# Analysis of Choroidal Structure Changes in Diabetic Patients without Diabetic Retinopathy: A Longitudinal Study

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## ABSTRACT

**Purpose:** To identify changes in choroidal structure in diabetic patients without diabetic retinopathy (DR) that led to choroidal thickening after one year of follow-up, by differentiating alterations in the stromal and vascular areas of the choroid.

**Methods:** Prospective observational cohort study in which 125 diabetic patients without DR were included, from October 2014 to December 2015. Patients received a complete ophthalmologic evaluation including spectral domain optical coherence tomography scans using enhanced depth imaging mode in the first visit (V1) and in a second visit after 12 months (V2). A 1500µm subfoveal choroidal area (total choroidal area [TCA]) was segmented into luminal area (LA) and stromal area (SA) using an image binarization technique. To assess the vascular status of the choroid, the choroidal vascularity index (CVI) was calculated as the proportion of LA to TCA. Generalized linear mixed-effects regression models were used.

**Results:** Of the 125 patients, 103 completed the study, nine of which developed DR (8.7%). SA was significantly higher at V2 than at V1 ($p$=0.010), while CVI was significantly lower ($p$=0.028). TCA and SA were negatively associated with axial length ($p$=0.010 and $p$=0.011, respectively) and positively with choroidal thickness CT ($p$<0.001). CVI was positively associated with axial length ($p$=0.001) and spherical equivalent ($p$<0.001). The presence of DR increased the CVI by 1.60% ($p$=0.042).

**Conclusion:** After 12 months of follow-up, diabetic patients without DR at baseline appear to have a thicker choroid at the expense of decreased vascularity and stromal thickening. These structural changes may be due to ischemic processes in choroidal vasculature, with subsequent hyperpermeability and increased extracellular matrix deposition, and may represent the primary event in diabetes before the onset of DR.

**Keywords:** choroidal thickness; choroidal vascularity index; diabetic retinopathy; image binarization; optical coherence tomography.

## RESUMO

**Objetivos:** Identificar as alterações na estrutura da coroideia em doentes diabéticos sem retinopatia diabética (RD) que levaram ao aumento da espessura da coroideia após um ano de seguimento, através da diferenciação entre alterações das áreas estromal e vascular da coroideia.

**Material e Métodos:** Estudo prospetivo observacional de coorte que incluiu 125 doentes diabéticos sem RD, decorrido entre outubro de 2014 e dezembro de 2015. Os doentes foram submetidos a exame oftalmológico completo incluindo tomografia de coerência ótica de domínio espetral em modo de enhanced depth imaging na primeira visita (V1) e numa segunda visita após 12 meses (V2). Através de uma técnica de binarização, segmentou-se uma área de coroideia subfoveal de 1500µm de largura (área coroideia total [ACT]) em área luminal (AL) e estromal (AS). Para avaliar o estado vascular da coroideia, calculou-se o índice de vascularização coroideia (IVC) como a proporção de AL para ACT. Foram utilizados modelos de regressão lineares generalizados de efeitos mistos.

**Resultados:** Dos 125 doentes, 103 completaram o estudo, nove dos quais desenvolveram RD (8.7%). A AS foi significativamente superior em V2 comparado com V1 ($p$=0.010), enquanto que o IVC foi significativamente inferior ($p$=0.028). A ACT e a AS revelaram-se negativamente associadas com o comprimento axial ($p$=0.010 e $p$=0.011, respetivamente) e positivamente associadas com a espessura da coroideia EC ($p$<0.001). O IVC encontrou-se positivamente associado com o comprimento axial ($p$=0.001) e com o equivalente esférico ($p$<0.001). A presença de RD aumentou o IVC em 1.60% ($p$=0.042).

**Conclusão:** Após 12 meses de seguimento, os doentes diabéticos sem RD de base parecem ter coroideias mais espessas à custa da diminuição da vascularização e aumento da espessura estromal. Estas alterações estruturais podem ser devidas a processos de isquémia da vasculatura coroideia, com consequente hiperpermeabilidade e aumento da deposição de matriz extracelular, e podem representar o evento primário da diabetes previamente ao desenvolvimento de RD.

**Palavras-chave:** espessura coroideia; índice de vascularização coroideia; retinopatia diabética; binarização de imagem; tomografia de coerência ótica.

## INTRODUCTION

The prevalence of type 2 diabetes mellitus (DM) is increasing worldwide. It is well known that DM affects all large and small vessels in the body, diabetic retinopathy (DR) being one of its most frequent and serious complications, making it a major cause of global blindness[1]. While DR has historically been the major study subject of diabetic microvascular complications in the eye and the appointed cause of decreased vision in these patients, recent studies also suggest the presence of a diabetic choroidopathy[2–6]. Several alterations on choroidal vasculature have been reported in diabetic patients, and it has also been hypothesized that these

changes can clarify the unexplained loss of visual acuity in diabetic eyes without evidence of DR[3,7].

