REPEATABILITY OF CHOROIDAL THICKNESS MEASUREMENTS ASSESSED WITH SWEPT-SOURCE OPTICAL COHERENCE TOMOGRAPHY IN HEALTHY AND DIABETIC INDIVIDUALS

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Purpose: To assess the intrasession repeatability of choroidal thickness measurements obtained using swept-source optical coherence tomography in Type 2 diabetic (T2D) patients and healthy controls.

Methods: This was a single-center, prospective, observational, cross-sectional study with consecutive inclusion of 33 healthy subjects and 43 T2D patients. Subjects underwent three consecutive swept-source optical coherence tomography scans in a single session. After automatic delineation of the choroid, subfoveal choroidal thickness, and thickness at 500-μm intervals up to 2,500 μm nasal and temporal from the fovea were measured using the software caliper by the same operator. Intraclass correlation coefficients (ICCs), coefficients of variation, and test-retest variability were calculated.

Results: Mean subfoveal choroidal thickness in healthy subjects and in T2D patients was 229.97 ± 79.9 and 192.67 ± 74.3 μm, respectively (P = 0.013). All intrasession intraclass correlation coefficients were higher than 0.95 and 0.99, respectively. Coefficients of variations were less than 4.4% and 1.8%, respectively. Test-retest variability ranged from 0.76 μm to 11.12 μm and 0.64 μm to 6.29 μm, respectively. No significant differences were found in the intrasession repeatability of any choroidal measurement between healthy subjects and T2D patients.

Conclusion: Swept-source optical coherence tomography provided excellent intrasession repeatability of choroidal thickness measurements in healthy subjects and T2D patients.

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Optical coherence tomography (OCT) has rapidly evolved since its development in the early 1990s, with ongoing improvements allowing for a better understanding of ocular structures such as the optic nerve, retina, and choroid.[1] Continuous improvement has been made to the scanning speed, sensitivity, and depth for generating high-resolution cross-sectional imaging of OCT, providing the opportunity to explore this vascular tissue in better detail. Enhanced depth imaging (EDI) spectral domain (SD) OCT system[2–4] and more recently the incorporation of technology for deep range image swept-source (SS) OCT has permitted a more precise study of the choroid.[5] Swept-source optical coherence tomography uses a longer-wavelength light source than SD-OCT, which allows deeper penetration in the choroid than EDI SD-OCT and provides better layer segmentation of the sclerochoroid interface, without affecting resolution in the retina.[6,7] Moreover, the automatic segmentation software of SS-OCT makes the measurements more accurate and reproducible.[8] Although Adhi et al[9] reported no differences in macular choroidal thickness measurements

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between SS- and EDI SD-OCT, Tan et al[10] found that manual segmentation of SD-OCT may differ by more than 50 μm compared with the automated segmentation of SS-OCT.

The better visualization has led to more intensive investigations of this vascular structure that seems to have a significant role in different retinal pathologies.[11] Diabetic retinopathy (DR) is a neurovascular disease with a high prevalence and high socioeconomic impact. Recent studies revealed associations between an abnormal choroidal thickness and diabetes.[12–18] Evaluating choroidal changes may help clinicians to make better therapeutic decisions and to monitor the effect of treatments. For example, recent studies in patients with wet age-related macular degeneration and diabetes reported that choroidal thickness may predict the response to antiangiogenic agents.[19,20] Assessment of the accuracy of the measurements to compare them over time and differentiate true anatomic changes from the actual variability of the measurements is thus critical. Although other studies have evaluated the choroidal thickness SS-OCT's reproducibility in healthy eyes,[21–25] eyes of diabetic patients have not been evaluated. The purpose of this study was to evaluate the repeatability of choroidal thickness measurements using SS-OCT in healthy individuals and in patients with Type 2 diabetes (T2D).

Material and Methods

The Clinical Research Ethics Committee of Aragón (CEICA) approved the study protocol, which adhered to the tenets of the Declaration of Helsinki. This study was performed in a retrospective observational cross-sectional manner. All individuals from December 2015 to July 2016 who met the inclusion criteria and provided written informed consent were consecutively recruited for the study. Healthy patients were selected from healthy volunteers and study-naive patients with T2D were recruited from the Retina Unit of Miguel Servet University Hospital at Zaragoza (Spain).

