

1 Early microvascular and neural changes in Patients with Type 1 and Type 2 Diabetes
2 Mellitus with no Clinical Signs of Diabetic Retinopathy

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4 [Stela Vujosevic MD, PhD](#), ¹ [Andrea Muraca MD](#),¹ [Micol Alkabes MD](#), ¹ [Edoardo Villani](#)

Formattato: Italiano (Italia)

5 MD, ^{2,3} [Fabiano Cavarzeran ScD](#), ⁴ [Luca Rossetti MD](#),⁵ [Stefano De Cilla' MD](#), ^{1,6}

6

7 1. [University Hospital Maggiore della Carita'](#), Ophthalmology Clinic, Novara, Italy

8 2. [Department of Clinical Science and Community Health](#), University of Milan, Milan, Italy

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9 3. [Eye Clinic San Giuseppe Hospital](#), Milan, Italy.

10 4. [Department of Neuroscience](#), University of Padova, Padova, Italy

11 5. ~~Eye Clinic~~, Eye Clinic, University of Milan, San Paolo Hospital, Milan, Italy

12 6. [Ophthalmology Unit](#), Department of Health Science, University East Piedmont "A.

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13 [Avogadro"](#), Novara, Italy

14

15 Corresponding Author: [Stela Vujosevic MD, PhD](#), FEBO

16 [University Hospital Maggiore della Carita'](#), Ophthalmology Clinic,

Formattato: Italiano (Italia)

17 Novara, Italy

18 Corso Mazzini 36, 28100 Novara

19 Phone: +390321805401

20 Email: stela.vujosevic@gmail.com

21

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28

29 **Abstract:**

30 Purpose:To assess early modifications in inner retinal layer (IRL) thickness and OCT
31 angiography(OCT-A) parameters in patients with diabetes mellitus(DM) Type 1 and 2
32 without clinical signs of diabetic retinopathy (DR).

33 Methods:90 eyes of 90 subjects (24 Type 1 DM, 36 Type 2 DM and 30 healthy controls)
34 were prospectively evaluated with SD-OCT, swept-source OCT–A and color fundus photo
35 (on the same day). Retinal nerve fiber layer(NFL), ganglion cell layer(GCL+) and
36 NFL+GCL+ (GCL++)thickness were automatically determined by the instrument in the 1, 3
37 and 6 central millimeters. On OCT-A, the following parameters were evaluated: area of
38 foveal avascular zone(FAZ), number of microaneurysms, presence of: regular/irregular
39 FAZ, capillary loss and capillary network irregularities in the superficial(SCP) and deep
40 capillary plexuses(DCP).

41 Results:GCL++ was thinner in DM Type 1 versus controls within 3mm. GCL+ ($p=0.0099$)
42 and GCL++ ($p=0.0367$) were significantly thicker in DM type 1 vs DM Type 2 in 1 central
43 mm.The area of FAZ was significantly larger: in DM Type 1 vs controls in both SCP and
44 DCP and in DM Type 1 vs Type 2 only in DCP($p<0.05$ for all); the number of
45 microaneurysms was higher in DM Type 1 vs controls in both SCP and DCP ($p<0.01$ for
46 all); and in DM type 2 vs controls only in DCP($p=0.007$). Perifoveal capillary loss in SCP
47 and IRL thickness had the highest correlation in both DM types.

48 Conclusions:There are specific neural and microvascular modifications even before clinical
49 signs of DR in DM type 1 and 2. In DM Type 1,both SCP and DCP were affected, whereas
50 in DM Type 2 mostly DCP was affected. IRL thickness and perifoveal capillary loss in the
51 SCP had the highest correlation. This data may help in characterization of patients at
52 preclinical stage of DR.

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55 Introduction:

56 Diabetic retinopathy (DR) is the most important ocular complication in diabetes mellitus
57 (DM). ¹ Chronic hyperglycemia leads to increased oxidative stress, inflammation and
58 hypoxia, all inducing alterations of the neurovascular unit of the retina. ² The neurovascular
59 unit of the retina consists of retinal neurons and glial cells, endothelial cells, and pericytes.
60 ² All of these cell types undergo specific modifications induced by DM, even before the
61 onset of clinical signs of DR, as reported by the experimental studies in animals and
62 humans with DM. ³⁻⁸

63 With recent advent of non invasive retinal imaging techniques such as spectral domain
64 optical coherence tomography (SD-OCT) and SD-OCT angiography, changes in thickness
65 of single retinal layers as well as modifications of macular capillary network have been
66 evaluated in patients with DM with or without DR. A decrease in retinal nerve fiber layer
67 and ganglion cell layer has been reported in patients with DM, even without clinical signs
68 of DR. ⁹⁻¹³ Alterations of the macular capillary network (especially foveal avascular zone
69 enlargement and presence of areas of capillary loss in the perifoveal area in deep capillary
70 plexus and or superficial capillary plexus) have been reported in patients with DM without
71 clinical signs of DR, when compared to subjects with no DM. ¹⁴⁻¹⁶

72 There are very limited data on the direct evaluation and comparisons between
73 microvascular changes detected on OCT angiography and retinal layer thickness changes
74 in patients with DM and no clinical signs of DR. ^{15, 16} Dimitrova et al, evaluated changes in
75 deep capillary plexus (DCP) and choroid in patients with DM Type 2 and no clinical signs
76 of DR. ¹⁵ Simonett et al. evaluated a population of patients with DM Type 1 and no clinical
77 signs of DM. ¹⁶ In this study we aimed to compare changes in inner retinal layer thickness
78 and early microvascular changes detected on OCT angiography in patients with DM Type
79 1 and DM Type 2 and no clinical signs of DR and compare to healthy controls.