The development of enhanced depth imaging (EDI) protocol for spectral domain optical coherence tomography (SD-OCT) facilitated the evaluation of the choroid in a noninvasive fashion and allowed to perform quantitative analysis of several parameters, the most common one being choroidal thickness (CT). Several studies have been conducted to assess CT in diabetic patients, most of which with DR[8–18]. However, as the CT changes are disputable among authors, there has not been a consensus about this subject yet[6].

Taking into account the variability of CT results in the evaluation of the choroid, the choroidal vascularity index (CVI) has been proposed as a marker for the choroid vascular health[19–25]. This parameter is obtained by applying an image binarization technique on EDI SD-OCT B-scans, which allows to distinguish between the vascular and stromal components of the choroid. It has been reported that CVI, unlike CT, is not significantly affected by physiological variables[19–21,23,26,27].

Our group has previously reported that diabetic patients without DR showed a thicker choroid and a thinner retina, particularly in inner layers, after one year of follow-up[28]. To the best of our knowledge, there are no longitudinal studies assessing image binarized EDI SD-OCT scans in diabetic patients without DR. In this study, our aim is to further identify and analyze the changes in choroidal structure that led to thickening after one year of follow-up, by differentiating alterations in the stromal and vascular areas of the choroid.

## MATERIALS AND METHODS

### Study design

This analysis was conducted as part of a prospective observational cohort study focusing on the identification of changes in CT, and in all retinal layers of diabetic patients without DR, after one year of follow-up, using EDI SD-OCT[28]. The data used for this study was collected between October 2014 and December 2015.

Board approval was obtained from the institutional ethics committee, and patients' informed consent was obtained. In addition, this research adhered to the principles of the Declaration of Helsinki.

### Study subjects

As part of the Portuguese nation-wide population screening program for DR, two-hundred and fifty consecutive type 2 diabetic patients were sent by primary care centres, and observed for inclusion and exclusion criteria. Diagnosis of type 2 DM was made following the guidelines of the Portuguese General Health Direction[29]. Type 2 diabetic patients without DR with normotensive eyes and with the ability to understand the study were included. The exclusion criteria were the following: refractive error > 5 diopters (D) or/and axial length > 25 mm in the studied eye, known diagnosis of DR or other retinal diseases, ocular hypertension or glaucoma, uveitis, neurodegenerative disease, and significant media opacities that precluded fundus imaging.

### Study procedures

After screening based on the inclusion and exclusion criteria, a total of 125 type 2 diabetic patients without DR were enrolled in the study.

Our study protocol has been described previously[28]. Briefly, participants performed two study visits: V1 (month 0) and V2 (month 12). At both visits all participants underwent comprehensive ophthalmological examination including best-corrected visual acuity (BCVA), anterior segment examination, intraocular pressure (IOP) measurement using Goldmann applanation and dynamic contour tonometries, dilated fundoscopic examination with fundus photography and SD-OCT. At V1, ultrasonic biometry was also performed to measure axial length. Furthermore, blood pressure (BP) was assessed and blood samples were collected for a fasting glucose test and glycosylated hemoglobin (HbA1c) quantification at each visit. One eye per patient was randomly selected for the study.

***Visual acuity.*** Best-corrected visual acuity at distance for each eye was measured using Snellen charts, which was then converted to logarithm of the minimum angle of resolution (logMAR) for analysis.

***Intraocular pressure.*** Intraocular pressure was measured with Goldmann applanation and dynamic contour tonometries, before pupillary dilation. A Pascal tonometer was used for this last measurement and IOP values with quality score $\leq 2$ were accepted.

***Fundus photography.*** After pupillary dilation and fundoscopic examination with a 90 D Volk® lens, two fundus photographs were taken: one centered on the fovea and another centered on the optic disc, using a Topcon TRC-50DX (Type IA)® camera.

***Spectral-domain optical coherence tomography imaging.*** Tomographic images were obtained using the Spectralis® SD-OCT (Heidelberg Engineering, Heidelberg, Germany, software version 6.0) after pupillary dilation, at the same time of day from 2 PM to 4 PM. All subjects had good media clarity and good quality scans were obtained, without artefacts and with signal strength > 20 (maximum = 40). Scans were performed using the EDI mode which sets the choroid closer to the zero-delay line and provide better visualization of the choroid, according to a previously reported method[30]. CT was measured from the outer portion of the hyperreflective line (corresponding to the retinal pigment epithelium [RPE]) to the hyporeflective line (corresponding to the sclerochoroidal interface). These measurements were made in the subfoveal choroid and at 500 µm temporal and nasal to the fovea. The average of these three measurements was used as an independent variable.

