

**Longitudinal microvascular and neuronal retinal evaluation in patients with diabetes mellitus type 1 and 2 and good glycemic control**

**Abbreviated title: 3-year OCT-A and OCT evaluation in no DR**

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**Summary statement (max 50 words):** This study showed longitudinal changes in microvascular macular parameters of patients with DM2, no DR and good glycemic control, which could be interpreted as an attempt to preserve a balance between superficial and deeper layers in order to maintain retinal function in the earliest phases of diabetic retinal disease.

## Abstract

**Purpose:** To evaluate microvascular and neuronal changes over 3 years in patients with type 1/2 diabetes mellitus (DM1/DM2), good metabolic control and no signs of diabetic retinopathy (DR).

**Methods:** In this prospective, longitudinal study, 20 DM1, 48 DM2 and 24 controls underwent macular OCT and OCT-A at baseline and after 3 years. Following parameters were considered: thickness of the central macula (CMT), retinal nerve fiber layer (NFL), ganglion cell (GCL+/GCL++) complex; perfusion and vessel density (PD/VD) and fractal dimension (FD) at the superficial and deep capillary plexuses (SCP/DCP); choriocapillaris flow deficits (CC-FD); foveal avascular zone (FAZ) metrics. MATLAB and ImageJ were used for OCT-A scans analyses.

**Results:** Mean HbA1c was  $7.4 \pm 0.8\%$  in DM1 and  $7.2 \pm 0.8\%$  in DM2 at baseline, with no change at 3 years. No eye developed DR. In longitudinal analyses, PD at SCP ( $p=0.03$ ) and FAZ area and perimeter ( $p<0.0001$ ) significantly increased in DM2 compared to other groups. No longitudinal changes occurred in OCT parameters. In comparisons within groups, DM2 had a significant thinning of GCL++ in the outer ring, decreased PD at DCP and CC-FD, an increase in FAZ perimeter and area in DCP; DM1 had an increase in FAZ perimeter in DCP ( $p<0.001$  for all comparisons).

**Conclusion:** Longitudinal data showed significant microvascular retinal changes in DM2. No changes were detected in neuronal parameters and in DM1. Longer and larger studies are needed to confirm these preliminary data.

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**Keywords:** diabetic retinal disease; diabetes mellitus type 1; diabetes mellitus type 2; glycemic control; microvasculopathy; neurodegeneration; optical coherence tomography; optical coherence tomography-angiography; perfusion density; pre-clinical diabetic retinopathy.**Introduction**

Diabetic retinopathy (DR) was recently defined as a “highly tissue-specific neurovascular complication” of both type 1 and type 2 diabetes mellitus (DM1/DM2)<sup>1</sup> and subclinical evidence of both neuronal and microvascular damage before clinically evident DR has been widely reported.<sup>2-7</sup>

Even if DM, and especially DM2, prevalence is increasing,<sup>8</sup> most evidence indicates that patients with DM can significantly reduce the risk of diabetes-related complications by maintaining an optimal long term metabolic control with low glycated haemoglobin (HbA1c) variability.<sup>9-11</sup> Current recommendations indicate a target HbA1c level  $\leq 6.5\%$  in DM1 and  $\leq 7\%$  in DM2 to minimize the risk of long-term vascular complications.<sup>12</sup> Moreover, a good glycemic control has also been proved to reduce the risk of DR progression and vision loss due to DR complications.<sup>13,14</sup> Some studies reported a 7 to 14-15% increase in the risk of progression from no or non proliferative DR to proliferative DR when HbA1c increases by 1% per year depending on the initial stage of disease<sup>13</sup> and the expected time to progress to the next stage of DR reduces contextually.<sup>15</sup>

Optical coherence tomography angiography (OCT-A) has offered a non-invasive and depth-resolved evaluation of microvascular changes in the macula and peripapillary region in DR. Moreover, a growing body of scientific evidence exists on the usefulness of OCT-A in the detection of microvascular impairment in patients with

DM and no clinical signs of DR on fundus examination/color fundus photography (CFP).<sup>3,4,5,6</sup> However, longitudinal data obtained by means of OCT-A in patients with DM are still limited to few recent papers with a variable period of observation ranging from 2 to 5 years.<sup>16-19</sup> In particular, to the best of our knowledge, only one previous study performed a sub-analysis on a cohort including only patients with no signs of DR at baseline<sup>16</sup> and comparative longitudinal studies between patients with DM1 and DM2 have not yet been performed.