80 **Material and Methods:**

81
82 **Patients:**

83 This is a prospective, cross-sectional, comparative and consecutive case-control series of
84 90 subjects (90 eyes), consisting of normal subjects (no DM) and patients with DM with no
85 clinical signs of DR. All subjects underwent blood pressure measurement, anamnestic
86 collection of data including type of DM, value of haemoglobin A1c (HbA1c) complete
87 ophthalmologic examination with best corrected visual acuity determination (BCVA),
88 intraocular pressure (IOP) measurement, slit lamp fundus examination with 90D lens, color
89 fundus photo of the macula, spectral domain optical coherence tomography (SD-OCT),
90 and OCT angiography performed on the same day. Inclusion criteria were: patients with
91 DM (type 1 and type 2, confirmed by the Diabetologist) with no clinical signs of DR
92 determined on slit lamp fundus examination and confirmed on color fundus photo of the
93 macula; healthy subjects (control group) and patients with DM older than 18 years; no
94 history of ocular hypertension (IOP>21mmHg), glaucoma, uveitis, or neurodegenerative
95 disease (for example Alzheimer disease, Parkinson, etc...); patients/subjects that
96 accepted to participate. Major exclusion criteria were: previous intraocular treatment
97 (laser, intravitreal injections, vitreo-retinal surgery); cataract surgery within 6 months; any
98 antiinflammatory/steroids topical therapy; refractive error $\geq \pm 6D$; any stage of DR or
99 diabetic macular edema; and significant media opacity that precluded good quality fundus
100 imaging and examination. The study was conducted in accordance with the tenets of the
101 Declaration of Helsinki. All patients accepted to participate and signed the consent form.

102 **Visual acuity:**

103 Best-corrected distance visual acuity (BCVA) for each eye was measured by a certified
104 tester using standard ETDRS protocol at 4 m distance with a modified ETDRS distance
105 chart illuminator (Precise vision, Bloomington, IL). Visual acuity was scored as the total
106 number of letters read correctly (ETDRS score) and (also expressed in logMar).

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107 **Imaging:**

108 **Spectral domain optical coherence tomography and OCT-angiography:**

109 Spectral domain optical coherence tomography (SD-OCT) and OCT angiography were
110 performed using the swept source OCT, DRI OCT Triton plus (Topcon Medical Systems
111 Europe, Milano, Italy). This instrument uses a 1050nm wavelength, with a scanning speed
112 of 100.000 A-Scans/second. Following scan-patterns were performed: a linear B-scan
113 (12mm length) centered on the fovea at 0°; 3D Macula map covering central area of 7mm
114 x 7mm; and OCT-angiography maps covering central 3mmx3mm area and 6mmx6mm
115 area.

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116 **SD-OCT Segmentation and measurement:**

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117 DRI OCT Triton plus OCT instrument with software version 10.07.003.03 allows for
118 automatic segmentation of following retinal layers from 3D map: retinal nerve fiber layer
119 (NFL) from inner limiting membrane (ILM) to NFL; ganglion cell layer (GCL)+ complex –
120 from NFL/GCL interface to inner plexiform layer (IPL)/inner nuclear layer (INL); GCL++
121 complex- from ILM/NFL interface to IPL/INL; outer retina- from IPL/INL interface to
122 interdigitation zone (Verhoeff's membrane); ¹⁷ (full) retina thickness- from ILM to
123 interdigitation zone. Automatic evaluation of the specific retinal layer thickness and full
124 retinal thickness was performed in 9 ETDRS areas and evaluated as (central subfield
125 retinal thickness (CSF) – circular area with 1 mm diameter centered on the fovea; inner
126 rings (mean value of 4 inner quadrants with diameter of 3mm; and outer rings (mean value
127 of 4 outer quadrants with 6 mm diameter). After automated segmentation, each scan was
128 checked for the presence of segmentation errors, and in that case a manual correction
129 was performed.

130 **OCT angiography scans evaluation:**

131 OCT angiography (OCT-A) scans obtained within 3mmx3mm were used for both
132 quantitative and qualitative evaluation. All evaluations were separately performed at the
133 level of SCP and DCP. The built-in software segmentation algorithm was used to define
134 SCP and DCP on *En face* OCT angiograms. The SCP slab was obtained from the ILM to
135 15.6 micron above the IPL/INL interface. The DCP slab was obtained from the IPL/INL
136 interface (above 15.6 micron) to the IPL/INL interface (below 70.2 micron). Only good
137 quality OCT-A images were considered for analyses, excluding those that had presence of
138 artifacts such as double vessel pattern, dark areas from blinks, or motion artifacts, and
139 those with signal strength index below 50.

