CHANGES IN TOTAL AND INNER RETINAL THICKNESSES IN TYPE 1 DIABETES WITH NO RETINOPATHY AFTER 8 YEARS OF FOLLOW-UP

ISABEL PINILLA, MD, PhD,† MIRIAM IDOIPE, MD, PhD,‡ LORENA PERDICES, BD, PhD,§ ANA SANCHEZ-CANO, OD, PhD,§ JAVIER ACHA, MD, PhD,*** MARIA ISABEL LOPEZ-GALVEZ, MD, PhD,†† NICOLAS CUENCA, MD, PhD,‡‡ EMILIO ABECIA, MD, PhD,‡ EMILIO ABECIA, MD, PhD,‡ EMILIO ABECIA, MD, PhD,‡ EMILIO ABECIA, MD, PhD,‡

Purpose: To evaluate changes in retinal layer thickness in patients with Type 1 diabetes with no diabetic retinopathy after 8 years of follow-up.

Methods: Ninety Type 1 diabetes and 60 control eyes were studied. Changes in the retinal nerve fiber layer, ganglion cell layer, and inner nuclear layer thicknesses in all Early Treatment Diabetic Retinopathy Study areas were evaluated.

Results: The mean ages were 42.93 ± 13.62 and 41.52 ± 13.05 years in the diabetic and control group, respectively. In 2009, total retinal thickness was higher in diabetic patients; differences were statistically significant in all except the nasal areas. In both groups, the mean foveal thickness remained the same during the 8 years. Among diabetic patients, there was a significant reduction in total retinal thickness in all areas excluding the outer temporal one; controls only in the inferior areas. The thickness loss was due to the thinning of the inner retinal layers (inner nuclear layer, ganglion cell layer, and retinal nerve fiber layer). The controls showed a significant diminution in the retinal nerve fiber layer and in the ganglion cell layer areas. The inner nuclear layer showed a diminution in the diabetes mellitus group.

Conclusion: Before the onset of diabetic retinopathy, Type 1 diabetes patients experience a diminution of their inner retinal layer thicknesses over time, supporting the hypothesis of retinal neurodegeneration.

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Although diabetic retinopathy (DR) is the major cause of visual loss in Type 1 diabetes patients, it is not the only cause of visual dysfunction. It has been proven that functional changes affecting the retinal cells exist before the microvascular changes. This dysfunction is secondary to a neurodegenerative process and to the loss of retinal neurons. Diabetic neurodegeneration has been diagnosed using different functional indicators including color perception, diminished contrast sensitivity, reduction in dark adaptation, electroretinogram changes such as reduction in oscillatory potential or abnormal multifocal pattern electroretinogram, abnormal electrophysiological tests, and defects in the retinal nerve fiber layer (RNFL) with concurrent defects in the visual field. Neuronal changes can be studied and quantified using spectral domain optical coherence tomography (SD-OCT) thickness and volume. Optical coherence tomography allows for the visualization of the retinal layers, their segmentation, and a quantitative mapping of the studied layer.

Neurodegeneration can be studied by looking for changes in the different neurons or in the RNFL. The loss of retinal neurons generates a secondary loss of the synaptic circuitry that transmits information to the central nervous system.

Various authors have demonstrated a diminution of retinal macular thickness before the development of DR, mainly due to a reduction of the ganglion cell layer (GCL)–inner plexiform layer and RNFL in both Type 1 and Type 2 diabetic patients. To the best of our knowledge, no one has yet demonstrated, in a retrospective study with a long follow-up time, inner retinal layer (IRL) changes in a diabetic population not
yet showing vascular changes. Sohn et al. showed that the loss of the RNFL and GCL–inner plexiform layer during 4 years of follow-up using Stratus OCT in a Type 1 diabetic population with no or minimal diabetic lesions correlates with similar findings in animal models and postmortem immunohistochemistry.