On this basis, the purpose of the present study was to evaluate and compare microvascular and neuronal changes occurring over a period of 3 years in the macula in patients with DM1 and DM2 and good metabolic control and no clinical signs of DR at baseline and healthy controls by means of OCT and OCT-A.

## **Methods**

### *Population and study design*

The present study was a 3-year prospective, longitudinal, observational case-control comparative evaluation of healthy volunteers and patients with DM1 and 2 with no clinically detectable signs of DR at the initial visit. The period of enrollment lasted from July to December 2017 and all study visits were performed at the Medical Retina Service, University Hospital Maggiore della Carità, Novara, Italy. Healthy volunteers were selected among the Hospital staff and care-givers of non-diabetic patients. The study followed the tenets of the Declaration of Helsinki and was

approved by the institutional Ethics Committee; all study participants signed written informed consent.

Inclusion criteria were: patients of age  $\geq 18$  years with DM1/DM2 and no signs of DR on dilated fundus examination; subjects with normal screening glucose test performed  $\leq 6$  months from the beginning of the study for the control group (glucose test was considered normal if fasting plasma glucose was  $< 100$  mg/dL); and well controlled systemic blood pressure (with or without treatment) with values  $< 140/90$  mmHg on the day of examination. Subjects were excluded in case of: any concomitant ocular or systemic disease that could confound the results; any intraocular surgery within 6 months in the study eye; refractive error higher than 4 diopters; and low quality imaging. Only one eye per patient (the right eye) was included; the left eye was chosen only in case of poor quality imaging in the right eye. A total of 120 eyes/subjects (26 controls, 24 DM1, 70 DM2) fulfilled all the eligibility criteria and were enrolled in the study.

Anamnestic data were obtained by subject report and review of past medical records. Full eye examination and non-invasive retinal imaging with CFP (single, macula-centred 45-degree image), swept-source OCT and OCT-A were performed at baseline and after 3 years.

### *Image acquisition protocol*

All images were acquired using swept-source DRI-OCT/OCT-A Triton Plus (Topcon Medical Systems Europe, Milan, Italy). Detailed description of the characteristics of this device has been previously reported.<sup>20</sup> The study acquisition protocol included: a

6-mm radial OCT scan centered on the fovea, and 3-dimensional 3 x 3 mm OCT-A map of the macula.<sup>20</sup> The same images were acquired at baseline and after 3 years using the instrument follow-up function.

#### *OCT evaluation*

The following parameters were evaluated on structural OCT using the instrument automatic segmentation tool to identify single layers: central macular thickness (CMT); thickness of retinal nerve fiber layer (NFL), segmented between inner limiting membrane (ILM) and NFL; thickness of ganglion cell layer (GCL+) complex, between NFL/GCL interface and inner plexiform layer (IPL)/inner nuclear layer (INL) interface; thickness of GCL++ complex, between ILM/NFL and IPL/INL interface. Thickness values were recorded in the central 1-mm subfoveal (SF) area, in the inner ring (IR; calculated as mean value of 4 inner quadrants with 3-mm diameter), and in the outer ring (OR; calculated as mean value of 4 outer quadrants with 6-mm diameter), using a 6-mm standard ETDRS grid as reference.

#### *OCT-A evaluation*

Perfusion density (PD), vessel density (VD) and fractal dimension (FD) were evaluated both at the superficial and deep capillary plexuses (SCP/DCP) using MATLAB image processing toolbox and custom scripts (version 2017b, MathWorks, Natick, MA). Foveal avascular zone (FAZ) area, perimeter and circularity index (CI) on SCP and DCP were calculated using ImageJ software, version 1.51 (<http://image>



j.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA). Choriocapillaris flow deficits (CC-FD) were evaluated with MATLAB software excluding CC-FD with diameter  $< 24 \mu\text{m}$ .<sup>21</sup> Original automatic segmentation was used for SCP and DCP analyses, while CC outer boundary was manually moved from 10.4 to 20.8  $\mu\text{m}$  below Bruch's membrane.<sup>20</sup> Only images free of artifacts and with signal strength index above 50 were evaluated.