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140 Following parameters were evaluated: 1) area of the foveal avascular zone (FAZ)
141 delimited using the tool caliper area available within software IMAGENET 6 (Version
142 1.17.9720, Topcon Medical Systems, Inc, Oakland, NJ). After manual delimitation of the
143 FAZ , the software automatically calculates the area in square milimeters (mm²); 2)
144 number of microaneurysms counted within 3mm area; 3) presence of regular (oval or
145 round shaped with regular and clear contour) ¹⁸ or irregular (asymmetrical) FAZ 4)
146 presence of area of capillary loss/reduced capillary density; 5) alterations of the capillary
147 network (presence of tortuosity and/or beadings).

148 All measurements and evaluations were performed by two graders, independently,
149 masked to the clinical data of patients, on 3 mm scan area, zooming the image in order to
150 have greater magnification and evaluate more easily details, and (if necessary) using also
151 the reverse mode (in which vessels are black and the background is white, for better
152 definition of details). In case of disagreement the experienced medical retina specialist
153 made a final adjudication.

154 **Statistical methods:**

155

156 The summary of parameters has been made by means of usual methods used for
157 descriptive statistics: mean value, standard deviation, and range for continuous numeric
158 variables; frequency distribution, absolute and relative (percentage), for qualitative
159 variables.

160

161 Sample characteristics (age, systolic pressure, diastolic pressure, HbA1c, BCVA, and IOP)
162 have been compared between patients with DM and controls by means of t-Student test.
163 Comparisons among groups (DM Type 1 and DM Type 2, and controls) have been made
164 by means of one-way Analysis of Variance (ANOVA) followed by post-hoc Bonferroni test
165 for multiple comparisons.

166 OCT parameters:

167 Mean values of retinal thickness (full retinal thickness, NFL thickness, GCL+ thickness,
168 GCL++ thickness, and Outer Retinal thickness) have been compared between patients
169 with DM and controls, and among DM type 1, DM type 2, and controls by means of three
170 factor with interaction ANOVA for repeated measures analysis, adjusted for age.
171 Comparison between DM Type 1 and DM Type 2 has been adjusted also for the duration
172 of DM. Retinal thickness determined in the 9 ETDRS quadrants was summarized into
173 three values referred as CSF (central sub-field quadrant), inner ring (average value of
174 inner superior, inner nasal, inner inferior, and inner temporal quadrant values), and outer
175 ring (average value of outer superior, outer nasal, outer inferior, and outer temporal
176 quadrant values). Factors of the model were Group (patients with DM and Controls in one
177 analysis, DM Type 1, DM Type 2, and Controls in the other), Sector (CSF, inner ring, and
178 outer ring), and the interaction Group by Sector. However particular interest has been
179 placed to significance of Group factor (which tested differences among overall mean
180 retinal thicknesses) and to interaction factor (which tested differences among retinal
181 thickness profiles). Comparisons have been adjusted for patient's age and in case of

182 significant result, post-hoc tests for multiple comparisons with Bonferroni correction were
183 applied.

184
185 **OCT Angiography parameters:**

186 Mean values of FAZ area extension and number of microaneurysms in the SCP and DCP
187 have been compared among groups (patients with DM vs. controls, and DM Type 1 vs.
188 DM Type 2 vs. controls) by means of one-way ANOVA analysis adjusted for patient's age
189 and blood pressure and followed by Bonferroni post-hoc test for multiple comparisons.

190 Association between Group and presence of regular/irregular FAZ, presence of area of
191 capillary loss/reduced capillary density, and presence of tortuosity/beadings have been
192 assessed by means of logistic regression model adjusted for patient's age and blood
193 pressure. Association was expressed in terms of Odds-Ratios and relative 95% Wald
194 confidence interval.

195
196 **Correlations among OCT and OCT Angiography parameters:**

197 Multiple linear regression models with stepwise selection of independent variables have
198 been used to assess the relationship between retinal thickness (dependent variable) – one
199 model for each parameters: full retina thickness, NFL layer, GCL+, GCL++, and Outer
200 retinal thickness – and OCT Angiography parameters. Patient's age and blood pressure
201 (systolic and diastolic) were taken into account. Three models have been estimated, one
202 for each of the following sectors: CSF, inner ring, and CSF plus inner ring. Qualitative
203 variables have been coded as follows before entering the model: FAZ regularity,
204 0=regular, 1=irregular; Capillary loss, 0=absent, 1=present, Tortuosity/Beadings,
205 0=absent, 1=present.

206
207 All the analyses have been performed by means of SAS® v.9.3 (SAS, Cary, NC) statistical
208 software. All statistical tests have been considered significant when $P < 0.05$.