Subjects were eligible if they were adults with a refractive error of less than 6 spherical diopters and/or 2 diopters cylinder, axial length $\leq$26 mm, and euthyroid. Exclusion criteria included opacity of the optical media that could interfere with the quality of the OCT (signal/noise ratio $<$70/100), preexisting retinal, choroidal, or optic nerve pathology, previous ocular treatment with laser (focal or panretinal photocoagulation) or intravitreal agents, inflammatory diseases or active or recent infection (ocular and/or systemic), systemic treatment with corticosteroids, immunosuppressive drugs or biologic therapies, pregnancy, and puerperium.

A full ophthalmologic examination was performed in all patients including clinical history, best-corrected visual acuity (BCVA, decimal scale), examination of the anterior segment using a slit-lamp, Goldmann applanation tonometry, and ophthalmoscopy of the posterior segment. Optical biometry (IOLMaster 500, Carl Zeiss Meditec AG, Jena, Germany) was used to measure axial length. In addition, a fasting blood sample was obtained from an arm vein to determine plasma glucose levels.

Diabetic Retinopathy Grading

Naive T2D patients were diagnosed based on the criteria of the American Diabetes Association,[26] and all were negative for anti-glutamic acid decarboxylase antibody. This group was divided according to the degree of DR into five subgroups based on the Diabetic Retinopathy Severity Scale criteria[27]: no DR, mild nonproliferative DR (NPDR), moderate NPDR, severe NPDR, and proliferative DR. Diabetic macular edema was assessed by clinical examination and SS-OCT imaging.

Choroidal Thickness Measurements Using Swept-Source Optical Coherence Tomography

Each SS-OCT (Topcon 3D deep range imaging OCT Triton [plus]; Topcon Corporation, Tokyo, Japan) scan comprised a 12-mm horizontal line scan protocol centered between the optic disc and the fovea with 1,024 A-scans for each 96 B-scan. The images were obtained by an experienced technician after pupil dilation with tropicamide 1% and phenylephrine 2.5% and performed at the same time of day in all patients (between 4:00 PM and 7:00 PM). Scan acquisition was realized with low ambient light looking at the internal fixation point to obtain the best alignment. Subjects underwent three consecutive SS-OCT scans in a single session. Between scans, patients sat back away from the device and rested at least for 2 minutes. The on-board segmentation algorithm (Topcon Advanced Boundary Segmentation; TABS) was used to automatically segment the choroidal layer from the outer edge of the hyper-reflective retinal pigment epithelial line to the inner edge of the sclera. After automatic delineation of the choroid on the B-scan, 11 thickness measurements were manually obtained using a caliper: 5 measurements nasal (N1, N2, N3, N4, and N5) and temporal (T1, T2, T3, T4, and T5) to the fovea were obtained at 500-μm intervals along with the subfoveal (SF) measurement (Figures 1 and 2). Each location was measured by the same operator within 2 weeks. The operator was masked to the patients' identity and clinical history. Automated segmentation errors of choroidal layers were defined as