The purpose of our study was to assess IRL thickness changes in Type 1 diabetic patients with no retinopathy changes after 8 years of follow-up. The aim was to demonstrate the presence of a progressive neurodegeneration before the signs of DR.

**Methods**

One hundred twenty-two eyes from 122 diabetes mellitus (DM) patients with no DR were studied by SD-OCT in 2009. The eyes were reexamined by SD-OCT looking for changes in macular thickness. Patients were studied at the Miguel Servet University Hospital and Lozano Blesa University Hospital in Zaragoza, Spain. All of the procedures were conducted in accordance with the principles of the Helsinki declaration, and the experimental protocol was approved by the local ethics committee (CEICA). Detailed consent forms were obtained from each patient.

The criteria for inclusion in the diabetic group were a Type 1 diabetes diagnosis and no retinal changes evident by biomicroscopy. The control group included healthy age-matched subjects. All controls had a best-corrected visual acuity greater than 20/25 on the Snellen chart, with refractive errors between +3.75 and −6.75 diopters.

Exclusion criteria were the presence of any sign of DR or retinopathy of other origin, glaucoma, or intraocular pressure over 21 mmHg assessed by Goldmann tonometry, optic nerve pathology, ocular inflammation, or any ocular surgery or procedure including laser therapy, ocular traumatism, anterior segment pathology, or media opacification. All the diabetic patients were followed up by the endocrinology unit during this time, and blood samples were analyzed every 6 months. Arterial blood pressure and lipid values were maintained under extreme control.

All of the subjects underwent a complete ophthalmic exploration including medical, ocular, and family history, best-corrected visual acuity with the Early Treatment Diabetic Retinopathy Study (ETDRS) chart, slit-lamp examination, intraocular pressure measurement with Goldmann applanation tonometry, and fundoscopy examination.

Each individual was imaged using the Spectralis OCT (Heidelberg Engineering, Inc, Heidelberg, Germany) device. Volume fast macula scanning was performed. The subject was asked to look into the internal fixation target for measurement with Tru-Track eye tracking technology. A second examination (2017) was scheduled for follow-up. Spectralis OCT provides a circular macular map analysis, which is composed of 9 sectorial thickness measurements in 3 concentric circles, with diameters of 1, 3 (inner), and 6 (outer) mm, forming the 9 areas corresponding to the ETDRS. The following regional macular thicknesses were analyzed: fovea (1 mm, R1), temporal inner (T1), superior inner (S1), nasal inner (N1), inferior inner (I1), temporal outer (T2), superior outer (S2), nasal outer (N2), and inferior outer (I2). The Spectralis software version was 6.8.1.0. Segmentation was performed automatically by the device in both images; measurements included the inner and outer retina divided by the external limiting membrane (ELM) and the different IRLs including the RNFL, GCL, inner plexiform layer, and inner nuclear layer (INL) (Figure 1). The IRL thickness was measured from the internal limiting membrane (ILM) to the ELM, and the outer retinal layer (ORL) thickness was measured from the ELM to the Bruch membrane (BM). The scan quality and segmentation accuracy were assessed before the analysis, and scans with poor quality were rejected. The threshold for image quality was at least 25 over 40 dB.

All of the statistical analysis was performed using SPSS (SPSS 22.0, SPSS, Chicago, IL). Temporal parameters (2017 vs. 2009) were compared by the nonparametric Wilcoxon test, and the mean values by groups within the same year were analyzed by the Kolmogorov–Smirnov test. A $P$ value $<0.05$ was considered statistically significant.

**Results**

From the 122 Type 1 diabetes patients studied in 2009, 32 patients failed to complete the study (four of
them died from causes that were not related to their diabetes, all of them from hematologic neoplasms). In addition, 16 eyes developed retinal changes before 2017 and were excluded, and thus, 12 patients did not continue with the study. Ultimately, 90 eyes from 90 patients were analyzed. Sixty eyes from 60 normal age-matched subjects served as a control group. The mean age in the control group was 42.41 ± 13.56 years (range 26–68); the mean in the diabetic group was 41.52 ± 13.05 years (range 22–65). Female subjects accounted for 46.7% of the Type 1 diabetes group and 65% of the control group.