209 **Results:**

210 Of 90 examined subjects (90 eyes), 60 were patients with DM with no clinical signs of DR
211 and 30 were healthy subjects (with no DM). Of 60 patients with DM, 24 patients had DM
212 Type 1 and 36 patients had DM Type 2 (of which 9 patients were on insulin whereas 27
213 were oral hypoglycemic drugs). Systemic and ocular findings (mean age, HbA1c, duration
214 of DM, blood pressure, BCVA and IOP values) are reported in Table 1.
215

216 Table 2 shows mean values of retinal thickness (automatically measured by the
217 instrument) of full retina, NFL, GCL+ complex, GCL++ complex and outer retina divided by
218 rings (CSF, inner ring and outer ring) and groups (normal subjects, all patients with DM
219 and separately DM type 1 and DM type 2). After adjusting for age there was a different
220 thickness profile in GCL++ complex among three examined groups (control, patients with
221 DM type 1 and DM type 2), $p=0.049$, (ANOVA) in the three different rings. Such a model
222 estimated that GCL++ complex in the inner ring was thinner in patients with DM Type 1
223 ($114.9\mu\text{m}\pm 8.3$) versus controls with borderline statistical significance ($118.0\mu\text{m}\pm 8.2$,
224 Bonferroni post-hoc test for multiple comparison, $p=0.0981$).

225 After adjusting for age and DM duration, GCL+ complex ($49.4\mu\text{m}\pm 7.3$ vs. $43.6\mu\text{m}\pm 9.6$,
226 $p=0.0099$) and GCL++ complex ($57.2\mu\text{m}\pm 10.8$ vs. $50.5\pm 10.2\mu\text{m}$, $p=0.0367$) were
227 significantly thicker in patients with DM type 1 vs DM Type 2 in the CSF (Table 2).

228 Table 3 shows data of parameters evaluated on OCT angiography, adjusted for patient's
229 age and blood pressure. All evaluated parameters both quantitative and qualitative were
230 significantly different in patients with DM versus controls in both SCP and DCP. (Table 3).

231 In particular, the area of FAZ was significantly larger: in patients with DM Type 1 versus
232 controls in both SCP ($p=0.05$) and DCP ($p<0.001$) and in patients with DM Type 1 versus
233 DM Type 2 only in DCP ($p<0.0001$); the number of microaneurysms was higher in patients
234 with DM Type 1 versus controls in both SCP ($p<0.001$) and DCP ($p<0.0001$); and in
235 patients with DM type 2 versus controls only in DCP ($p=0.007$). (Table 3) Logistic
236 regression analysis (adjusted for patient's age and blood pressure), showed greater

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237 association between all evaluated qualitative parameters in patients with DM type 1 and
238 almost all (exception for irregularities in the SCP) for patients with DM type 2 versus
239 control group. (Table 3)

240 Table 4 shows statistically significant correlations between OCT and OCT angiography
241 parameters. Statistically significant correlations to retinal thickness include almost all
242 parameters evaluated on OCT angiography in the SCP in patients with DM type 1,
243 whereas only the presence of areas of capillary loss and FAZ area in the SCP in patients
244 with DM type 2. In particular, the area of FAZ in SCP inversely correlated to NFL thickness
245 in the CSF in patients with DM type 2 whereas, the area of FAZ in DCP inversely
246 correlated to NFL thickness in the CSF in patients with DM type 1.

247 The highest correlations were found between perifoveal capillary loss in the SCP and
248 GCL+ and GCL++ thickness in the inner ring in patients with DM Type 1 and perifoveal
249 capillary loss in the SCP and NFL thickness in the CSF and the inner ring in patients with
250 DM Type 2.

251 **Discussion:**

252 In this study we report on early microvascular and retinal thickness changes in patients
253 with DM and no clinical signs of DR, evaluating further and separately patients with DM
254 Type 1 and DM Type 2 vs healthy subjects. All quantitative and qualitative parameters
255 evaluated on OCT-A in the SCP and DCP were significantly different in patients with DM
256 versus healthy controls. In particular, in patients with DM and no clinical signs of DR, FAZ
257 area was larger and irregular, the number of microaneurysms was higher, areas of
258 perifoveal capillary loss and perifoveal capillary network irregularities (such as tortuosity
259 and beadings) were more numerous in both SCP and DCP when compared to healthy
260 subjects. Simonett et al. evaluated patients with DM type 1 and no DR (9 eyes) or mild non
261 proliferative DR (19 eyes) and reported decreased parafoveal vessel density (that could be
262 compared to parafoveal capillary nonperfusion) only in the DCP, and no changes in the