***Image binarization.*** The central scan passing through the foveal region was selected for image binarization. The modified Sonoda et al protocol by Agrawal et al[23,31] was used for segmentation (Figure 1). Image binarization was performed using a public domain software, ImageJ (version 1.51j8, provided by the National Institutes of Health, Bethesda, MD, USA; https://imagej.nih.gov/ij). After uploading the 1x1 pixel image of the OCT to ImageJ, the scale was set by converting pixels to microns using the horizontal scale present at the bottom of the image, so that a 1500 µm linear line centered at the fovea could be drawn parallel to the RPE. The images were then converted to 8-bit images to allow binarization with the application of Niblack's auto local threshold tool. Using the drawn line for guidance, the polygon tool was used to select the subfoveal choroid area with a 1500 µm width. The upper border was marked on the outer portion of the RPE and the lower border was marked below the line of light pixels at the sclerochoroidal interface. Only the 1500 µm width subfoveal area was used in this study as a representative segment of the macular region due to the segmental nature of the choroidal blood supply described by Hayreh[32] and because this single foveal scan's choroidal vascularity is representative of total macular choroidal vascularity in healthy subjects as reported by Agrawal et al[24]. This area (total choroidal area [TCA]) was added to the region of interest (ROI) manager. Subsequently, the area of vascularity was highlighted by converting the image back to Red Green Blue (RGB) and adjusting colour threshold. The selected area was added to the ROI manager and merged with TCA, and the resulting composite third area was also added to the ROI manager. TCA, the area of dark pixels (luminal area [LA]) and the area of light pixels (stromal area [SA]) were calculated. To determine the vascularity status of the choroid, CVI was calculated as the proportion of LA to TCA.

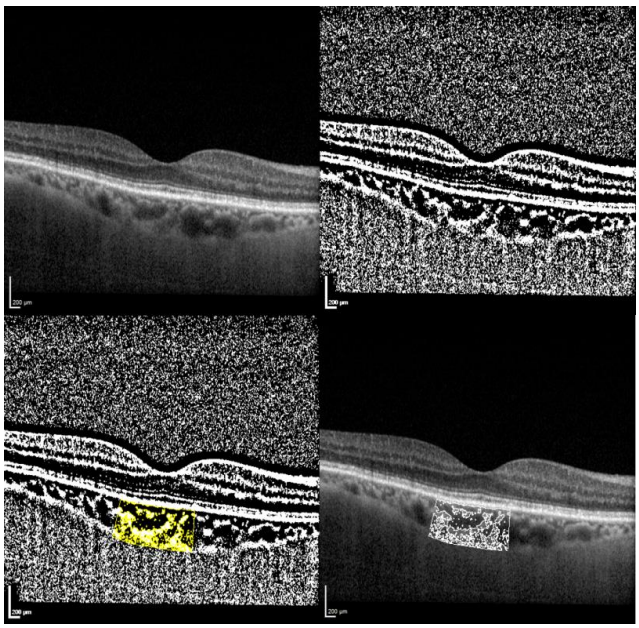

**Figure 1** - Enhanced depth imaging spectral domain optical coherence tomography scans conversion to binarized images. (Top left) A scan of a diabetic patient. (Top right) Image binarization is performed using Niblack's auto local threshold. (Bottom left) The region of interest of the choroid is demarcated. (Bottom right) An overlay of the region of interest of the binarized image and the optical coherence tomography scan which shows that the traced areas coincide with the dark areas of the scan (assumed as the vascular or luminal areas).

***Intra-observer and inter-observer variability.*** Thirty of the 175 images were segmented three times by one grader (R.F.) after an interval of one and two weeks to determine intra--subject variability. The same set of images was also segmented by two additional observers (D.H.F. and J.T.F.) to determine inter-subject variability.

***Mean arterial pressure.*** Blood pressure was measured in seated position by an automatic sphygmomanometer. Systolic and diastolic blood pressures (SBP and DBP) were recorded

and mean arterial pressure (MAP) was calculated using the following formula: MAP = DBP +1/3 (SBP – DBP).

## Statistical analysis

An exploratory analysis was carried out for all variables. Continuous variables were described using mean and standard deviation (SD), median and interquartile range (IQR: 25$^{th}$ percentile-75$^{th}$ percentile), as appropriate, as well as frequencies (percentages) for categorical variables.

