Retinal Neurodegeneration in Diabetic Patients Without Diabetic Retinopathy

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Purpose. To compare the thickness of all retinal layers between a nondiabetic group and diabetic patients without diabetic retinopathy (DR).

Methods. Cross-sectional study, in which all subjects underwent an ophthalmic examination including optical coherence tomography. After automatic retinal segmentation, each retinal layer thickness (eight separate layers and overall thickness) was calculated in all nine Early Treatment Diabetic Retinopathy Study (ETDRS) areas. The choroidal thickness (CT) also was measured at five locations. Generalized additive regression models were used to analyze the data.

Results. A total of 175 patients were recruited, 50 nondiabetic subjects and 125 diabetic patients without DR, stratified into three groups according to diabetes duration: group I (<5 years, $n = 55$), group II (5–10 years, $n = 39$), and group III (>10 years, $n = 31$). Overall, groups I and III of diabetic patients had a decrease in the photoreceptor layer (PR) thickness, when compared with the nondiabetic subjects in six ETDRS areas ($P < 0.0007$). Patients with more recent diagnosis (group I) had thinner PR than those with moderate duration (group II). Interestingly, patients with longer known disease (group III) had the thinnest PR values. There were no overall differences in the remaining retinal parameters.

Conclusions. Retinal thickness profile is not linear throughout disease duration. Even in the absence of funduscopic disease, PR layer in diabetic patients seems to differ from nondiabetic subjects, thus suggesting that some form of neurodegeneration may take place before clinical signs of vascular problems arise.

Keywords: diabetic retinopathy, optical coherence tomography, retinal layers, neurodegeneration

Diabetes mellitus (DM) is increasing worldwide and, accordingly, diabetic retinopathy (DR) is the leading cause of legal blindness among working-aged adults.[1] Of 415 million people worldwide living with diabetes in 2015, more than one-third will develop DR in their lifetime.[2] More than 93 million people currently suffer some sort of eye damage from diabetes.[3]

In Portugal, the PREVADIAB study found a diabetes prevalence of 11.7%.[4] If "pre-diabetes" is also considered, then approximately one-third (34.9%) of the population aged 20 to 79 years is affected.[4] The RETINODIAB study, an epidemiologic study that determines the prevalence and progression incidence rates of DR based on a national screening community program in Portugal, identified a 16.3% prevalence rate of DR and a 4.6% incidence rate of any DR in the first year, in patients without retinopathy at baseline.[5,6]

The International Clinical Classification of DR is based in the observation of microvascular changes. The first recognizable vascular abnormalities are microaneurysms and small hemorrhages, followed by more severe signs of vascular leakage, such as hard exudates and larger hemorrhages; vascular dropout, such as cotton wool spots; and more widespread hemorrhages and neovascularizations.[7] However, retinal neurodegenerative changes have been described as including apoptosis of several populations of retinal cells (photoreceptors, bipolar cells, ganglion cells, and astrocytes) with consequent reduction in thickness of the different retinal layers, in the earliest stages of DR or even when DR cannot be detected by ophthalmologic examination.[8–11]

Recently, optical coherence tomography (OCT) has been introduced into clinical practice as the most noninvasive and objective method to visualize the retina, showing an amount of detail that resembles histological specimens.[12,13] Initially, OCT was applied to detect complications of DR (edema macular or epiretinal membrane).[14] Later on, it allowed quantitative and qualitative measurements of retinal thickness and segmentation of all intraretinal layers.[15–18] The new Spectralis spectral-