The mean length of diabetes evolution at the 2017 exploration time was 24.88 ± 8.42 years (range 9–40 years). The diabetic population showed a mean HbA1c value of 7.73 ± 1.22% in 2009 and 7.76 ± 1.06% in 2017. Blood pressure and lipid values were maintained in normal limits.

In 2017, ophthalmological evaluation of the control group showed 20.113 ± 0.097 logarithm of the minimum angle of resolution (logMAR) (26/20 Snellen) mean best-corrected visual acuity with 100% ETDRS chart, +0.21 ± 0.18 logMAR (12/20 Snellen) with 2.5% ETDRS chart, and +0.32 ± 2.27 logMAR (10/20 Snellen) with 1.25% ETDRS chart. Ophthalmological evaluation of the diabetic group showed −0.13 ± 0.11 logMAR (27/20 Snellen) with 100% ETDRS chart, 0.35 ± 0.16 logMAR (9/20 Snellen) with 2.5% ETDRS chart, and 0.35 ± 2.87 logMAR (9/20 Snellen) with 1.25% ETDRS chart. At follow-up, the mean intraocular pressure was 16.59 ± 2.27 mmHg and 16.82 ± 2.87 mmHg in the control and diabetic groups, respectively.

In 2009, total retinal thicknesses were higher in the diabetic group than in the control group; differences were statistically significant in all the areas (P < 0.05), including the central retina, except in the parafoveal nasal (N1). These differences were mainly due to IRL thickness, and in the ORL, there was only difference in I2. In 2017, the only areas in which statistically significant differences were found between the diabetic and control group were the central retina (R1) and in

Table 1. Total Retina Thickness, IRL (From ILM to ELM), and ORL (From ELM to BM) in DM vs. Controls, for the Two Time Points (2017 and 2009)

<table>
<thead>
<tr>
<th></th>
<th>Total Retina</th>
<th>IRLs (ILM–ELM)</th>
<th>Outer Retinal Layers (ELM–BM)</th>
<th>Total Retina</th>
<th>IRLs (ILM–ELM)</th>
<th>Outer Retinal Layers (ELM–BM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Foveal center (ETDRS central region: R1, 1 mm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Inner circle (ETDRS paracentral region: 3 mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior S1</td>
<td>0.005*</td>
<td>0.046*</td>
<td>0.884</td>
<td>0.030*</td>
<td>0.030*</td>
<td>0.143</td>
</tr>
<tr>
<td>Temporal T1</td>
<td>0.024*</td>
<td>0.045*</td>
<td>0.423</td>
<td>0.269</td>
<td>0.236</td>
<td>0.660</td>
</tr>
<tr>
<td>Inferior I1</td>
<td>0.009*</td>
<td>0.016*</td>
<td>0.766</td>
<td>0.276</td>
<td>0.250</td>
<td>0.977</td>
</tr>
<tr>
<td>Nasal N1</td>
<td>0.056</td>
<td>0.033*</td>
<td>0.649</td>
<td>0.213</td>
<td>0.109</td>
<td>0.947</td>
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<tr>
<td><strong>Outer circle (ETDRS pericentral region: 6 mm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior S2</td>
<td>0.006*</td>
<td>0.035*</td>
<td>0.363</td>
<td>0.004*</td>
<td>0.010*</td>
<td>0.939</td>
</tr>
<tr>
<td>Temporal T2</td>
<td>0.027*</td>
<td>0.053</td>
<td>0.460</td>
<td>0.259</td>
<td>0.070</td>
<td>0.989</td>
</tr>
<tr>
<td>Inferior I2</td>
<td>0.001*</td>
<td>0.003*</td>
<td>0.044*</td>
<td>0.009*</td>
<td>0.005*</td>
<td>0.051</td>
</tr>
<tr>
<td>Nasal N2</td>
<td>0.238</td>
<td>0.369</td>
<td>0.090</td>
<td>0.440</td>
<td>0.633</td>
<td>0.178</td>
</tr>
</tbody>
</table>

