography revealed macular edema, no ischemia in the fovea, and no hard exudates in or near the fovea. The pupil profile had a normal shape. The squares represent a male type II diabetic subject, aged 44 years. Duration of his diabetic condition was 21 years, visual acuity was 0.63. He had macular edema and ischemia in the fovea, but no hard exudates. The directional component of the reflectance was clearly reduced in amplitude. A female type I diabetic subject aged 58 presents a more extreme example (crosses). Duration of her diabetic condition was 27 years and visual acuity was 0.4. No edema or exudates were present in or near the fovea, there was no ischemia in the fovea.

Four reflectance spectra, for the same subjects as in Fig. 6.1, are shown in Fig. 6.2. Reflectance at the lowest wavelengths was substantially lower for two diabetic subjects (squares and crosses), indicative of absorption in the lens being higher.
Figure 6.3: (A) The amplitudes of the directional component $A'$ and (B) the diffuse background $B'$ corrected for absorption in the lens versus age in normal subjects (circles) and patients (squares). Symbols depict the mean of five measurements. Error bars indicate the standard deviation within a series of five measurements. The directional component decreased with age and was reduced in the diabetics group. The diffuse component increased with age and was higher in the diabetics group.

that the first diabetic subject (squares) and the control subject (circles) were of similar age. Absorption by the macular pigment caused a decline from 520 nm towards lower wavelengths. The absorption edge was clearly present in all four subjects. Between 540 and 580 nm a plateau was present. The level of reflectance in this wavelength region is a measure for reflectance of the photoreceptors. As expected, the plateau was reduced for the subjects with a decreased directional reflectance (squares and crosses).

Photoreceptor integrity was assessed from the profiles as were shown in Fig. 6.1.
The corrected amplitudes $A'$ and $B'$ for the directional reflectance and the non-directional background reflectance are plotted versus age in Figs. 6.3A and B for the diabetics (squares) and controls (circles). Differences between the two groups were assessed with univariate analysis of variance with age as a covariate. The directional reflectance $A'$ (Fig. 6.3A), adjusted for absorption in the lens with Eq. (6.2), decreased with $(9.4 \pm 3.4) \times 10^{-3}$ percentage reflection year$^{-1}$ ($P = 0.01$, regression parameters are presented as mean $\pm$ standard error). There was no significant difference in the slope for diabetics and controls ($P = 0.1$). The directional reflectance was lowered by 0.36 percentage reflection in the diabetics ($P < 0.001$). The non-directional background reflectance $B'$ (Fig. 6.3B), also adjusted for absorption in the lens, increased with $(11 \pm 4) \times 10^{-3}$ percentage reflection year$^{-1}$ ($P < 0.05$). There was no significant difference in the slope for diabetics and controls ($P = 0.3$). The background reflectance was 0.23 percentage reflection higher in the diabetics ($P < 0.05$).

Figures 6.4A and B depict the directionality $\rho$ and the position of the maximum of the directional reflectance $x_c$ versus age for the diabetics (squares) and controls (circles). The results of two diabetic eyes for $\rho$ were discarded, because they had virtually no directional component and $\rho$ could not be assessed with reasonable accuracy. Again, univariate analysis of variance with age was applied. Directionality (Fig. 6.4A) did not change with age ($P = 0.4$). For the diabetics mean $\rho$ was $0.15 \pm 0.07$ mm$^{-2}$, in the controls it was $0.17 \pm 0.04$ mm$^{-2}$, which was not significantly different ($P = 0.3$, population means are given $\pm$ the standard deviation). The position of the maximum (Fig. 6.4B) did not change with age ($P = 0.6$). The mean position was $0.56 \pm 0.89$ mm nasal in the diabetics and $0.95 \pm 0.56$ mm nasal in the controls. The mean position of the maximum and its standard deviation were not significantly different in the two groups ($P = 0.2$, Levine’s test for equality of variances: $P = 0.2$).