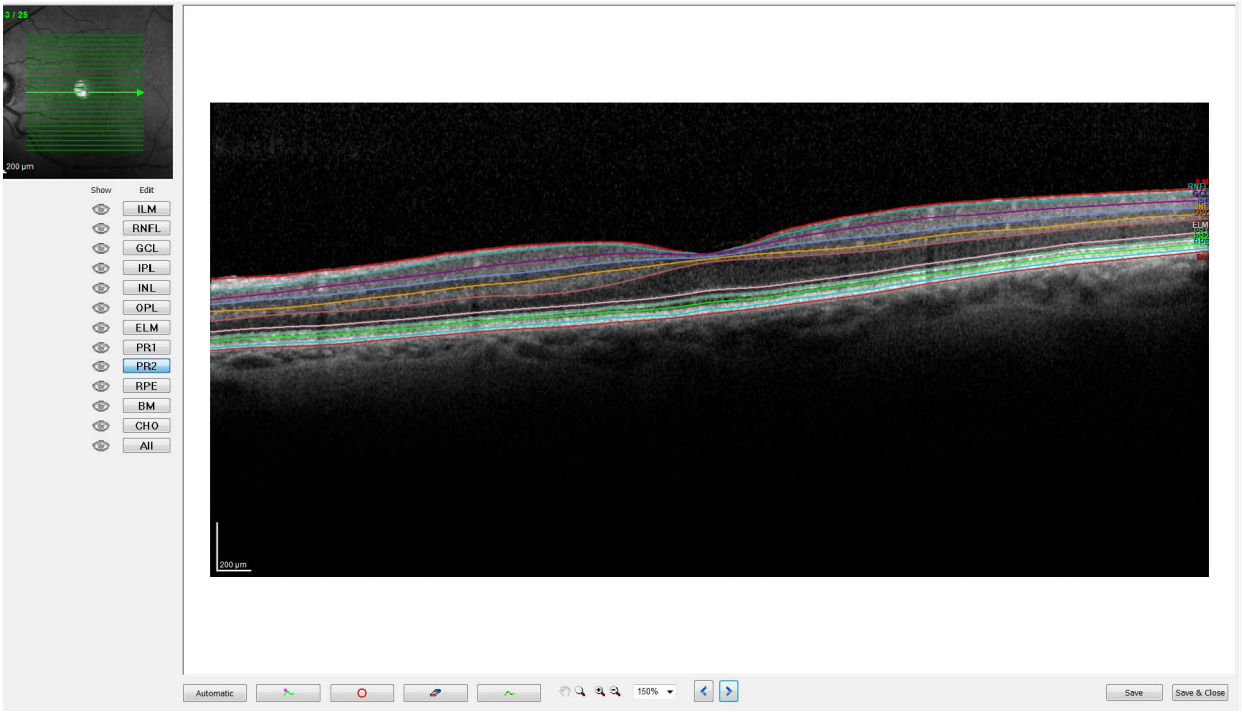

Figure 1. Retinal layer segmentation.

domain (SD)-OCT automatic segmentation software (software version 6.0; Heidelberg Engineering, Heidelberg, Germany) demonstrated excellent repeatability and reproducibility of each of the eight individual retinal layer thickness measurements.[19] Potentially, OCT might detect early retinal changes, and thus help define which diabetic patients may be at risk to develop DR. Ultimately, it could be used to plan preventive therapy before the development of vascular lesions detectable by ophthalmoscopy.[20] However, until now, the smaller scale, mostly pilot studies or only focusing on specific retinal layers on this topic in OCT image analysis did not show a temporal relationship between DM duration or arising DR and the changes observed in retinal layers.

The present study aimed to address this unmet need, by comparing the thickness of all retinal layers, measured with SD-OCT, between nondiabetic subjects and diabetic patients without DR.

Materials and Methods

Patients

This cross-sectional study was conducted at the Ophthalmology Department of the Central Lisbon Hospital Center, between October and December of 2014. Two groups of patients were recruited: group 1 with 50 nondiabetic subjects, and group 2, with 125 type 2 diabetic patients without DR, classified according to diabetes duration: group I (<5 years), group II (5–10 years), and group III (>10 years). Per protocol, the diagnosis of type 2 DM was made following the guidelines of the Portuguese General Health Direction.[21] The inclusion criteria were to be a type 2 diabetic patient without DR, with normotensive eyes, and with ability to understand the study. The exclusion criteria were the following: refractive error >5 diopters or/and axial length >25 mm in the studied eye, known diagnosis of DR or other retinal diseases, glaucoma or ocular hypertension, uveitis, neurodegenerative disease, and significant media opacities that precluded fundus imaging.