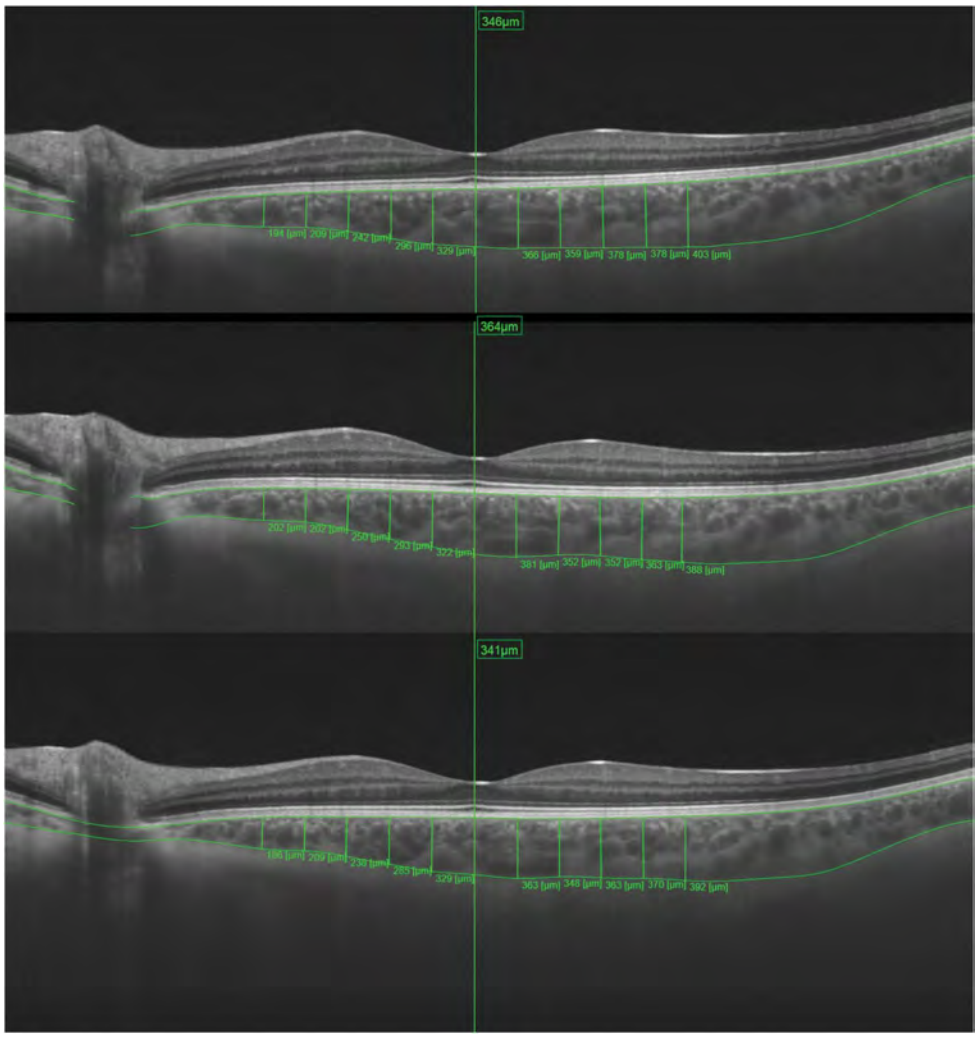

Fig. 1. Choroidal measurements obtained on the 3 different captured scans at SF level, and at 500-μm intervals up to 2,500 μm nasal and temporal from the fovea in a healthy individual.

instances in which the software determined choroidal boundaries that clearly deviated from the true anatomical boundaries. The automated segmentation errors were manually corrected by the same experienced observer. Scans with a lower quality (<70/100) were discarded.

Statistical Analysis

Statistical analyses were performed using IBM SPSS (version 23.0; IBM Corporation, Somers, NY) statistical software. All the variables followed a normal distribution as verified by the Kolmogorov–Smirnov test. For description of the clinical characteristics of the groups, the mean and standard deviation were used. The intraclass correlation coefficient (ICC), coefficient of variation (COV), and test-retest variability (TRTV) were calculated for each choroidal thickness.

Intraclass correlation coefficient is a statistic that condenses the reproducibility of a parameter for a given group of subjects. A large ICC suggests small fluctuations among repeated measurements in the same individual. The ICC value can range from 0 to a maximum of 1.

Coefficients of variation were calculated as the standard deviation divided by the average of the measured values expressed as a percentage. Test-retest variability in choroidal thickness was calculated as two times the standard deviation of the three repeated measurements for each choroidal thickness variable.

Differences between quantitative parameters were tested by Student's t test, and qualitative variables were compared by the chi-square test. The mean of the three scans was used for comparison of the choroidal measurements. For all analyses, $P < 0.05$ was considered statistically significant.