Statistically significant differences found between the DM group and control group are shown in bold and with an asterisk (*) for the ETDRS regions evaluated (foveal center, inner circle, and outer circle). Differences were mainly found in the IRL.
external vertical sectors (S2 and I2). These differences were located in the IRL (Tables 1 and 2).

Table 2 shows that the mean retinal subfoveal thickness remained the same during the 8-year follow-up period. The values for the control and diabetic group were 277.63 ± 17.96 μm versus 286.60 ± 23.90 μm in 2009, respectively, and 279.28 ± 16.36 μm versus 288.28 ± 28.59 μm in 2017, respectively.

In the diabetic patients, there was a significant reduction in total retinal thickness in all of the ETDRS areas excluding the outer temporal (T2) area; the control group showed a thickness decrease only in both inferior areas (I1 and I2). The thickness loss was mainly due to the thinning of the IRL that was significantly reduced in all areas of the diabetic patients excluding the central and outer temporal (T2) areas (Table 3), as reflected in the total retinal thickness values (Table 1). Globally, the thickness diminution was seen in RNFL, GCL, and INL, and the regions achieving statistically significant differences between evaluations are shown in gray on the map of the different ETDRS areas (Figure 2). The control group showed preserved IRL thickness in all areas except the outer vertical areas (S2 and I2), and ORL remained the same in all areas except S1 in controls and T2 in the DM group (Table 4).

Loss of GCL and RNFL was found in both diabetic and healthy subjects (Figure 2). Looking at the parafoveal central areas (N1, S1, T1, and I1) where the GCL bodies are located, the control group showed loss in both the S1 and I1 zones, and diabetic patients showed loss in all of these sectors. Looking at the RNFL in the perifoveal areas, where the fibers are predominantly located, the control group again showed loss in the I2 and S2 regions, as well as in N2, whereas the diabetic patients showed loss in the T2, I2, and N2 sectors.

The loss of the different layers by ring during the 8 years of follow-up was calculated. In the control group, the mean GCL loss was −0.076 μm/year in the parafoveal ring; no loss of RNFL was found in the perifoveal areas during this time. Inner nuclear layer loss in the control group was −0.053 μm/year in the perifoveal area with no changes in the parafoveal area. Diabetic patients lost −0.182 μm/year in the GCL of the parafoveal ring, and they did not show loss of the mean perifoveal RNFL. The INL of the diabetic patients lost −0.111 μm/year and −0.126 μm/year in the perifoveal and parafoveal areas, respectively.

The outer retinal layer (comprising the ELM and the retinal pigment epithelium) was mainly preserved in both groups during the 8 years of follow-up.

Table 2. Mean Values of the Total Retinal Thickness of the Areas Evaluated and Statistical Comparison of the Results by Group and Year