The study was approved by our institutional ethics committee and informed consent was obtained from patients. The principles of the Declaration of Helsinki were respected.

The ophthalmological examination included determination of best-corrected visual acuity with Snellen scale and after conversion to logMar, anterior segment examination, Goldmann applanation tonometry and dynamic contour tonometry with Pascal digital tonometer, indirect ophthalmoscopy, and ultrasonic biometry. Last, an SD-OCT was obtained and, randomly, one eye of each subject was included in this study.

Spectral-Domain OCT Imaging and Layer Segmentation

Tomographic images were obtained using the Spectralis SD-OCT (software version 6.0; Heidelberg Engineering), after pupillary dilation, by a single, well-trained technician (G.A.), as described previously.[22] Only good-quality scans with well-focused images, without overt misalignment, continuous scan patterns without missing or blank areas, without artifacts, and a signal strength better than 20 (40 = maximum) were included in the analyses. The fast macular thickness OCT protocol was performed with measurements 20 × 20-degree raster scans (consisting of 25 high-resolution scans). The automatic real-time function was set to nine frames per B-scan. An internal fixation light was used to center the scanning area on the fovea while the eye-tracking system was activated.

The new Spectralis automatic segmentation software was used to obtain individual retinal layer thickness measurements including overall retinal thickness (RT), retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), retinal pigment epithelium (RPE), and photoreceptor layer (PR) (Fig. 1). The OCT images obtained by a technician were assessed by an ophthalmologist (J.F.) masked to the patients' diagnosis, that verified the automatic segmen-

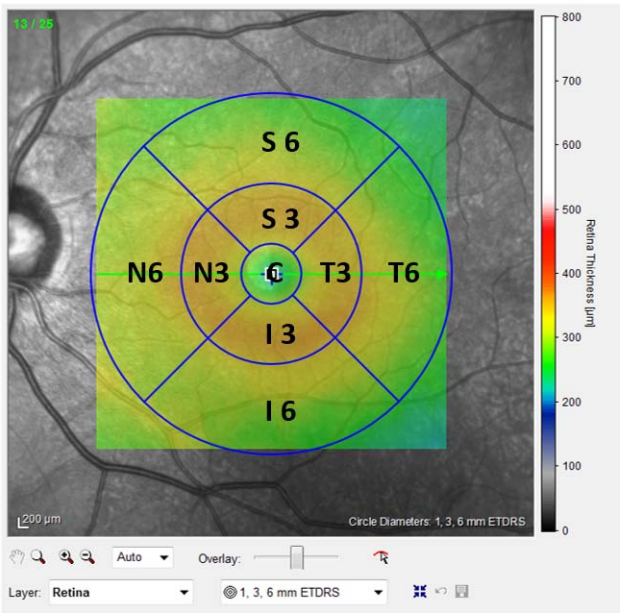

Figure 2. Representative Spectralis SD-OCT scans of macular thickness map (ETDRS protocol).

tation and corrected with manual segmentation when it was not defined correctly.

In all layers, the thickness values were calculated for the nine Early Treatment Diabetic Retinopathy Study (ETDRS) areas.[23] An ETDRS plot consists of three concentric rings of 1-, 3-, and 6-mm diameter centered at the fovea. The two outer rings are divided into quadrants by two intersecting lines. Each sector was designated C, S3, S6, T3, T6, I3, I6, N3, and N6, according to Figure 2. The ETDRS grid was positioned automatically by the Spectralis OCT software, enabling the capture and extraction of the macular thickness values.

The fast macular thickness OCT protocol scans were then again performed in enhanced depth imaging mode according to the previously reported method.[24] The choroidal thickness (CT) was manually measured from the outer portion of the hyperreflective line (corresponding to the RPE) to the hyporeflective line (corresponding to the sclerochoroidal interface). These measurements were made in the subfoveal choroid and at 1000 µm superior, inferior, nasal, and temporal of the fovea (five locations).