Analysis of the spectra as in Fig. 6.2 with van de Kraats et al.’s fundus reflectance model$^3$ provided, among other parameters, the optical density of the macular pigment at 460 nm and the aging component of the optical density of the lens at 420 nm. Both parameters are plotted versus age in Figs. 6.5A and B for the diabetics (squares) and controls (circles). Again, univariate analysis of variance with age was applied. Macular pigment (Fig. 6.5A) did not change with age ($P = 0.6$). For the diabetics mean macular pigment was $0.38 \pm 0.17$, in the controls it was $0.44 \pm 0.14$. This 14% reduction was not significantly different ($P = 0.3$). The optical density of the lens (Fig. 6.5B) in the controls increased with $(7.5 \pm 2.5) \times 10^{-3}$ year$^{-1}$ ($P < 0.01$). In the diabetics there was an additional increase of $(7.8 \pm 3.5) \times 10^{-3}$ year$^{-1}$ ($P < 0.05$). When corrected for age, the lens density in both groups was not significantly different ($P = 0.5$).
Figure 6.4: (A) The directionality of the directional component $\rho$ and (B) the position of the maximum reflectance $x_c$ for normal subjects (circles) and patients (squares). Nasal (N) and temporal (T) side are indicated. Symbols depict the mean of five measurements. Error bars indicate the standard deviation within a series of five measurements. Both the directionality and the maximum position were similar in normal and diabetic eyes.

6.4 Discussion

The strong reduction of the amplitude of the directional reflection in the diabetics group indicates changes in the integrity of the foveal cone-photoreceptors. Surprisingly, the directionality of the cones was not reduced. Macular pigment was not significantly lower in diabetics. Lens density increased at a substantially higher rate in diabetics, compared with the normal age-related increase in the controls.

The amplitude of the directional component $A'$ in the controls decreased with
Figure 6.5: (A) Macular pigment density (at 460 nm) and (B) the aging component of the lens density (at 420 nm) versus age in normal subjects (circles) and patients (squares). Symbols depict the mean of five measurements. Error bars indicate the standard deviation within a series of five measurements. Macular pigment density in the diabetic and normal eyes was not significantly different. The optical density of the lens in the controls increased with age in both groups, in the diabetics this effect was stronger.

age, even after correction for absorption losses in the lens. This indicates that the degradation of the receptors is a normal age-related phenomenon. The anatomical alterations in the cone outer segments in normal aged retinas reported by Marshall support this finding. The non-directional background $B'$ slightly increased with age. Possibly, we have applied an over-correction for absorption in the eye lens. In that case, the actual decrease of the directional component with age might be stronger than our present estimate. After correction for age, the amplitude $A'$ was
lower in diabetes. For as far as this reduction is of concern, we agree with Lardenoye et al., that the integrity of the cone-photoreceptors is altered in diabetes. This finding is also in agreement with Elsner et al., who found cone photopigment bleaching abnormalities in diabetes, and with Weiner et al., who observed changes in foveal cone electroretinography. In contrast to Lardenoye et al., we did not find a decrease in the directionality.

How can the alteration of the optical Stiles–Crawford effect in diabetes be understood? The first requisite for a normal directional reflectance is a precise alignment of the individual receptor cells toward a common location near the center of the pupil. Second, it requires a sharply peaked back reflectance from each single receptor. Whether considered a sign of the antenna or waveguide properties of the receptor cells, or a directional reflectance from the stack of the disks in the outer segments of the photoreceptors, this demands healthy photoreceptors. Because only the amplitude dropped, and because there were no major changes in the maximum position, a common tilt or random disruption of the orientation of all the receptors can be excluded. At least part of the receptor population was still commonly aligned near the center of the pupil. Because the cells are closely packed, it seems impossible that the cells still aligned, and those deviating, are mixed. As a first possible explanation for the reduced amplitude, we propose that groups of cells are deviating, while other groups remain aligned. Alternatively, the amplitude might also drop when a fraction of the cells no longer acts as a directional reflector, whatever the cause. Possibly, the age-related alterations in the outer segments as reported by Marshall, occur at an earlier age in the diabetics. However, the difference in age between the two linear regression lines was 38 years, a rather large value. It seems likely that diabetic retinopathy inflicts additional damage to the photoreceptors. Again, we can conclude on the basis of the high directionality that the cells are probably not massively affected. Recently, Roorda and Williams demonstrated assessment of the waveguide properties of single photoreceptors. It would be worthwhile to examine the affection of the photoreceptor reflectance in diabetes with this technique, to narrow down the possible causes of the abnormalities.