<table>
<thead>
<tr>
<th>Area</th>
<th>2009 Mean ± SD (Min–Max)</th>
<th>2017 Mean ± SD (Min–Max)</th>
<th>Control 2009</th>
<th>Control 2017</th>
<th>DM 2009</th>
<th>DM 2017</th>
<th>P Wilcoxon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foveal center (ETDRS)</td>
<td>286.60 ± 23.90 (224–346)</td>
<td>277.63 ± 17.69 (226–302)</td>
<td>288.28 ± 28.59 (237–393)</td>
<td>279.28 ± 16.36 (234–305)</td>
<td>0.118</td>
<td>0.043</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Inner circle (ETDRS)</td>
<td>327.54 ± 20.66 (261–364)</td>
<td>315.27 ± 18.61 (251–361)</td>
<td>328.80 ± 20.66 (261–361)</td>
<td>315.27 ± 18.61 (251–361)</td>
<td>0.004*</td>
<td>0.004*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Pericentral region: 6 mm</td>
<td>327.54 ± 19.28 (281–392)</td>
<td>315.27 ± 18.61 (251–361)</td>
<td>328.80 ± 20.66 (261–361)</td>
<td>315.27 ± 18.61 (251–361)</td>
<td>0.004*</td>
<td>0.004*</td>
<td>&lt;0.001*</td>
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<td>Outer circle (ETDRS)</td>
<td>327.54 ± 19.28 (281–392)</td>
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<td>0.004*</td>
<td>0.004*</td>
<td>&lt;0.001*</td>
</tr>
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</table>
| Statistically signifi cant differences were found in all described areas with P < 0.05.

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Discussion

In our study, we observed an early impairment of the neurosensorial retina in Type 1 diabetic patients with no DR signs, manifesting as a diminution of the total inner retina. At the beginning of the study, we observed that the diabetic group presented a thicker total retina due to an increase in IRL thickness, but these differences disappeared during the study follow-up. This increase in retina thickness has been explained by different theories: changes in the blood-retinal barrier, modification in different cell types such as endothelial cells or pericytes, Müller cells, or other glial cells; all these changes could be related to the hyperglycemia. Various authors have shown a reduced GCL in diabetic patients with no DR, both in Type 1 and 2 diabetes. Demirkaya et al found a loss of GCL and RNFL in a small number of diabetic patients with no signs of DR (n = 19). Scarinci et al demonstrated an early impairment of the neurosensorial retina with an increased INL yet a decrease in the GCL, in Type 1 diabetic patients with no changes or minimal impairment. In our patients, we found loss at the GCL level not only in the diabetic group but also in the vertical areas of the control subjects. Loss of thickness at the RNFL has been previously reported in patients with no or minimal retinal changes. In our retrospective study, we have demonstrated that Type 1 diabetic patients lose internal retina, including all IRL from the ILM to the ELM. Ganglion cell layers and their fibers are lost not only in diabetic patients before the onset of the retinopathy changes but also in healthy subjects in this age range after 8 years. Demirkaya et al demonstrated the loss in RNFL and GCL related to age using SD-OCT. Van Dijk used the SD-OCT algorithm (Iowa Reference Algorithm) to describe the same finding in Type 1 and 2 diabetic patients with no or minimal diabetic lesions. Looking at the parafoveal areas (N1, S1, T1, and I1) where most ganglion cell bodies are located, the control group showed GCL loss mainly in the vertical areas (S1 and I1); diabetic patients showed loss in all of the paracentral areas. The total rate of parafoveal loss in both groups was higher in the diabetic than in the control group (−0.182 μm/year vs. −0.076 μm/year). The retinal nerve fiber layer is located in pericentral ETDRS areas. Looking at these peripheral areas, both healthy and diabetic subjects showed fiber loss in almost all of the areas. Vertical sectors (S2 and I2) are the ones that are more affected in healthy subjects. The retinal nerve

<table>
<thead>
<tr>
<th>Table 3. Mean Values of IRL (From ILM to ELM) Thickness of the Areas Evaluated and Statistical Comparison of the Results by Group and Year</th>
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</thead>
<tbody>
<tr>
<td>DM</td>
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<tr>
<td>-----</td>
</tr>
<tr>
<td>Total Macular Thickness (μm)</td>
</tr>
<tr>
<td>Mean + SD (Min–Max)</td>
</tr>
<tr>
<td>Foveal center (ETDRS central region; R1; 1 mm)</td>
</tr>
<tr>
<td>Superior S1</td>
</tr>
<tr>
<td>Inferior I1</td>
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<tr>
<td>Nasal N1</td>
</tr>
<tr>
<td>Superior S1</td>
</tr>
<tr>
<td>Inferior I1</td>
</tr>
<tr>
<td>Nasal N1</td>
</tr>
</tbody>
</table>