### *Statistical analyses*

The means of the experimental groups were reported as arithmetic mean (standard deviation) for continuous variables in the text and Tables. Categorical variables were reported as experimental percentages.

OCT and OCT-A variables at baseline were compared among different study groups using one-way Analysis of Covariance (ANCOVA) to control for the confounding effect of age, which was treated as the continuous predictor. The means of populations were estimated as least square means, which are the best linear estimates for the marginal means in the ANCOVA design. In case of an overall statistically significant difference among study groups, pairwise comparisons among the different groups were done using Scheffé's test

Significant differences in OCT and OCT-A variables within DM1 and DM2 groups between baseline and end of observation were assessed using paired t-test with the Bonferroni's correction for multiple comparisons.

The longitudinal analyses of OCT and OCT-A variables parameters in the three study groups were led using ANOVA with repeated measures. In case of an overall

statistically significant difference among study groups, pairwise comparisons among the different groups were done using Scheffé's test.

The statistical analyses were performed using Statistica software version 6.0 (Statsoft Inc., Tulsa, OK, USA), using a two-sided type I error rate of  $p \leq 0.05$ .

## Results

Ninety-two eyes/patients completed the 3-year follow-up and were then included in the final analyses: 24 controls (mean age:  $44.1 \pm 10.2$  years), 20 DM1 (mean age:  $37.8 \pm 12.8$  years) and 48 DM2 (mean age:  $64.1 \pm 9.6$  years). 28 patients (2 controls, 4 DM1 and 22 DM2) were lost to follow-up and then excluded from all study analyses. Table 1 shows main demographic and clinical data of the study population at baseline. All patients maintained a good glycemic control throughout the study (mean HbA1c:  $7.4 \pm 0.8\%$  at baseline,  $7.1 \pm 0.9\%$  after 3 years in DM1;  $7.2 \pm 0.8\%$  at baseline,  $6.8 \pm 1.2\%$  after 3 years in DM2). No eye developed clinically evident DR during the follow-up.

Significant differences in OCT and OCT-A parameters among the 3 study groups obtained by one-way ANCOVA analyses performed at baseline and adjusting for age are reported in Table 2.

As for comparisons between baseline and the end of observation within groups and considering a level of statistical significance of 0.001 after Bonferroni's correction, DM2 group had a significant thinning of GCL++ in the OR, a significant reduction of PD at the DCP and CC-FD, and an increase in FAZ perimeter and area in the DCP;

DM1 group had only an increase in FAZ perimeter in the DCP ( $p < 0.001$  for all comparisons). DM2 showed a decreasing trend in NFL in the IR ( $p = 0.05$ ) and in the OR ( $p = 0.002$ ), in GCL++ in the IR ( $p = 0.002$ ) and in VD at the DCP ( $p = 0.006$ ) and an increasing trend in PD and VD in the SCP ( $p = 0.002$  and  $p = 0.008$  respectively). DM1 showed a decreasing trend in SF-NFL ( $p = 0.03$ ), GCL++ in the OR ( $p = 0.01$ ), PD at the DCP ( $p = 0.002$ ) and FAZ CI at the DCP ( $p = 0.01$ ), however none of these comparisons were significant after Bonferroni's correction. Comparisons within DM1 and DM2 groups are summarized in Table 3.

In longitudinal analyses with comparisons among the 3 study groups, a significant difference was found in the variation of PD at the SCP ( $p = 0.018$ ), due to an increase in DM2 group at post hoc analysis ( $p = 0.03$ ); in addition, FAZ area and perimeter at the DCP significantly increased in DM2 compared to DM1 and controls ( $p < 0.0001$ ). No significant longitudinal changes were observed in OCT parameters. Tables 4 and 5 show the variation of all evaluated parameters over 3 years in each study group.

## Discussion

In the present study, OCT-A and OCT were used to investigate diabetic retinal microvascular and neuronal impairment in the macula of patients with DM1 and DM2 and no DR over a period of 3 years. The results of longitudinal analyses among the three study groups show a significant change only in PD at the SCP, due to an increase in this parameter in eyes with DM2 compared to DM1 and controls, and in FAZ metrics, due to an increase of both area and perimeter in DM2 compared to DM1

and controls. No significant longitudinal changes were observed in OCT parameters. It is important to note that no eye/patient included in the study developed DR and that both patients' groups with DM1 and DM2 had good glycemic control at the baseline visit and maintained it during the entire period of observation.