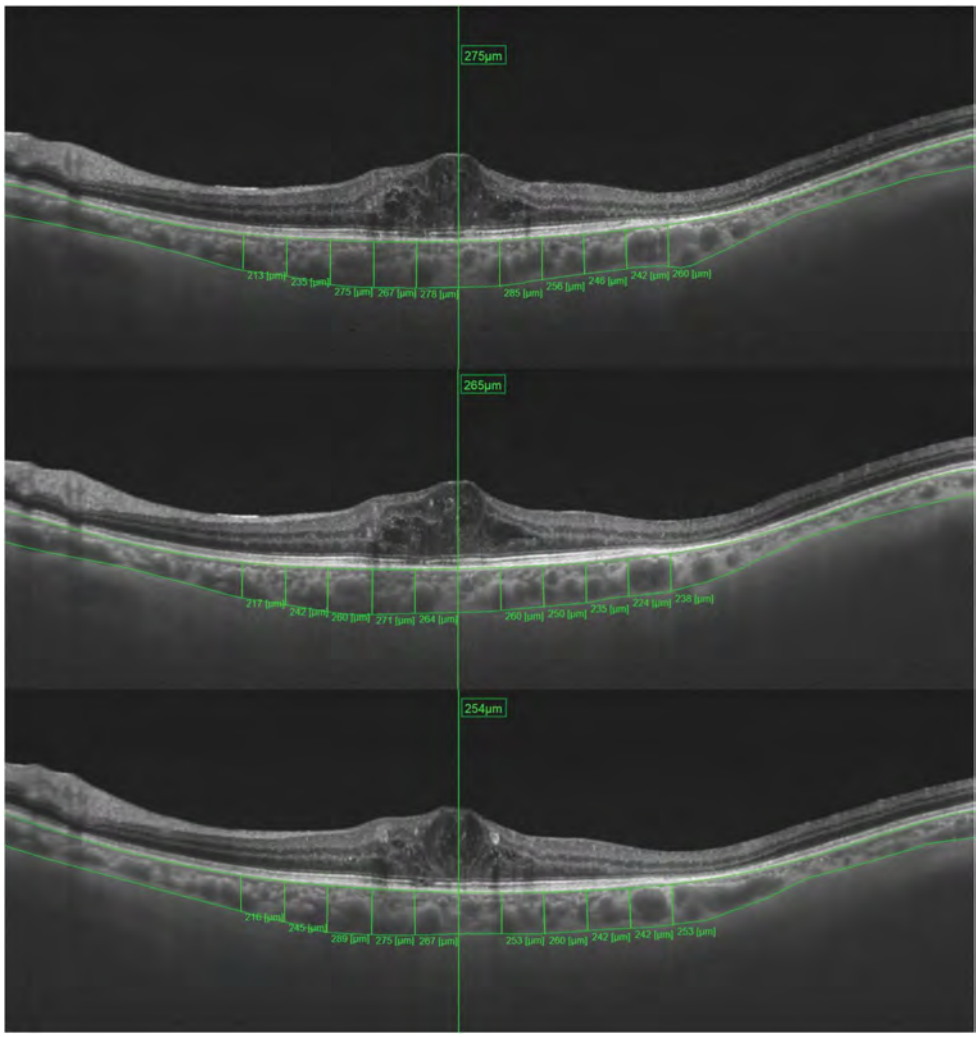

Fig. 2. Choroidal measurements obtained on the 3 different captured scans at SF level, and at 500-μm intervals up to 2,500 μm nasal and temporal from the fovea in a diabetic patient with diabetic macular edema.

Results

Overall, 110 eyes of 76 Caucasian individuals were included in this prospective study. All participants completed the study. The healthy group comprised 50 eyes of 33 patients, and the T2D group included 60 eyes of 43 patients: 7 eyes without DR, 13 eyes with mild NPDR, 32 eyes with moderate NPDR, 5 eyes with severe NPDR, and 3 eyes with proliferative DR. Diabetic macular edema was diagnosed in 30 eyes (50%). Mean patient age in the healthy and T2D groups was 68.02 ± 8.8 and 66.28 ± 7.8 years, respectively (Table 1). Overall, no statistically significant differences were detected between healthy controls and the T2D group regarding age, intraocular pressure, quality of scan, accuracy of automatic layer segmentation, or laterality. The groups differed significantly regarding sex, BCVA, and choroidal thickness in SF, N1, T1, T2, T3, T4, and T5 measurements.