To assess the intra-observer repeatability and inter--observer reproducibility of TCA and LA, intraclass correlation coefficients (ICCs) and corresponding 95% confidence intervals were calculated using linear mixed effects regression models. Larger values of ICCs indicate lower inter/intra observer variability, attaining a maximum of 1.

Univariable and multivariable linear mixed effects regression models were used to identify the variables which explained the variability of TCA, SA, and CVI in diabetic patients after one year of follow-up. The variables time (V1 and V2), gender, age, IOP-Goldmann, axial length, spherical equivalent, MAP, IOP, BCVA, and average choroidal thickness (average of the three measurements: subfoveal choroid, 500 µm temporal, and nasal to the fovea) were considered in this analysis. Those variables attaining a p-value <0.25 in the univariable analysis were selected as candidates for the multivariable models. Normality assumption of the residuals was verified using Kolmogorov--Smirnov goodness-of-fit test with Lilliefors correction.

Statistical analysis was performed using R software (R: A Language and Environment for Statistical Computing, R Core Team, R Foundation for Statistical Computing, Vienna, Austria, year = 2017, http://www.R-project.org). A level of significance α=0.05 was considered.

# RESULTS

## Patient demographics and clinical characteristics

A total of 125 type 2 diabetic patients without DR (63 males) with a mean age of 66.9 (9.3) years were eligible. As per protocol, these patients were asked to return for a second visit after 12 months. Of the initial 125 patients, 19 did not present to the second visit (18 were lost to follow-up and one had died). Of the remaining 106 patients, three presented epiretinal membranes in OCT scans and were thus excluded from further analysis. Overall, the attrition rate was 17.6% (n=22). Of the 103 patients who completed the study, nine developed DR (8.7%) (Figure 2).

Demographic and clinical characteristics from both visits are described in Table 1.

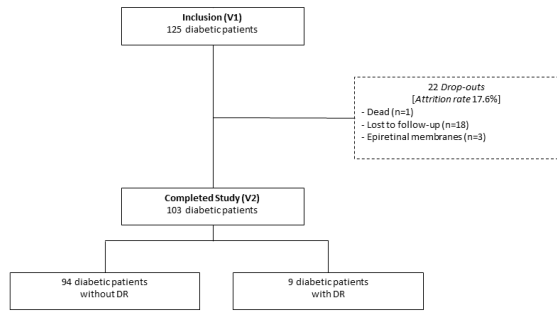


**Figure 2 -** Flow chart of patients during follow-up.

**Table 1 -** Patients' demographic and clinical characteristics in the two visits

| | Visit 1 (V1) | Visit 2 (V2) | |
|---|---|---|---|
| | **Diabetic patients (n=125)** | **Diabetic patients without DR (n=94)** | **Diabetic patients with DR (n=9)** |
| **Male gender, n (%)** | 63.00 (50.40) | 49.00 (52.10) | 4.00 (44.40) |
| **Age, years** | 66.90 (9.33) | 66.54 (8.76) | 75.33 (8.46) |
| **BCVA, logMAR** | 0.05 (0.10) | 0.05 (0.08) | 0.02 (0.03) |
| **IOP - Goldmann, mmHg** | 16.28 (3.08) | 16.43 (2.86) | 17.22 (2.33) |
| **IOP - Pascal, mmHg** | 19.06 (3.56) | 18.83 (3.00) | 18.27 (2.45) |
| **OPA** | 2.80 (2.30-4.20) | 2.95 (2.38-4.13) | 2.70 (2.15-4.15) |
| **Spherical equivalent** | 0.63 (1.53) | 0.60 (1.45) | 0.13 (1.38) |
| **Axial length, mm** | 23.11 (0.81) | 23.14 (0.79) | 23.17 (1.21) |
| **State of lens - phakic, n (%)** | 119.00 (95.20) | 91.00 (96.80) | 7.00 (77.80) |
| **Diabetes duration, months** | 60.00 (30.00-126.00) | 72.00 (36.00-132.00) | 180.00 (90.00-276.00) |
| **MAP, mmHg** | 97.00 (91.50-108.00) | 98.00 (92.75-103.00) | 101.00 (96.50-105.50) |
| **HbA1c, %** | 6.40 (6.00-7.00) | 6.50 (6.00-7.11) | 7.40 (6.55-8.05) |
| **Glycemia, mg/dL** | 137.00 (118.00-156.00) | 130.00 (112.00-145.00) | 136.00 (112.50-175.00) |

Results are expressed as mean (SD) or median (P25-P75). BCVA, best-corrected visual acuity; DR, diabetic retinopathy; HbA1c, glycated hemoglobin; IOP, intraocular pressure; MAP, mean arterial pressure; OPA, ocular pulse amplitude.