Baseline comparisons of microvascular parameters among the 3 study groups are in line with previously published cross-sectional studies on the topic, reporting signs of disease before it becomes clinically detectable.<sup>3-6</sup> In fact, compared to healthy controls, patients with both DM1 and DM2 showed a significant alteration of FAZ metrics and a decrease in FD, which is an index of loss of small vascular branches, both in the SCP and DCP. Moreover, reduced perfusion at the SCP was detected in eyes with DM1 compared to controls. Previous studies evaluating retinal neurodegeneration (DRN) in DM reported a significant thinning of NFL and GCL and an increase in INL thickness compared to controls.<sup>2,3,22-24</sup> However, in the present study no significant neurodegenerative changes have been observed nor in DM1 or in DM2. Despite a great availability of data from cross-sectional studies, whether microvasculopathy precedes DRN or vice versa in diabetic retinal disease (DRD) is still debated and only few longitudinal studies have investigated these aspects so far. The European Consortium for the Early Treatment of Diabetic Retinopathy study postulated the existence of two different phenotypes in the early phases of DRD: a neurodegenerative phenotype and a primarily microangiopathic phenotype.<sup>25</sup> However, it has to be considered that these two mechanisms are not completely independent of each other but can have a mutual interference (for example, the loss of ganglion cells, which are a source of vascular endothelial growth factor, could

contribute to microvascular dysfunction)<sup>26</sup> and can follow different pathways in DM1 and DM2.

In this study, in the longitudinal analysis, a comparison among DM1, DM2 and controls was performed and only eyes with DM2 showed significant changes. Recently, *Aschauer* et al. conducted a 2-year longitudinal study evaluating microvascular changes and DRN progression in eyes with DM2 (including patients with  $HbA1c \leq 8\%$  and eyes with no DR or mild/moderate DR) detecting a reduction in perfusion at the SCP and an increase in FAZ parameters, while DCP perfusion did not change significantly.<sup>16</sup> Moreover they found a parallel decline in GCL and IPL thickness.<sup>16</sup> These results were not confirmed in the present study, despite a longer duration of follow-up. In fact, no significant longitudinal changes have been detected in eyes with DM2 as for neuronal parameters, and perfusion in the SCP interestingly increased. More in detail, a decrease in OR-GCL++ thickness within the DM2 group was observed, but no differences were detected among groups. The analyses within single groups also showed an increase in perfusion in DCP and a decrease in perfusion in the CC in the DM2 group, with no differences among groups. However, the inclusion of patients with initial stages of DR and the lack of a control group in the analysis performed by *Aschauer* et al. makes it difficult to compare to the present study. Moreover, even if baseline mean HbA1c values are comparable between the two studies, *Aschauer* et al. do not report on the change of this parameter during the period of observation.

An early increase in retinal blood flow in the SCP in DM with no DR, as observed in the present study, has been already reported in recent studies<sup>5-7</sup> and interpreted as a compensatory effect to initial capillary occlusion, maybe due to regulatory

mechanisms separate from those of deeper vessels and capable of preserving flow at this level.<sup>6</sup> Similarly, *Ashraf et al.* found no correlation between changes observed in the SCP and in the DCP in different DR stages thus suggesting that these changes may be at least partially independent.<sup>27</sup> The early compensatory mechanism previously proposed and involved in the early increase in SCP perfusion probably consisted in dilation of pre-existing capillaries, while recruitment of reserve capillary segments could have contributed to a minimum extent.<sup>5,6</sup> This hypothesis is further supported by the fact that we detected an increase in PD, evaluated on binary images, and not in VD, evaluated on skeletonized images and thus not influenced by vessel caliber as the process of skeletonization reduces all vessels' diameter to 1 pixel. Moreover, previous studies also reported an increase in vessel diameter index, defined as the average vessel caliber, in patients with DM and no DR.<sup>5,28</sup> Conversely to the increase in blood flow observed in the SCP, a decrease in PD was detected in the DCP (in the analysis within group), which could be explained by a greater susceptibility of the deeper vessels to damage mainly due to its anatomy and location in the retina; in fact, DCP has a greater distance from the larger arterioles, while it is near to the outer retinal layers, characterized by highly active metabolism and oxygen demand and has a more complex anatomical architecture.<sup>26</sup> These early changes in perfusion of the different capillary plexuses in the initial phases of DRD result in a balance between capillary loss and compensatory dilation. It has been hypothesized that, with disease progression, nonperfusion increases and exceeds the local compensatory mechanisms with consequent progressive decline in retinal blood flow.<sup>5</sup> An opposite explanation of the present results, previously postulated by *Onishi et al.*, is that dilated SCP vessels with decreased resistance could exert a sort of "steal phenomenon" on DCP,