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263 FAZ area were found in neither SCP nor DCP. ¹⁶ Dimitrova et al. documented decreased
264 parafoveal vessel density in the SCP and DCP and FAZ area increase in the SCP in
265 patients with DM (mostly type 2) and no DR when compared to control subjects. ¹⁵
266 In the present study, specific changes are documented separately in patients with DM
267 Type 1 and DM Type 2. All quantitative and qualitative parameters evaluated on OCT
268 angiography resulted altered in both SCP and DCP in patients with DM type 1 vs controls.
269 In DM type 2, the only quantitative parameter found different, compared to controls, was
270 the higher number of microaneurysms in the DCP. Thus, the other evaluated quantitative
271 parameters (the area of the FAZ in SCP and DCP and the number of microaneurysms in
272 SCP) were not found different in patients with DM type 2 vs controls. Moreover, the area of
273 the FAZ was significantly larger in patients with DM type 1 vs patients with DM type 2 in
274 the DCP. Besides modifications in quantitative parameters, also all evaluated qualitative
275 parameters were found different in patients with DM type 1 vs controls, and almost all
276 (except the presence of capillary irregularities in the SCP) in patients with DM type 2
277 compared to controls. Although data from the present study cannot be directly correlated
278 to the data available in the literature on OCT angiography parameters in patients with DM
279 and no clinical signs of DR, due to different population of patients (considering together
280 patients with DM type 1 and 2, or only separately patients with DM type 1 but also with
281 some signs of DR), some similarities or differences could be drawn. De Carlo et al.
282 reported on increased prevalence in changes to the FAZ (increased FAZ area and
283 presence of FAZ remodeling) and capillary nonperfusion in patients with DM (considering
284 together type 1 and type 2) and no clinical signs of DR. ¹⁴ The same authors reported that
285 vascular tortuosity was present in similar percentage in both controls and patients with
286 DM, concluding that this microvascular abnormality may be a variant of normal, and thus
287 cannot be used as the OCT-A screening parameter for retinal vascular change in DM. ¹⁴
288 However, the authors did not report on blood pressure data and differences between the

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289 groups. On the contrary, in the present study, after adjusting for blood pressure differences
290 both venous beading and vascular tortuosity were found more frequent in patients with DM
291 type 1 and DM type 2 versus non diabetic subjects. Thus the significance of microvascular
292 abnormalities in patients with DM and no clinical signs of DR may warrant further
293 evaluation.

294 Results from the present study confirm data reported by Dimitrova et al, who found
295 changes in parafoveal vessel density in both SCP and DCP in patients with DM (mostly
296 type 2) and no clinical signs of DR. ¹⁵ Simonett et al, evaluated a cohort of 28 patients with
297 DM type 1 and no or minimal signs of DR using an automated algorithm software. ¹⁶ These
298 Authors reported a decrease in parafoveal vessel density only in the DCP. ¹⁶ Choi et al,
299 using an ultrahigh speed swept source OCT angiography prototype, documented retinal
300 microvascular abnormalities (such as capillary dropout, dilated capillary loops, tortuous
301 capillary branches, patches of reduced capillary perfusion, irregular FAZ contours, and/or
302 FAZ enlargement) in both SCP, intermediate and DCP, in 18 of the 51 eyes with DM and
303 no clinical signs of DR (with no specification of DM type). ¹⁹ Moreover, these Authors
304 reported focal or diffuse choriocapillaries flow impairment in almost half of the evaluated
305 patients without DR.

306 Data from the present study may indicate that both SCP and DCP are precociously altered
307 in patients with DM type 1 and no clinical signs of DR, whereas in patients with DM type 2,
308 DCP is more precociously involved. To the best of our knowledge, no data are currently
309 available on direct comparison between patients with DM type 1 and type 2 at pre-clinical
310 stage of DR.

311

312 Patients with DM type 1 had thinner inner parafoveal retinal thickness (GCL++ complex in
313 the inner ring) when compared to normal subjects (when adjusted for age). Scarinci et al,
314 recently reported a significant thinning of the GCL layer in patients with DM type 1 and no

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315 DR. ¹³ Van Dijk et al documented no significant difference in any layer thickness in the
316 macula in patients with DM but no DR compared to normal controls and a decrease in the
317 NFL, GCL and IPL thickness in the pericentral area of the macula, in patients with minimal
318 DR compared to controls ¹⁰ Vujosevic and Midena documented decreased NFL thickness
319 in the macular area in patients with no DR or with non proliferative DR, mostly with DM
320 type 2. ¹¹ In the present study, after adjusting for age and DM duration, GCL+ and GCL++
321 complex thicknesses were significantly higher in patients with DM type 1 vs DM Type 2 in
322 the CSF. The exact mechanism leading to this finding is not known. One hypothesis may
323 implicate a protective role of more intensive insulin treatment on retinal ganglion cells
324 already reported in experimental studies, thus thicker inner retina in DM type 1.^{20, 21}
325 Another hypothesis may see reported differences in complication characteristics in
326 patients with DM type 2 versus DM type 1, that included more prevalent peripheric
327 neuropathy and cardiovascular disease in DM type 2, independent of disease duration,
328 whereas no differences were found for the presence of DR. ²² This may explain thinner
329 inner retina in DM type 2 when corrected for age difference and DM duration in the present
330 study. ²²

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331 In conclusion this study documents very early and specific microvascular and neuronal
332 changes both in patients with DM type 1 and DM type 2, when compared to non diabetic
333 controls. OCT-A allows for detection of microvascular changes even before clinical signs
334 of DR are present. Thus, OCT-A may help in earlier diagnosis and new classification of
335 DR. Further, larger studies are needed to better evaluate and correlate microvascular and
336 neural changes in the retina in DM.