## Analysis of choroidal structure

Using image binarization, the intra-observer repeatability (ICC: 0.98; 95%CI: 0.96 to 0.99 for TCA and ICC: 0.98; 95%CI: 0.96 to 0.99 for LA), and inter--observer reproducibility (ICC: 0.98 to 0.99 for TCA and ICC: 0.99 to 1.00 for LA) were excellent for both TCA and LA.

Univariable analysis of the choroidal structure parameters showed that TCA and SA were higher at V2 and that CVI was lower for diabetic patients without DR (Table 2).

Time, average CT and axial length remained in the final multivariable models for TCA and SA (for univariable analysis of TCA and SA see Supplementary Tables 1 and 2, respectively). Results showed that after one year, the TCA and SA mean values increased 0.04 $mm^2$ and 0.02 $mm^2$, respectively, but only SA reached statistical significance (coefficient estimates=0.04 and 0.02; p=0.079 and 0.010, respectively). These two parameters were also positively associated with average CT, and for each µm increase in average CT, there was a mean increase of 0.04 $mm^2$ and 0.01 $mm^2$ (coefficient estimates=0.04 and 0.01, respectively; p<0.001). Regarding axial length, for each additional millimeter, TCA and SA mean values decreased 0.04 $mm^2$ and 0.02 $mm^2$, respectively (coefficient estimates=-0.04 and -0.02; p<0.001 and 0.011, respectively) (Table 3).

In the multivariable analysis for CVI, time, axial length, RD and spherical equivalent remained in the final model (for univariable analysis, see Supplementary Table 3). Results showed that after one year, CVI mean values decreased 0.54% (coefficient estimate=-0.54; p=0.028), for each mm increase of axial length there was a mean increase of 0.92% (coefficient estimate=0.92; p=0.001), the presence of DR increased 1.60% of CVI mean values (coefficient estimate=1.60; p=0.042), and for each diopter increase of the spherical equivalent there was a CVI mean increase of 0.62% (coefficient estimate=0.62; p<0.001). There were no other statistically significant associations between TCA, SA and CVI and any other factors including the group (Table 3).

**Table 2 -** Choroidal parameters in the two visits

| | Visit 1 (V1) | Visit 2 (V2) | |
|---|---|---|---|
| | **Diabetic patients (n=125)** | **Diabetic patients without DR (n=94)** | **Diabetic patients with DR (n=9)** |
| **Total choroidal area (TCA), $mm^2$** | 1.18 (0.32) | 1.27 (0.35) | 1.25 (0.34) |
| **Stromal area (SA), $mm^2$** | 0.42 (0.13) | 0.46 (0.14) | 0.44 (0.14) |
| **Choroidal vascularity index (CVI), %** | 65.03 (2.98) | 64.38 (2.58) | 65.28 (1.80) |
| **Average choroidal thickness, µm** | 254.24 (63.66) | 268.99 (66.80) | 259.00 (58.45) |

Results are expressed as mean (SD); DR, diabetic retinopathy; Average choroidal thickness, average of the three measurements: subfoveal choroid, 500 µm temporal, and nasal to the fovea.

**Table 3 -** Multivariable regression models results for TCA, SA and CVI.

| Model | Coefficient estimate | 95% CI | p-value |
|---|---|---|---|
| **ΔTCA V2-V1** | 0.036 | -0.004 | 0.076 |
| **Axial length** | -0.043 | -0.076 | -0.011 |
| **Average CT** | 0.041 | 0.037 | 0.045 |
| | | | |
| **ΔSA V2-V1** | 0.019 | 0.005 | 0.034 |
| **Axial length** | -0.019 | -0.033 | -0.004 |
| **Average CT** | 0.015 | 0.013 | 0.017 |
| | | | |
| **ΔCVI V2-V1** | -0.540 | -1.019 | -0.061 |
| **Axial length** | 0.918 | 0.367 | 1.470 |
| **Diabetic retinopathy** | 1.603 | 0.063 | 3.142 |
| **Spherical equivalent** | 0.624 | 0.307 | 0.941 |

ΔTCA V2-V1, ΔSA V2-V1 and ΔCVI V2-V1 correspond to the mean difference of TCA, SA and CVI measurements between V2 and V1, respectively; CT, choroidal thickness; CVI, choroidal vascularity index; SA, stromal area; TCA, total choroidal area; CI, confidence interval; p-values were obtained by generalized linear mixed effects regression models.