thus not counteracting but contributing to progressive ischemia of deeper retinal layers.<sup>6</sup>

In this report, an increase in perfusion in the CC was also observed in DM2 after an initial impairment compared to DM1 and controls detected at baseline. It is well known that also CC is impaired in DRD and this could result in dysfunctional RPE and outer retina;<sup>29</sup> however, we can speculate that the increased blood flow observed in the CC in the earliest phases of disease could be another compensatory mechanism in response to DCP impairment, in particular due to the high demand of oxygen supply to the non-vascularized outer retina. Similarly, *Lupidi et al.* recently reported a negative correlation between DCP and CC perfusion density in the very initial stages of DR, hypothesizing that, in case of DCP insufficiency, CC blood flow could increase to maintain sufficient oxygen supply.<sup>30</sup>

As for DM1, a recent 2-year longitudinal study on patients with DM1 and mild DR detected a progressive decrease in parafoveal perfusion in the DCP compared to healthy controls.<sup>17</sup> In the present study, despite a borderline significant reduction in PD in the SCP at baseline, the longitudinal analysis did not show a corresponding decreasing trend over time, however the small number of eyes in this group could have reduced the strength of the analysis.

Limitations of this study include a relatively small number of patients, impossibility to separately analyze the intermediate capillary plexus due to intrinsic limits of the instrument, and the lack of intermediate follow-up visits between baseline and the end of observation with possible intermediate reversible changes gone undetected. Moreover, possible confounders as body mass index and axial length have not been considered in the quantitative analyses performed. However, its major strengths

consist in its prospective longitudinal design, that at least in part compensates for the presence of potential confounders, and in the inclusion of only patients with DM1/DM2 with pre-clinical DRD.

In conclusion, in the present study we reported longitudinal data on eyes with DM1 and DM2 and no signs of DR throughout the study and we found significant microvascular changes in DM2 patients, while no changes have been detected in neuronal parameters and in DM1 patients. During 3 years no patient developed clinically detectable DR in a cohort of DM type 1 and 2 with good glycemic control. Microvascular changes in eyes with DM2 consist in decreased perfusion in the DCP along with a probably compensatory increased perfusion in the SCP and CC. It can be assumed that, as disease worsens due to poor glycemic control and/or progressive increase of DM duration, the capillary loss could exceed the compensatory effect of pre-existing capillaries dilatation leading to frank ischemia. Changes in eyes with DM1 and DRN could need more time to manifest in a population of patients with good glycemic control. Longer and larger longitudinal studies are needed to confirm these preliminary data and to better evaluate eyes with DM1 (and the specific role of insulin as a potentially protective factor for DR onset) and DRN progression in both DM groups.



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**Table 1. Main demographic and clinical data of the study population.**

<b>Group</b>	<b>Age (years)</b>	<b>BCVA (ETDRS score)</b>	<b>DM duration (years)</b>	<b>HbA1c (%)</b>
<b>Controls (24)</b>	44.1±10.2	85±0.0		
<b>DM1 (20)</b>	37.8±12.8	84.9±0.3	14.6±10.6	7.4±0.8
<b>DM2 (48)</b>	64.1±9.6	82.9±5.2	7.33±6.4	7.2±0.8

Values are reported as mean ± SD.