Statistical Analysis

Demographics and clinical characteristics of patients were described using the mean (SD) or median (interquartile range: 25th percentile–75th percentile) for continuous variables, and the frequencies (percentages) for categorical variables. Generalized additive regression models were used to identify the variables that explain the variability of thickness of retinal layers considering diabetic and nondiabetic groups. All the multivariable regression models included age, IOP–Pascal, axial length, and sex to adjust the association among the four groups, classified according to diabetes duration, and the layer thickness. The continuous covariates were modeled with splines due to their nonlinear association with the thickness of all retinal layers. In particular, multivariable regression models of RPE and PR layers in sectors C, S3, I3, N3, and T3, also considered the variable CT subfoveal, 1000 µm superior, inferior, nasal, and temporal of the fovea, respectively. Normality assumption of the residuals was verified using Kolmogorov-Smirnov goodness-of-fit test. A level of significance of $\alpha = 0.05$ was considered. Bonferroni adjustment for multiple testing was applied. Data were analyzed using the Statistical Package for the Social Science for Windows (released 2013, IBM SPSS Statistics for Windows, Version 22.0; IBM Corp., Armonk, NY, USA) and R (R: A Language and Environment for Statistical Computing, R Core Team, 2014; R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org.)

Results

Patient Demographics and Clinical Characteristics

A total of 125 diabetic patients with type 2 DM without DR (63 males), and 50 nondiabetic subjects (20 males), were included in this study. The diabetic patients were classified into three groups, according to the duration of diabetes: group I (up to 5 years, $n = 55$), group II (5–10 years, $n = 39$), and group III (more than 10 years, $n = 31$). The remaining demographic,

Table 1. Demographic and Clinical Characteristics of the Patients by Group

Clinical Characteristics	Diabetic Patients, $n = 125$			Nondiabetic Subjects, $n = 50$
	Group I, $n = 55$	Group II, $n = 39$	Group III, $n = 31$	
Diabetes duration, y	<5	5–10	>10	
Male sex, *n* (%)	26 (47)	23 (59)	14 (45)	20 (40)
Age, y	65.82 (9.88)	66.95 (10.14)	68.74 (6.97)	69.18 (8.55)
BCVA, logMAR	0.04 (0.07)	0.06 (0.13)	0.07 (0.10)	0.02 (0.05)
IOP–Goldmann, mm Hg	15.73 (3.08)	16.80 (2.98)	16.61 (3.14)	14.79 (2.76)
IOP–Pascal, mm Hg	19.03 (3.19)	19.02 (4.08)	19.07 (3.60)	18.25 (3.10)
OPA	2.80 (2.20–4.50)	2.50 (2.30–3.20)	3.20 (2.30–4.50)	3.10 (2.40–3.95)
Spherical equivalent	0.50 (0.00–1.50)	0.38 (−0.13–1.50)	0.50 (−0.38–1.63)	0.31 (−1.00–1.56)
Axial length, mm	23.01 (0.82)	23.16 (0.81)	23.22 (0.78)	22.51 (1.12)
State of lens–phakic, *n* (%)	53 (96)	37 (95)	29 (94)	41 (82)
Mean arterial pressure, mm Hg	97.00 (91.00–110.00)	97.00 (90.00–104.00)	99.00 (93.00–109.00)	97.50 (92.75–105.00)
HbA1c, %	6.20 (5.80–6.80)	6.40 (6.00–7.20)	6.80 (6.30–7.50)	–
Glycemia, mg/dL	125.00 (114.00–145.00)	142.00 (122.00–161.00)	153.00 (133.00–168.00)	–

Results are expressed as mean (SD) or median (interquartile range: 25th percentile–75th percentile). BCVA, best-corrected visual acuity; IOP, intraocular pressure; OPA, ocular pulse amplitude.