**Supplementary Table 1 -** Univariable regression analysis, dependent variable: total choroidal area.

| Model | Coefficient estimate | 95% CI | | p-value |
|---|---|---|---|---|
| **ΔTCA V2-V1*** | 0.085 | 0.054 | 0.117 | <0.001 |
| **Gender†** | 0.002 | -0.122 | 0.127 | 0.972 |
| **Age** | -0.010 | -0.017 | -0.004 | 0.003 |
| **BCVA** | -0.109 | -0.919 | 0.700 | 0.791 |
| **IOP (Goldmann)** | 0.012 | -0.008 | 0.031 | 0.232 |
| **IOP (Pascal)** | -0.008 | -0.025 | 0.009 | 0.357 |
| **OPA** | 0.029 | -0.008 | 0.066 | 0.119 |
| **Spherical equivalent** | 0.011 | -0.032 | 0.054 | 0.625 |
| **Axial length** | -0.133 | -0.204 | -0.062 | <0.001 |
| **Diabetes duration** | -0.001 | -0.001 | 0.002 | 0.148 |
| **Diabetic retinopathy** | -0.031 | -0.251 | 0.189 | 0.783 |
| **Average CT** | 0.044 | 0.040 | 0.047 | <0.001 |
| **MAP** | -0.002 | -0.004 | 0.001 | 0.128 |
| **SBP** | -0.002 | -0.003 | -0.001 | 0.028 |
| **DBP** | -0.001 | -0.004 | 0.002 | 0.535 |
| **HbA1c** | 0.004 | -0.040 | 0.049 | 0.850 |
| **Glycemia** | -0.001 | -0.002 | 0.000 | 0.309 |
| **Lens status‡** | 0.122 | -0.167 | 0.410 | 0.409 |
| **ACE inhibitors** | 0.018 | -0.112 | 0.148 | 0.787 |
| **ARBs** | -0.010 | -0.139 | 0.120 | 0.886 |
| **Beta blockers** | -0.012 | -0.172 | 0.148 | 0.883 |
| **Diuretics** | 0.036 | -0.088 | 0.160 | 0.569 |
| **Calcium channel blockers** | 0.078 | -0.070 | 0.227 | 0.301 |
| **Alpha-2 agonists** | -0.544 | -1.169 | 0.082 | 0.089 |
| **Oral antihyperglycemic agents** | -0.066 | -0.516 | 0.385 | 0.776 |
| **Insuline** | -0.189 | -0.477 | 0.098 | 0.196 |
| **Statins** | 0.063 | -0.071 | 0.197 | 0.358 |
| **Thiazides** | -0.235 | -0.684 | 0.213 | 0.304 |

*ΔTCA V2-V1 corresponds to the mean difference of TCA measurements between V2 and V1; †Reference category: female gender; ‡Reference category: phakic; ACE, angiotensin-converting enzyme; ARBs, angiotensin II receptor blockers; BCVA, best corrected visual acuity; CT, choroidal thickness; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; IOP, intraocular pressure; MAP, mean arterial pressure; OPA, ocular pulse amplitude; SBP, systolic blood pressure; TCA, total choroidal area; CI, confidence interval; p-values were obtained by generalized linear mixed effects regression models

**Supplementary Table 2 -** Univariable regression analysis, dependent variable: stromal area.

| Model | Coefficient estimate | 95% CI | | p-value |
|---|---|---|---|---|
| **ΔSA V2-V1*** | 0.357 | 0.022 | 0.050 | <0.001 |
| **Gender†** | 0.001 | -0.049 | 0.051 | 0.970 |
| **Age** | -0.004 | -0.006 | -0.001 | 0.010 |
| **BCVA** | 0.037 | -0.291 | 0.365 | 0.823 |
| **IOP (Goldmann)** | 0.004 | -0.004 | 0.012 | 0.292 |
| **IOP (Pascal)** | -0.003 | -0.010 | 0.004 | 0.410 |
| **OPA** | 0.012 | -0.004 | 0.273 | 0.131 |
| **Spherical equivalent** | -0.003 | -0.020 | 0.015 | 0.750 |
| **Axial length** | -0.052 | -0.081 | -0.024 | <0.001 |
| **Diabetes duration** | 0.000 | -0.001 | 0.001 | 0.081 |
| **Diabetic retinopathy** | -0.026 | -0.115 | 0.063 | 0.564 |
| **Average CT** | 0.016 | 0.014 | 0.018 | <0.001 |
| **MAP** | -0.001 | -0.002 | 0.001 | 0.210 |
| **SBP** | -0.001 | -0.001 | 0.001 | 0.072 |
| **DBP** | 0.000 | -0.001 | 0.001 | 0.615 |
| **HbA1c** | 0.000 | -0.019 | 0.019 | 0.975 |
| **Glycemia** | 0.000 | -0.001 | 0.001 | 0.476 |
| **Lens status‡** | 0.040 | -0.077 | 0.157 | 0.500 |
| **ACE inhibitors** | -0.001 | -0.053 | 0.052 | 0.971 |
| **ARBs** | 0.004 | -0.049 | 0.056 | 0.891 |
| **Beta blockers** | -0.006 | -0.071 | 0.059 | 0.857 |
| **Diuretics** | 0.015 | -0.036 | 0.065 | 0.567 |
| **Calcium channel blockers** | 0.023 | -0.038 | 0.083 | 0.461 |
| **Alpha-2 agonists** | -0.209 | -0.463 | 0.045 | 0.107 |
| **Oral antihyperglycemic agents** | -0.044 | -0.227 | 0.138 | 0.635 |
| **Insuline** | -0.070 | -0.187 | 0.046 | 0.238 |
| **Statins** | 0.022 | -0.033 | 0.076 | 0.433 |
| **Thiazides** | -0.078 | -0.260 | 0.104 | 0.400 |