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447 Figures' Legend:

448 Figure 1.

449 Right eye of a patient with diabetes mellitus type 1 and no clinical signs of
450 diabetic retinopathy. First row: Left - color fundus photo; middle-OCT-
451 angiography (OCT-A) of the superficial capillary plexus (SCP); right- OCT-
452 angiography of the deep capillary plexus (DCP); modifications of the foveal
453 avascular zone-FAZ including enlargement and presence of irregular form
454 presence of area of capillary loss/reduced capillary density, and alterations of
455 the capillary network are present both the SCP and DC; Second row: OCT linear
456 scan in the macula showing automatic segmentation (green lines) of the nerve
457 fiber layer; ganglion cell layer (GCL)+ complex – from NFL/GCL interface to
458 inner plexiform layer (IPL)/inner nuclear layer (INL); GCL++ complex- from
459 ILM/NFL interface to IPL/INL; outer retina- from IPL/INL interface to
460 interdigitation zone.

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462 **Figure 2.**

463 Right eye of a patient with diabetes mellitus type 1 and no clinical signs of
464 diabetic retinopathy. First row: Left - color fundus photo; middle-OCT-
465 angiography (OCT-A) of the superficial capillary plexus (SCP); right- OCT-
466 angiography of the deep capillary plexus (DCP); enlargement of the foveal
467 avascular zone as well as the presence of microaneurysm is better observed in
468 the DCP. presence of area of capillary loss/reduced capillary density, and
469 alterations of the capillary network are present both the SCP and DC; Second
470 row: OCT linear scan in the macula showing automatic segmentation (green
471 lines) of the nerve fiber layer; ganglion cell layer (GCL)+ complex – from

472 NFL/GCL interface to inner plexiform layer (IPL)/inner nuclear layer (INL);
473 GCL++ complex- from ILM/NFL interface to IPL/INL; outer retina- from IPL/INL
474 interface to interdigitation zone.

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500 **Table 1** – Sample characteristics. Mean (SD, range)

| <i>Variable</i> | <i>Controls (N=30)</i> | <i>Diabetics (N=60)</i> | <i>Diabetes Type I (N=24)</i> | <i>Diabetes Type II (N=36)</i> |
|-----------------|----------------------------|---------------------------------------|---------------------------------------|--|
| Age | 44.4 (14.8, 21-72) | 57.4 ^a (15.4, 19-81) | 36.9 (12.1, 19-53) | 64.2 ^{e,f} (8.9, 41-81) |
| HbA1c | n.a. | 7.3 (1.0, 5.8-10.3) | 7.2 (0.9, 5.9-9.1) | 7.3 (1.1, 5.8-10.3) |
| Duration | n.a. | 9.9 (8.7, 0.5-40) | 16.3 (11.3, 2-40) | 7.7 ^d (6.4, 0.5-21) |
| SBP | 120.2 (8.9, 90-130) | 126.1 ^b (10.0, 100-150) | 119.2 (11.6, 100-140) | 128.5 ^{g,h} (8.3, 120-150) |
| DBP | 76.7 (7.8, 60-90) | 77.3 (5.8, 60-85) | 73.3 (8.1, 60-85) | 78.6 ⁱ (4.2, 70-85) |
| ETDRS | 85.0 (0.0, 85-85) | 83.6 (4.3, 55-88) | 84.9 (0.3, 84-85) | 82.8 ^j (5.4, 55-88) |
| IOP | 14.6 (2.1, 12-19) | 13.9 ^c (1.2, 12-18) | 13.2 ^k (1.1, 12-15) | 14.3 ^l (1.0, 13-18) |

Legend: Duration=duration of diabetes (years); SBP=systolic blood pressure (mmHg); DBP=diastolic blood pressure (mmHg); ETDRS=number of letters; IOP=intraocular pressure (mmHg); n.a.=not applicable. Diabetics vs. Controls t-Student test: a) $P=0.0010$; b) $P=0.0161$; c) $P=0.0315$. Bonferroni post-hoc test: d) Type I vs. Type II, $P=0.0020$; e) Type II vs. Controls, $P<0.0001$; f) Type II vs. Type I, $P<0.0001$; g) Type II vs. Controls, $P=0.0029$; h) Type II vs. Type I, $P=0.0092$; i) Type II vs. Type I, $P=0.0425$; j) Type II vs. Controls, $P=0.0326$; k) Type I vs. Controls, $P=0.0020$; l) Type II vs. Type I, $P=0.0143$.