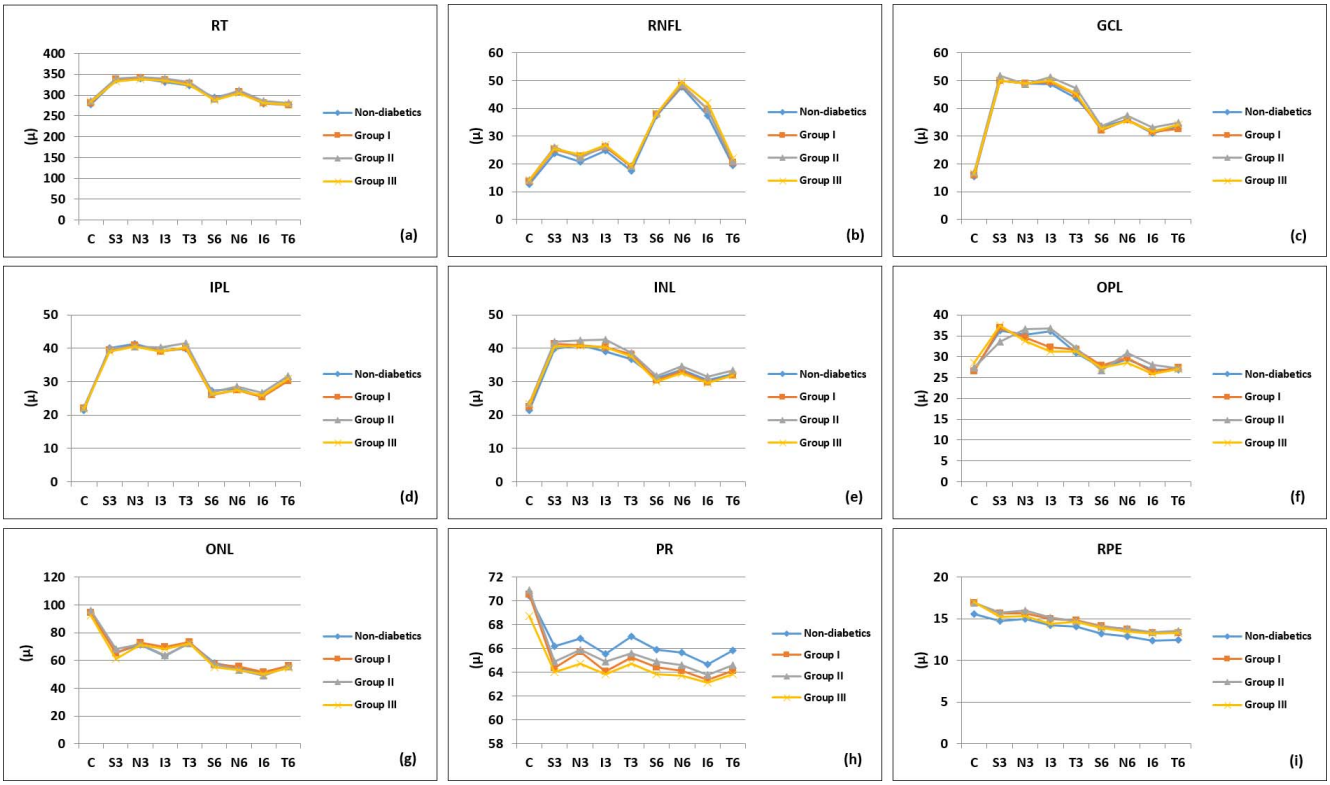

Figure 3. Graphs showing retinal layer thickness in all groups, determined automatically by SD-OCT in nine ETDRS areas in the macula. (**A**) RT; (**B**) RNFL; (**C**) GCL; (**D**) IPL; (**E**) INL; (**F**) OPL; (**G**) ONL; (**H**) PR; (**I**) RPE.

clinical and ophthalmologic characteristics are summarized in Table 1, and mean retinal layer thicknesses of groups are presented in Figure 3.