*ΔSA V2-V1 corresponds to the mean difference of SA measurements between V2 and V1; †Reference category: female gender; ‡Reference category: phakic; ACE, angiotensin-converting enzyme; ARBs, angiotensin II receptor blockers; BCVA, best corrected visual acuity; CT, choroidal thickness; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; IOP, intraocular pressure; MAP, mean arterial pressure; OPA, ocular pulse amplitude; SA, stromal area; SBP, systolic blood pressure; CI, confidence interval; p-values were obtained by generalized linear mixed effects regression models.

**Supplementary Table 3 -** Univariable regression analysis, dependent variable: choroidal vascularity index

| Model | Coefficient estimate | 95% CI | | p-value |
|---|---|---|---|---|
| **ΔCVI V2-V1*** | -0.549 | -1.020 | -0.078 | 0.022 |
| **Gender†** | 0.157 | -0.775 | 1.089 | 0.741 |
| **Age** | 0.021 | -0.031 | 0.073 | 0.424 |
| **BCVA** | -4.345 | -10.353 | 1.663 | 0.156 |
| **IOP (Goldmann)** | -0.010 | -0.165 | 0.146 | 0.903 |
| **IOP (Pascal)** | 0.074 | -0.066 | 0.214 | 0.300 |
| **OPA** | -0.111 | -0.440 | 0.218 | 0.508 |
| **Spherical equivalent** | 0.439 | 0.127 | 0.752 | 0.006 |
| **Axial length** | 0.594 | 0.040 | 1.148 | 0.035 |
| **Diabetes duration** | 0.005 | 0.000 | 0.011 | 0.058 |
| **Diabetic retinopathy** | 1.342 | -0.288 | 2.973 | 0.107 |
| **Average CT** | -0.060 | -0.123 | 0.003 | 0.063 |
| **MAP** | 0.005 | -0.022 | 0.032 | 0.726 |
| **SBP** | 0.004 | -0.012 | 0.021 | 0.592 |
| **DBP** | 0.002 | -0.030 | 0.033 | 0.933 |
| **HbA1c** | 0.150 | -0.263 | 0.562 | 0.477 |
| **Glycemia** | 0.001 | -0.010 | 0.012 | 0.797 |
| **Lens status‡** | 0.269 | -1.899 | 2.437 | 0.808 |
| **ACE inhibitors** | 0.748 | -0.213 | 1.709 | 0.127 |
| **ARBs** | -0.385 | -1.353 | 0.584 | 0.436 |
| **Beta blockers** | 0.034 | -1.168 | 1.236 | 0.956 |
| **Diuretics** | 0.125 | -0.807 | 1.057 | 0.793 |
| **Calcium channel blockers** | 0.416 | -0.700 | 1.533 | 0.465 |
| **Alpha-2 agonists** | 1.966 | -2.773 | 6.704 | 0.416 |
| **Oral antihyperglycemic agents** | 1.928 | -1.429 | 5.286 | 0.260 |
| **Insuline** | 0.333 | -1.835 | 2.501 | 0.763 |
| **Statins** | 0.060 | -0.947 | 1.067 | 0.907 |
| **Thiazides** | -0.814 | -4.188 | 2.560 | 0.636 |

*ΔCVI V2-V1 corresponds to the mean difference of CVI measurements between V2 and V1; †Reference category: female gender; ‡Reference category: phakic; ACE, angiotensin-converting enzyme; ARBs, angiotensin II receptor blockers; BCVA, best corrected visual acuity; CT, choroidal thickness; CVI, choroidal vascularity index; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; IOP, intraocular pressure; MAP, mean arterial pressure; OPA, ocular pulse amplitude; SBP, systolic blood pressure; CI, confidence interval; p-values were obtained by generalized linear mixed effects regression models.