**Table 2. Baseline significant OCT and OCT-angiography parameters.**

Parameter	Controls	DM1	DM2	p value*
SCP PD (%)	27.3 ± 2.1*	25.6 ± 2.2*	26.1 ± 2.4	*0.034
SCP FD	1.56 ± 0.08* <sup>§</sup>	1.45 ± 0.01*	1.45 ± 0.02 <sup>§</sup>	* <sup>§</sup> <0.001
DCP FD	1.58 ± 0.06* <sup>§</sup>	1.50 ± 0.01*	1.49 ± 0.01 <sup>§</sup>	* <sup>§</sup> <0.001
SCP FAZ perimeter (mm)	2.15 ± 0.39*	2.52 ± 0.63	2.74 ± 0.51*	*0.006
SCP FAZ CI	0.76 ± 0.12* <sup>§</sup>	0.63 ± 0.12*	0.66 ± 0.1 <sup>§</sup>	*<0.001 <sup>§</sup> 0.001
DCP FAZ perimeter (mm)	2.42 ± 0.36* <sup>§</sup>	2.90 ± 0.63* <sup>#</sup>	3.36 ± 0.66 <sup>§#</sup>	*<0.001 <sup>§</sup> <0.001 <sup>#</sup> 0.02
DCP FAZ CI	0.92 ± 0.02* <sup>§</sup>	0.72 ± 0.1*	0.76 ± 0.09 <sup>§</sup>	* <sup>§</sup> <0.0001

\*One-way ANCOVA analyses: comparison among controls, patients with DM1 and patients with DM2, controlling the confounding effect of age. Statistical significance was set at p = 0.05. Values are reported as mean ± SD.

SCP = superficial capillary plexus; PD = perfusion density; DCP = deep capillary plexus; FD = fractal dimension; FAZ = foveal avascular zone; CI = circularity index.

**Table 3. Changes in OCT and OCT-angiography parameters within DM1 and DM2 groups.**

Group	Parameter	t <sub>0</sub>	t <sub>1</sub>	p value*
DM1	SF-NFL (μm)	6.5 ± 3.3	4.9 ± 1.7	0.03
	OR-GCL++ (μm)	66.6 ± 5.2	66.0 ± 4.8	<b>&lt;0.001</b>
	DCP PD (%)	29.57 ± 1.9	28.0 ± 1.4	0.002
	DCP FAZ perimeter (mm)	2.90 ± 0.6	3.23 ± 0.6	<b>&lt;0.001</b>
	DCP FAZ CI	0.72 ± 0.1	0.66 ± 0.1	0.01
DM2	IR-NFL	26.9 ± 6.0	24.9 ± 5.7	0.05
	OR-GCL++	104.8 ± 7.4	102.1 ± 7.3	<b>&lt;0.001</b>
	OR-NFL	41.2 ± 4.8	39.1 ± 3.7	0.002
	IR-GCL++	116.5 ± 8.7	114.6 ± 6.9	0.002
	SCP PD (%)	26.1 ± 2.4	27.4 ± 2.3	0.002
	SCP VD (%)	11.2 ± 1.3	12.2 ± 2.5	0.008
	DCP PD (%)	28.7 ± 2.1	27.2 ± 2.1	<b>&lt;0.001</b>
	DCP VD (%)	14.3 ± 1.0	13.9 ± 0.9	0.006
	CC-FD (%)	14.4 ± 0.9	13.8 ± 1.1	<b>&lt;0.001</b>
	DCP FAZ area (mm <sup>2</sup> )	0.69 ± 0.23	0.84 ± 0.3	<b>&lt;0.001</b>
	DCP FAZ perimeter (mm)	3.36 ± 0.66	3.78 ± 0.7	<b>&lt;0.001</b>

\*Paired t-test analyses. Statistically significant difference values with the Bonferroni's correction are in bold. Values are reported as mean ± SD.

SF = subfoveal; NFL = nerve fiber layer; OR = outer ring; GCL = ganglion cell layer; DCP = deep capillary plexus; PD = perfusion density; FAZ = foveal avascular zone; CI = circularity index; IR = inner ring; SCP = superficial capillary plexus; VD = vessel density; CC-FD = choriocapillaris flow deficits.