Although our results corroborate the findings of Lardenoye et al., a critical note can be made on their analysis of the directional reflectance, which also concerns the work of DeLint et al. They both applied a fit with a simpler version of Eq. (6.1), with a single parameter $B$ accounting for the diffuse background component (see also Gorrand and Delori, and Burns et al.) A fit with such an equation works well in healthy subjects, but becomes unstable when the directional part of the reflectance decreases. When this occurs, the amplitude of the directional component $A$ increases and fits the entire signal in the pupil center, while the diffuse background parameter
6.4. DISCUSSION

B decreases or may even be negative. The directionality becomes very low. DeLint et al. made a comment on this, but continued using the spuriously low directionality values. Lardenoye et al. quoted values for the directionality as low as 0.01 mm$^{-2}$, indicative of an unstable fit. Analysis of our profiles with the more elaborated model as given by Eq. (6.1) was stable in all cases; this improves interpretation of the pupil profiles. We note that the apparatus used in the aforementioned studies was also built in our laboratory. The new instrument applied in the present study offers a number of benefits. Instead of the formerly used bitebar, a simple chinrest suffices. Previously, a series of images were required, which makes the technique vulnerable to errors in fixation or slight head movements during the series. The new technique merely requires capturing a single reflection image, obtained in a second. Our results illustrate the potential of the new instrument as a fast test on photoreceptor integrity in a clinical setting.

The reduction of macular pigment was approximately 14%. This difference is small compared to the natural variation in macular pigment, and was not significant. Davies and Morland found a mean reduction of 59% in a population of 34 diabetics. Our much smaller reduction is probably not related to a lack of diabetic retinal complications in our population. The clinical data in Table 6.1 provide evidence that many patients suffered from retinopathy in the foveal region. Duration of diabetes ranged from 6 to 28 years, mean 19 years, with a standard deviation of 6 years; in Davies and Morland’s patients duration ranged from 2 to 38 years, mean 13.8 years, standard deviation 10.3 years. Searching for other sources of the discrepancy, we wonder whether their subjects were capable of performing a complicated psychophysical test. The experimental errors were large; for half of their diabetics the confidence interval included zero, and 8 patients and 4 controls were not included in the macular pigment test for reasons unknown. We conclude that if a reduction in macular pigment in diabetics exists, it is probably small.

The density of the lens in healthy subjects strongly correlated with age. In the normal controls, the increase in lens density was $(7.5 \pm 2.5) \times 10^{-3}$ year$^{-1}$, which is of the same order as the increase of $8.66 \times 10^{-3}$ year$^{-1}$ for subjects aged less than 60 years in Pokorny et al.’s aging model. In the diabetics, the increase in lens density was $(15 \pm 4) \times 10^{-3}$ year$^{-1}$, a factor of two higher. Lutze and Bresnick also found a faster increase in lens density in diabetics compared to normals. The increase in lens density in young diabetics was similar to that of their controls above age 60 years. They concluded that the aging mechanism in diabetics and elderly must be similar. We do not agree with this conclusion. In Pokorny et al.’s aging model, subjects over age 60 have an increase in lens density of $28.9 \times 10^{-3}$ year$^{-1}$, almost twice the rate we found in our diabetics. Possibly, other factors besides an acceler-
tion of the normal aging process exist. Pigments with other spectral signatures might accumulate in the diabetic lens, or scattering of light might increase. In conclusion, the increased optical density of the lens reflects early changes in the diabetic lens. Possibly, these early changes are predictive for cataract formation. They could serve to monitor the affection of the lens by the disease or as a marker in clinical studies.