Analysis of Retinal Layer Thickness

In multivariable regression models, after adjusting for age, sex, IOP, and axial length, and correcting for multiple testing, no difference in the overall RT thickness throughout the ETDRS areas was found. Interestingly, the patterns of layer distribution were not the same in the two samples.

An exploratory analysis of the RT data showed a thicker RNFL, INL, and RPE in diabetic patients when compared with controls. This increase reached statistical significance in only a small number of locations (see detailed locations in Supplementary Tables S1–S3, for multivariable regression model results for RNFL, INL, and RPE thickness).

Interestingly, the PR layer was the most consistent finding, with a smaller thickness in diabetic patients when compared with their nondiabetic controls (Table 2). Nevertheless, the pattern of thickness in this layer differs with disease duration. Once we stratified diabetic patients according to this parameter, the thinner layers could be found in patients with both an early (group I) and longer known diabetes diagnosis (group III) ($P < 0.001$). On the other hand, the thinning in PR in diabetic patients with moderate duration (group II) did not reach statistical significance when compared with the healthy controls (Table 2).

The remaining layers (ONL, OPL, INL, and GCL) showed an overall tendency toward a thicker layer in diabetic retinas when compared with nondiabetic patients, but did not reach statistical significance.

Discussion

This cross-sectional study used SD-OCT to compare the retinal layer thickness between nondiabetic subjects and type 2 diabetic patients without DR and with different DM duration. Overall, the current analysis revealed a significant thinning of the PR layer in diabetic patients when compared with controls.

Photoreceptors are the most metabolically active neurons in the central nervous system,[25] although not usually regarded as important in the pathogenesis of early DR, perhaps due in part to the substantial distance between the photoreceptors and the retinal microvasculature that is affected by diabetes. However, a number of animal studies have reported that at least some photoreceptors degenerate in DM.[26–28] Furthermore, electrophysiology data suggest that photoreceptors and/or RPE also show variable impairments in diabetes.[29,30]

The vasculopathy of the choriocapillary layer that nourishes photoreceptors may be the cause of photoreceptor degeneration in diabetic patients. A diabetic choroidopathy in diabetic eyes without DR was identified in histological, animal, and human studies and characterized by changes in choroidal blood flow, impaired autoregulation,[31] and differences in the CT measured by OCT[32] accompanied by pathologic changes like degenerative capillaries and capillary dropouts[33] were described. Therefore, it is possible that these microvascular changes of the choroid may contribute to the photoreceptor degeneration described in this study. A further possible cause of photoreceptor degeneration may be the direct effects of hyperglycemia and hypoinsulinemia. Diabetes mellitus changes some elements in the insulin signalling pathway in the photoreceptors, impairing the important survival and neuroprotective signal.[34,35]

One interesting finding from our study was that the pattern of thinning PR layer was not uniform throughout disease