**Table 4. Longitudinal analyses of OCT parameters in the three study groups.**

Parameter (μm)		Controls	DM1	DM2
<b>CMT</b>	t <sub>0</sub>	253.3 ± 13.9	254.6 ± 14.9	242.3 ± 21.16
	t <sub>1</sub>	253.3 ± 14.26	251.5 ± 15.8	245.1 ± 24.3
	Δ%	<b>+0.0</b>	<b>+1.2</b>	<b>+1.1</b>
<b>SFNFL</b>	t <sub>0</sub>	4.2 ± 1.5	6.5 ± 3.3	5.2 ± 3.2
	t <sub>1</sub>	4.5 ± 1.4	4.9 ± 1.7	4.4 ± 2.8
	Δ%	<b>+8</b>	<b>-24.6</b>	<b>-15.3</b>
<b>IR-NFL</b>	t <sub>0</sub>	27.1 ± 1.7	26.7 ± 2.3	26.9 ± 6.0
	t <sub>1</sub>	27.2 ± 1.9	26.3 ± 1.5	24.9 ± 5.7
	Δ%	<b>+0.4</b>	<b>-1.6</b>	<b>-7.7</b>
<b>OR-NFL</b>	t <sub>0</sub>	42.5 ± 5.4	41.1 ± 3.9	41.2 ± 4.8
	t <sub>1</sub>	42.7 ± 5.5	40.2 ± 4.3	39.1 ± 3.7
	Δ%	<b>+0.3</b>	<b>-2.2</b>	<b>-5.1</b>
<b>SF-GCL+</b>	t <sub>0</sub>	51.3 ± 9.6	54.7 ± 6.1	47.3 ± 9.1
	t <sub>1</sub>	52.2 ± 9.4	53.2 ± 6.3	47.8 ± 9.6
	Δ%	<b>+1.8</b>	<b>-2.7</b>	<b>+1.2</b>
<b>IR-GCL+</b>	t <sub>0</sub>	93.9 ± 5.1	91.5 ± 6.5	88.5 ± 8.3
	t <sub>1</sub>	93.5 ± 5.3	91.3 ± 7.6	88.7 ± 5.2
	Δ%	<b>-0.4</b>	<b>-0.3</b>	<b>+0.2</b>
<b>OR-GCL+</b>	t <sub>0</sub>	65.7 ± 4.0	66.6 ± 5.2	63.8 ± 6.8
	t <sub>1</sub>	64.1 ± 4.1	66.0 ± 4.8	66.9 ± 27.7
	Δ%	<b>-1.6</b>	<b>-0.8</b>	<b>+4.9</b>
<b>SF-GCL++</b>	t <sub>0</sub>	55.4 ± 10.7	61.6 ± 8.8	52.4 ± 10.1
	t <sub>1</sub>	56.6 ± 10.4	58.2 ± 7.5	51.9 ± 11.0
	Δ%	<b>+2.2</b>	<b>-5.1</b>	<b>-0.7</b>
<b>IR-GCL++</b>	t <sub>0</sub>	120.9 ± 5.9	118.4 ± 7.4	116.5 ± 8.7
	t <sub>1</sub>	119.9 ± 7.6	117.2 ± 8.2	114.6 ± 6.9
	Δ%	<b>-0.8</b>	<b>-0.8</b>	<b>-1.7</b>
<b>OR-GCL++</b>	t <sub>0</sub>	108.3 ± 5.8	107.6 ± 7.9	104.8 ± 7.4
	t <sub>1</sub>	107.1 ± 6.4	106.0 ± 7.7	102.1 ± 7.3
	Δ%	<b>-1.1</b>	<b>-1.5</b>	<b>-2.6</b>

Values are reported as mean ± SD and percentage of variation.

CMT = central macular thickness; SF = subfoveal; NFL = nerve fiber layer; IR = inner ring; OR = outer ring; GCL = ganglion cell layer.