Statistically significant differences were found in all described areas with \( P < 0.05 \).
The fiber layer is expected to be lost first at the nasal region of the peripheral (N2) area, but this finding was common to both the control group and diabetic patients. Using the total value of the RNFL in the 4 pericentral areas, we did not find any loss during the 8 years, not only in the control group but also in the diabetic group as well. The distribution of the RNFL in vertical areas with less fibers and more separation between them make them more susceptible to the damage, as it happens in other diseases. Park et al. found that both RNFL and GCL in the superior quadrants had a higher fiber and cell loss. They explained their findings were due to a lower perfusion in this area. The biggest change in our studied groups was at the INL level: In the perifoveal area, the loss was $-0.053 \mu m/year$ versus $-0.111 \mu m/year$ in the control and diabetic group, respectively. Only the diabetic patients had loss in the parafoveal area, at a rate of $-0.126 \mu m/year$, whereas the controls remained unaltered in this area. These changes in the INL could be related to the loss of the GCL as a sign of the retrograde degeneration.

Sohn et al. had similar findings in a smaller population including minimal retinal changes in a 4-year follow-up period. The strength of their study was the ability to correlate the OCT findings with the IRL thinning in a diabetic rodent model. Their results are provided using Stratus, a time-domain OCT. Spectral domain optical coherence tomography has a much better resolution of the retinal structures in these small values of the retinal layers.

The loss of the IRL could support the perifoveal capillary loss seen in both Type 1 and 2 diabetic patients before the onset of diabetic retinal changes. Dimitrova et al. showed a significant decrease in both the superficial and deep capillary plexus using OCTA in diabetic patients before the onset of lesions (mainly Type 2 diabetic patients). The foveal avascular zone was increased in the superficial plexus supporting the inner vascular changes. Vujosevic et al. showed this capillary loss in the superficial capillary plexus and an increase in the foveal avascular zone supporting the changes in the IRL. The authors showed a decrease in the inner retinal thickness values, but the results did not reach significant levels.

Subfoveal thickness is generated by cones and Müller cell bodies. It has no relation to the amount of ganglion cells. We did not find glial cell diminution.

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**Fig. 2.** Thickness maps showing differences in the RNFL, the GCL, and the INL in the different ETDRS areas corresponding to a right eye, with temporal areas on the left and nasal areas on the right, in DM and the control group. Significant changes after 8 years of follow-up are shown in gray ($P < 0.05$).
at this level. The absence of changes during the 8 years of follow-up in our study supports the hypothesis that the number of cones at the foveal level is well maintained during a long time interval. Other explanations could be the Müller cell activation or thickening of the retinal pigment epithelium that appears with age, but the mean age of our population was relatively young for this retinal pigment epithelium aging explanation.

The IRL is a part of the central nervous system. Changes in the central nervous system have been found in long-standing Type 1 diabetic patients with decreased functional connectivity and cognition, suggesting that chronic hyperglycemia can damage neural function with long-term diabetic status, as seen in our study.

Patients included in our study had good glycemic control. Other concomitant diseases that could affect the results were excluded. Lipid levels and arterial tension were extremely well controlled, eliminating potential confounders. None of the diabetic patients showed kidney dysfunction or peripheral neuropathy.

### Conclusion

In conclusion, before the onset of DR, Type 1 diabetic patients can present an increased retinal thickness due to IRL thickening. Patients experience a diminution of their retinal thicknesses over time, mainly in their IRL, supporting the hypothesis of retinal neurodegeneration in the diabetic patient.

**Key words:** diabetic retinopathy, ganglion cell layer, inner nuclear layer, inner retinal layers, neurodegeneration, ophthalmology, retinal nerve fiber layer, retinal thickness, spectral domain optical coherence tomography, Type 1 diabetes mellitus.

### References