## DISCUSSION

In the present study, we evaluated choroidal structure changes in diabetic patients without DR in a one-year follow-up by measuring quantitative parameters, namely CT, TCA, SA, and CVI. A thickened choroid along with a higher SA and a lower CVI was evident, suggesting that the thickening of the choroid before the onset of DR is due to stromal thickening, with less contribution by the vascular components.

In recent years, we have assisted to a growing body of literature on diabetic choroidopathy[6,10,13–15,18,22,25,33–37]. To the best of our knowledge, our study is the first longitudinal study to examine TCA, SA and CVI in diabetic patients without DR. Most of the literature has focused on CT, with contradictory results: while many authors reported decreased CT in diabetic patients[8,9,11–13,17,18], others reported increased CT[13–17]. This inconsistency may be due to the fact that CT is affected by several physiological variables, namely age, axial length, refractive error, ocular perfusion pressure, IOP and BP[26,27].

As the pathophysiology of diabetic choroidopathy is not completely understood, it is still not clear if it represents a primary microvascular complication of DM independent of DR or if it occurs secondarily to retinal changes. As the choroid is responsible for the blood supplement to the outer retinal layers, it is logical to assume that changes in choroidal structure might play an important role in the development of DR. It has been shown in an animal model that choroidal blood flow deficit can be an early pathologic change in DR[38], and a study using laser Doppler flowmetry reported that choroidal blood flow significantly decreases in diabetic patients before the clinical manifestation of DR[39].

In our study, we found that CVI was significantly lower in the second visit, regardless of the presence of retinopathy. We postulate that the structural changes that occur in the choroids of diabetic patients may be due to ischemic changes in choroidal vasculature, with subsequent hyperpermeability and increased extracellular matrix deposition leading to higher SA and, accordingly, thicker choroids. We thus hypothesize that these changes may represent the primary event in diabetes before the onset of DR. An interesting find is that higher values of CVI were associated with the presence of DR. If before the onset of DR there seems to be less vascularized area, when the patients develop retinopathy, there may be inflammatory processes that then lead to dilation of the choroidal vessels, which may explain this association. However, the number of patients who developed DR was relatively small, which can bias this assumption.

A recent retrospective study by Kim et al[37] reported significantly reduced CVI values in diabetic patients,

regardless of DR stage, compared to healthy controls, the lower mean values being in the proliferative DR group ($p<0.001$). In agreement with our results, the authors also reported decreased CVI in diabetic patients without DR compared to healthy controls (67.07 [3.71]% vs 69.08 [2.290]%). Contrary to our results, Kim et al reported that, in the mild/moderate nonproliferative DR group, CT and CVI tended to diverge, with greater CT and significantly lower CVI compared with the no DR group[37]. As the disease progressed to severe nonproliferative DR and proliferative DR, the authors found a decreased CT and CVI, postulating that persistent choroidal hypoxia may lead to thinner and less vascular choroids, either due to choriocapillary loss or vascular constriction[37]. This adds to the body of evidence that diabetic choroidopathy may precede the retinal changes. Wang et al[25] also reported significantly reduced choroidal vascular density and volume in more advanced stages of DR, though they did not find significant differences in these indices in diabetic patients without DR when compared to controls. Tan et al[22] also found a reduced CVI in DR compared to controls (65.10 [0.20]% vs 67.20 [0.16]%, $p<0.001$), though the sample was relatively small and they did not perform the analysis according to the DR stage.

Our study has some limitations. First, the image binarization technique is based on the assumption that the dark areas represent the vascular areas and the light areas the stromal areas. While the findings of our, and earlier studies, reveal juxtaposition of the dark areas in the binarized images with the vascular choroidal components in the original OCT scans, there are no histopathological studies that prove this assumption. Second, our study measured a 1500µm width subfoveal area as a representative segment of the macular region. Although subfoveal CVI may be representative of total macular choroidal vascularity in healthy eyes[24], this may not be applicable in patients with choroidal disease, especially if the pathological process is localized. Third, the selection of the subfoveal area and CT measurement were done manually. However, as in our study, these manual measurements have already showed good inter-observer and intra-observer agreement[20,40].

In conclusion, the choroids of diabetic patients without DR seem to get thicker at the expense of increased stromal area and less vascularity after one year of follow-up, as they show increased SA and CT but a decreased CVI. This may represent a primary choroidal ischemic process that precedes the onset of DR. CVI, being a quantitative parameter to evaluate the choroidal vasculature, may prove to be a useful parameter to follow these patients.

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