**Table 5. Longitudinal analyses of OCT-angiography parameters in the three study groups.**

Parameter		Controls	DM1	DM2
SCP PD (%)	t <sub>0</sub>	27.3 ± 2.1	25.6 ± 2.2	26.1 ± 2.4
	t <sub>1</sub>	26.8 ± 1.6	26.6 ± 2.1	27.4 ± 2.3
	Δ%	<b>-1.9</b>	<b>+3.9</b>	<b>+5.1*</b>
DCP PD (%)	t <sub>0</sub>	28.6 ± 2.4	29.57 ± 1.9	28.7 ± 2.1
	t <sub>1</sub>	27.5 ± 1.9	28.0 ± 1.4	27.2 ± 2.1
	Δ%	<b>-3.9</b>	<b>-5.3</b>	<b>-5.6*</b>
SCP VD (%)	t <sub>0</sub>	11.9 ± 1.2	11.4 ± 1.1	11.2 ± 1.3
	t <sub>1</sub>	12.1 ± 1.1	11.7 ± 2.8	12.2 ± 2.5
	Δ%	<b>+1.6</b>	<b>+2.5</b>	<b>+8.6</b>
DCP VD (%)	t <sub>0</sub>	14.7 ± 1.1	14.8 ± 1.1	14.3 ± 1.0
	t <sub>1</sub>	14.4 ± 0.9	14.6 ± 0.7	13.9 ± 0.9
	Δ%	<b>-2.3</b>	<b>-1.6</b>	<b>-2.8</b>
CC-FD (%)	t <sub>0</sub>	13.6 ± 1.0	13.7 ± 0.7	14.4 ± 0.9
	t <sub>1</sub>	13.4 ± 1.0	13.5 ± 0.7	13.8 ± 1.1
	Δ%	<b>-0.9</b>	<b>-1.3</b>	<b>-3.9*</b>
SCP FD	t <sub>0</sub>	1.56 ± 0.1	1.45 ± 0.0	1.45 ± 0.0
	t <sub>1</sub>	1.57 ± 0.1	1.46 ± 0.0	1.45 ± 0.0
	Δ%	<b>+0.1</b>	<b>+0.7</b>	<b>+0.5</b>
DCP FD	t <sub>0</sub>	1.58 ± 0.1	1.50 ± 0.0	1.49 ± 0.0
	t <sub>1</sub>	1.58 ± 0.1	1.49 ± 0.0	1.48 ± 0.0
	Δ%	<b>-0.3</b>	<b>-0.2</b>	<b>-0.5</b>
SCP FAZ area (mm <sup>2</sup> )	t <sub>0</sub>	0.29 ± 0.1	0.33 ± 0.2	0.39 ± 0.12
	t <sub>1</sub>	0.30 ± 0.1	0.34 ± 0.2	0.41 ± 0.1
	Δ%	<b>+2.1</b>	<b>+4.7</b>	<b>+4.9</b>
SCP FAZ perimeter (mm)	t <sub>0</sub>	2.15 ± 0.4	2.52 ± 0.6	2.74 ± 0.5
	t <sub>1</sub>	2.17 ± 0.4	2.57 ± 0.7	2.85 ± 0.5
	Δ%	<b>+0.9</b>	<b>+1.8</b>	<b>+4.1</b>
SCP FAZ CI	t <sub>0</sub>	0.76 ± 0.1	0.63 ± 0.1	0.66 ± 0.1
	t <sub>1</sub>	0.76 ± 0.1	0.62 ± 0.1	0.63 ± 0.1
	Δ%	<b>+0.2</b>	<b>-0.7</b>	<b>-3.3</b>
DCP FAZ area (mm <sup>2</sup> )	t <sub>0</sub>	0.44 ± 0.1	0.49 ± 0.18	0.69 ± 0.23
	t <sub>1</sub>	0.47 ± 0.1	0.55 ± 0.2	0.84 ± 0.3
	Δ%	<b>+6.4</b>	<b>+13.3</b>	<b>+22.1*</b>
DCP FAZ perimeter (mm)	t <sub>0</sub>	2.42 ± 0.4	2.90 ± 0.6	3.36 ± 0.66
	t <sub>1</sub>	2.51 ± 0.3	3.23 ± 0.6	3.78 ± 0.7
	Δ%	<b>+3.5</b>	<b>+11.3</b>	<b>+12.6*</b>

<b>DCP FAZ CI</b>	<b>t<sub>0</sub></b>	0.92 ± 0.0	0.72 ± 0.1	0.76 ± 0.09
	<b>t<sub>1</sub></b>	0.92 ± 0.0	0.66 ± 0.1	0.73 ± 0.1
	<b>Δ%</b>	<b>+0.0</b>	<b>-8.5</b>	<b>-3.2</b>

Values are reported as mean ± SD and percentage of variation.

SCP = superficial capillary plexus; PD = perfusion density; DCP = deep capillary plexus; VD = vessel density; CC-FD = choriocapillaris flow deficits; FD = fractal dimension; FAZ = foveal avascular zone; CI = circularity index.

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