Table 2. Multivariable Regression Models Results for PR Thickness

Model*	Coefficient Estimate	*P*	95% Confidence Interval	
Dependent variable: PR layer thickness at sector S3				
CT	0.005	0.096	−0.001	0.011
Sex	0.339	0.373	−0.404	1.082
Group I	−2.096	< 0.001†	−3.067	−1.124
Group II	−1.582	0.004	−2.650	−0.514
Group III	−2.324	< 0.001†	−3.450	−1.197
Dependent variable: PR thickness at sector I3				
CT	0.000	0.880	−0.005	0.006
Gender	0.277	0.407	−0.375	0.928
Group I	−1.535	< 0.001†	−2.374	−0.695
Group II	−0.720	0.131	−1.650	0.211
Group III	−1.744	< 0.001†	−2.726	−0.762
Dependent variable: PR layer thickness at sector T3				
CT	0.005	0.181	−0.002	0.012
Gender	0.131	0.753	−0.684	0.946
Group I	−2.059	< 0.001†	−3.132	−0.987
Group II	−1.678	0.006	−2.854	−0.501
Group III	−2.448	< 0.001†	−3.684	−1.213
Dependent variable: PR layer thickness at sector N3				
CT	0.004	0.214	−0.002	0.010
Gender	0.525	0.180	−0.240	1.289
Group I	−1.311	0.010	−2.301	−0.321
Group II	−1.283	0.025	−2.390	−0.175
Group III	−2.048	< 0.001†	−3.203	−0.894
Dependent variable: PR layer thickness at sector S6				
Gender	0.354	0.290	−0.300	1.008
Group I	−1.685	< 0.001†	−2.531	−0.838
Group II	−1.229	0.011	−2.164	−0.295
Group III	−2.291	< 0.001†	−3.282	−1.301
Dependent variable: PR layer thickness at sector I6				
Gender	0.364	0.248	−0.250	0.978
Group I	−1.293	0.002	−2.091	−0.494
Group II	−0.842	0.062	−1.719	0.035
Group III	−1.456	0.003	−2.387	−0.524
Dependent variable: PR layer thickness at sector T6				
Gender	0.351	0.297	−0.307	1.009
Group I	−1.704	< 0.001†	−2.560	−0.848
Group II	−1.262	0.009	−2.202	−0.322
Group III	−1.984	< 0.001†	−2.981	−0.986
Dependent variable: PR layer thickness at sector N6				
Gender	0.582	0.050	0.004	1.160
Group I	−1.569	< 0.001†	−2.321	−0.818
Group II	−1.075	0.012	−1.900	−0.250
Group III	−1.920	< 0.001†	−2.797	−1.044

P values were obtained by generalized additive regression models.
* Reference categories: female sex and nondiabetic group.
† Statistical significance using Bonferroni correction.

duration, with patients with a moderate duration appearing to have a smaller difference in thickness than both early and longer known diabetes. This could be interpreted as a temporary cellular swelling due to a number of reasons, ranging from the diabetic-induced hypoxia,[36] oxidative stress with increased generation of superoxide, and other reactive oxygen species in the retina,[37] which induces the release of proinflammatory molecules and changes in retinal vasculature. Ultimately, the continuous cellular swelling is known to lead to a cellular atrophy,[36] potentially explaining the thinnest PR layers in the patients with longer disease duration. This nonlinear behavior is important, as it can explain the contradictory results in this field, as each study may be recruiting patients with a different disease duration. Additionally, it could be clinically relevant, as studies have suggested the importance of the PR layer in the development of DR, loss of PR reduced the severity of vascular degeneration in DR.[38,39] Further studies would be needed to interpret such findings.

The several clinical studies using SD-OCT to show changes that correspond to an early neurodegenerative process in DR typically analyzed the inner layers of the retina, and they either showed a decreased RNFL or GCL thickness in diabetic patients without DR[17,40,41] or did not find differences in any inner layer thickness between nondiabetic and type 1 or type 2 diabetic patients even without DR.[15,16] Vujosevic and Midena[17] studied both inner and outer layers but in opposition to this work they did not find any differences in the RPE and PR layer thickness. However, these authors have studied the RPE and PR layers together not individualizing them in two different layers.

This study had some limitations. First, despite being one of the largest studies in the field, including 125 diabetic patients without DR, subdividing into smaller groups for disease duration may have hampered our ability to subanalyze the RT. Nevertheless, our main outcome was the analysis of the overall RT between diabetic and nondiabetic subjects. Our interesting data regarding the subgroup analysis can provide a useful hint in future studies. Second, retinal measurements were done with automatic software. However, a manual correction was performed when the segmentation was inaccurate by an ophthalmologist masked to the patients' diagnosis. Third, our assumption for the length of disease duration is dependent on the clinical diagnosis, which may have underestimated the real time of diabetes.

In conclusion, diabetic patients without DR have a thinning of the PR layer, when compared with a nondiabetic group. There are early changes in outer retinal layers of diabetic patients even without clinical signs of DR that probably correspond to an inflammatory and apoptotic process of the retina as a neurovascular unit.

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