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531 **Table 2 – Retinal thickness. Mean (SD)**

| <i>Parameter</i> | <i>Controls (N=30)</i> | <i>Diabetics (N=60)</i> | <i>Diabetes Type I (N=24)</i> | <i>Diabetes Type II (N=36)</i> |
|------------------------|----------------------------|-----------------------------|---------------------------------------|--|
| Total thickness | | | | |
| CSF | 243.2 (21.4) | 238.4 (20.9) | 241.2 (20.1) | 236.5 (21.5) |
| Inner ring | 313.0 (12.2) | 307.7 (16.1) | 309.1 (16.5) | 306.8 (16.0) |
| Outer ring | 273.0 (12.9) | 267.0 (13.0) | 268.9 (12.8) | 265.8 (13.2) |
| NFL layer | | | | |
| CSF | 7.3 (3.6) | 7.3 (3.3) | 8.0 (3.6) | 6.8 (3.1) |
| Inner ring | 27.0 (2.0) | 26.9 (2.4) | 26.4 (1.3) | 27.1 (2.9) |
| Outer ring | 40.5 (2.9) | 39.6 (3.3) | 39.4 (2.7) | 39.8 (3.7) |
| GCL+ layer | | | | |
| CSF | 47.6 (8.7) | 46.0 (6.7) | 48.7 ^c (6.1) | 44.1 (6.5) |
| Inner ring | 92.7 (5.4) | 90.0 (6.7) | 90.2 ^a (7.2) | 89.8 (6.5) |
| Outer ring | 66.5 (3.8) | 65.2 (5.3) | 66.1 (4.4) | 64.6 (5.8) |
| GCL++ layers | | | | |
| CSF | 54.8 (12.0) | 53.2 (9.2) | 56.8 ^d (9.0) | 50.9 (8.6) |
| Inner ring | 119.7 (6.8) | 116.8 (8.2) | 116.7 ^b (7.9) | 116.9 (8.4) |
| Outer ring | 107.0 (5.2) | 104.8 (7.3) | 105.6 (6.5) | 104.3 (7.8) |
| Outer retina | | | | |
| CSF | 188.3 (11.5) | 185.2 (14.2) | 184.5 (13.2) | 185.6 (15.1) |
| Inner ring | 193.3 (7.2) | 190.9 (10.3) | 192.5 (10.9) | 189.9 (9.8) |
| Outer ring | 165.9 (8.6) | 162.2 (8.6) | 163.3 (9.9) | 161.5 (7.6) |

532 Legend: NFL: retinal nerve fiber layer; GCL+ - ganglion cell layer; GCL++- NFL and GCL+ ;

534 CSF: central subfield retinal thickness; inner ring- retinal thickness within central 3mm;

535 outer ring: retinal thickness within central 6mm.

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537 **Table 3** – OCT-Angiography parameters.

| <i>Parameter</i> | <i>Controls (N=30)</i> | <i>Diabetics (N=60)</i> | <i>Diabetes Type I (N=24)</i> | <i>Diabetes Type II (N=36)</i> |
|--|----------------------------|-----------------------------|---------------------------------------|--|
| <i>FAZ area¹</i> | | | | |
| Superficial plexus | 286.4 (137.0) | 359.3 ^a (129.1) | 377.2 ^e (150.6) | 347.5 (113.2) |
| Deep plexus | 363.7 (142.1) | 497.0 ^b (150.3) | 557.1 ^{f,g} (139.6) | 456.9 (145.9) |
| <i>Number of microaneurisms¹</i> | | | | |
| Superficial plexus | 0.10 (0.40) | 1.40 ^c (1.61) | 1.38 ^h (2.10) | 1.42 (1.20) |
| Deep plexus | 0.07 (0.37) | 2.07 ^d (1.89) | 1.83 ⁱ (2.16) | 2.22 ^j (1.71) |
| <i>Irregular quality²</i> | | | | |
| Superficial plexus | 17 (56.7) | 50 ^A (83.3) | 21 ^G (87.5) | 29 (80.6) |
| Deep plexus | 8 (26.7) | 50 ^B (83.3) | 23 ^H (95.8) | 27 ^M (75.0) |
| <i>Capillary loss²</i> | | | | |
| Superficial plexus | 12 (40.0) | 55 ^C (91.7) | 22 ^I (91.7) | 33 ^N (91.7) |
| Deep plexus | 7 (23.3) | 58 ^D (96.7) | 23 ^J (95.8) | 35 ^O (97.2) |
| <i>Presence of Tortuosity/Beadings²</i> | | | | |
| Superficial plexus | 1 (3.3) | 47 ^E (78.3) | 14 ^K (58.3) | 33 ^P (91.7) |
| Deep plexus | 3 (10.0) | 54 ^F (90.0) | 19 ^L (79.2) | 35 ^Q (97.2) |

538 Legend: 1) Mean (standard deviation); 2) Frequency (%). ANOVA adjusted for patient's age and
539 blood pressure, test vs. Control group: a) $P=0.0582$; b) $P=0.0003$; c) $P=0.0005$; d) $P<0.0001$. *Post-*
540 *hoc* Bonferroni test vs. Control group after ANOVA adjusted for patient's age and blood pressure:
541 e) $P=0.0189$; f) $P<0.0001$; g) vs. Type II group, $P=0.0012$; h) $P=0.0013$; i) $P<0.0.001$; j) $P=0.0118$.
542 Logistic regression analysis adjusted for patient's age and blood pressure, Odds-Ratios (95%CI)
543 vs. Control group: A) 4.0 (1.3 to 11.7); B) 22.0 (6.0 to 81.5); C) 13.1 (3.8 to 44.3); D) 87.5 (14.8 to
544 517.2); E) 97.1 (11.0 to 858.3); F) 168.1 (19.0 to +∞); G) 5.6 (1.3 to 23.0); H) 68.2 (7.7 to 606.9); I)
545 27.9 (4.4 to 177.1); J) 179.3 (12.6 to +∞); K) 68.3 (6.4 to 788.2); L) 352.4 (16.3 to +∞); M) 8.9 (1.9
546 to 42.4); N) 5.4 (1.0 to 29.8); O) 36.9 (3.5 to 389.5); P) 136.8 (11.7 to +∞); Q) 87.7 (7.3 to +∞).

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553 **Table 4** – Correlation between OCT parameters and OCT-Angiography parameters.

| Quadrant | Total thickness | NFL layer | GCL+ layer | GCL++ layer | Outer retina |
|-----------------|--------------------------------|---|---|--------------------------------|---|
| Controls | | | | | |
| CSF | FAZs (-0.145) | FAZd (-0.020) CAPd (+2.516) | FAZs (-0.036) FAZd (-0.023) | FAZs (-0.046) FAZd (-0.036) | FAZs (-0.073) QUAd (-6.692) |
| Inner | FAZs (-0.060) QUAs (-8.472) | FAZd (-0.008) | FAZs (-0.026) QUAs (-2.695) TORd (+7.891) | FAZs (-0.033) QUAs (-3.748) | FAZs (-0.030) QUAs (-4.653) |
| CSF+Inner | FAZs (-0.082) QUAs (-6.930) | FAZd (-0.011) | FAZs (-0.031) TORd (+7.016) | FAZd (-0.043) QUAs (-2.938) | FAZs (-0.038) QUAd (-3.968) |
| DM | | | | | |
| CSF | FAZs (-0.069) MICd (-3.487) | FAZs (-0.009) TORs (-2.552) | FAZs (-0.021) MICs (+1.427) MICd (-1.110) TORs (-6.681) TORd (+5.111) | FAZs (-0.031) TORs (-7.267) | FAZd (-0.036) MICd (-2.576) QUAd (+7.720) |
| Inner | MICd (-2.627) | FAZd (-0.003) CAPs (-2.307) | FAZs (-0.019) | FAZs (-0.023) | MICd (-1.764) |
| CSF+Inner | FAZs (-0.033) MICd (-2.456) | FAZd (-0.004) QUAd (+1.390) CAPs (-2.601) | FAZs (-0.020) | FAZs (-0.022) TORs (-3.818) | MICd (-2.075) |
| Type I | | | | | |
| CSF | FAZd (-0.083) | FAZd (-0.013) | FAZd (-0.027) | FAZd (-0.038) | MICs (-3.357) |
| Inner | MICs (-2.899) | QUAs (-1.835) TORs (-1.114) | CAPs (-10.273) | CAPs (-11.011) | - |
| CSF+Inner | MICs (-3.305) | MICs (-0.357) | CAPs (-8.936) | MICs (-1.522) | MICs (-1.783) |
| Type II | | | | | |
| CSF | FAZs (-0.119) MICd (-3.853) | FAZs (-0.009) CAPs (-4.401) | FAZs (-0.042) | FAZs (-0.056) | FAZs (-0.067) MICd (-3.749) |
| Inner | FAZd (-0.041) MICd (-2.995) | CAPs (-4.053) | FAZd (-0.022) | FAZd (-0.027) | MICd (-2.766) |
| CSF+Inner | FAZs (-0.067) MICd (-3.456) | CAPs (-4.388) | FAZd (-0.023) | FAZs (-0.038) | FAZs (-0.029) MICd (-2.912) |

Legend: In brackets statistically significant ($P < 0.05$) regression coefficients from the multiple regression model of each OCT parameter vs. Angio-OCT parameters (stepwise selection criterion) adjusted for patient's age and blood pressure (systolic and diastolic). FAZ = extension of area of the foveal avascular zone FAZ (mm^2); MIC = microaneurysms (number); QUA = quality of the FAZ area (0=regular, 1=irregular; CAP = capillary loss (0=no, 1=yes); TOR = presence of tortuosity/beadings (0=no, 1=yes); suffix 's' = superficial plexus; suffix 'd' = deep plexus. CSF = central subfield retinal thickness; Inner = the mean of superior, nasal, inferior and temporal inner quadrant thickness (central 3mm); CSF+Inner = the average of CSF and Inner quadrants' thickness.

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