Table 2 shows ICC and 95% confidence interval (CI), COV, and TRTV in the whole sample. All ICCs were higher than 0.98 ($P < 0.001$) with a 95% CI close to 1 in all positions. Coefficient of variation was <2% for all choroidal measurements, and the maximum variability (TRTV) observed was 6.78 μm in the N5 position.

In the healthy group, ICCs were excellent (>0.95; $P < 0.001$) for all choroidal measurements (Table 3). The SF choroidal thickness had the highest ICC (1; 95% CI 1–1; $P < 0.001$) and the N5 choroidal thickness exhibited the lowest ICC (0.965; 95% CI 0.944–0.979; $P < 0.001$). All COVs were under 4.5%, with the highest value (4.38%) in the N5 choroidal measurement and the lowest (0.20%) in the SF choroidal measurement. Test-retest variability ranged from 0.76 μm to 11.12 μm. The lowest value corresponded to the SF measurement and the highest to the N5 position.

Table 1. Clinical Characteristics in Healthy Subjects and Type 2 Diabetic Patients

	Healthy Subjects	T2D Patients	*P*
Age (years)	68.02 ± 8.8	66.28 ± 7.8	0.28*
BCVA (Snellen)	0.88 ± 0.2	0.69 ± 0.3	<0.001*
IOP (mmHg)	16.08 ± 2.4	16.85 ± 3.6	0.17*
Plasma glucose (mg/dL)	94.64 ± 12.5	143.07 ± 45.8	<**0.001***
Quality scan	94.34 ± 4.4	93.13 ± 5.2	0.20*
Choroidal thickness SF (μm)	229.97 ± 79.9	192.67 ± 74.3	**0.013***
Choroidal thickness N1 (μm)	228.83 ± 81.4	191.43 ± 75.3	**0.014***
Choroidal thickness N2 (μm)	218.11 ± 79.6	189.04 ± 78.1	0.06*
Choroidal thickness N3 (μm)	197.92 ± 81.1	171.04 ± 75.6	0.08*
Choroidal thickness N4 (μm)	173.56 ± 81.7	149.74 ± 70.0	0.10*
Choroidal thickness N5 (μm)	150.00 ± 75.4	129.42 ± 67.2	0.13*
Choroidal thickness T1 (μm)	225.08 ± 73.0	189.51 ± 67.4	**0.009***
Choroidal thickness T2 (μm)	222.95 ± 72.4	188.61 ± 68.7	**0.012***
Choroidal thickness T3 (μm)	223.53 ± 71.5	187.78 ± 68.3	**0.009***
Choroidal thickness T4 (μm)	218.19 ± 69.5	180.37 ± 63.0	**0.003***
Choroidal thickness T5 (μm)	213.23 ± 69.3	173.94 ± 63.1	**0.002***
Female-male (%)	39-11 (78%–22%)	21-39 (35%–65%)	<**0.001**†
Right/left (%)	26-24 (52%)	31-29 (51.7%)	0.97†
Accurate segmentation	45-5 (90%)	50-10 (80%)	0.31†
n	50	60	

Choroidal thicknesses and quality scan are expressed as the mean of the three scans. Inaccurate segmentation was considered when the three scans had automated segmentation errors. Significant differences are highlighted in bold print. Data are expressed in mean ± SD, except sex, laterality, accuracy of layer segmentation, and number of cases.

*Student's *t* test.

†Chi-square test.

BCVA, best-corrected visual acuity; IOP, intraocular pressure; n, number of cases; N1, nasal 500 μm from fovea; N2, nasal 1,000 μm from the fovea; N3, nasal 1,500 μm from the fovea; N4, nasal 2,000 μm from the fovea; N5, nasal 2,500 μm from the fovea; T1, temporal 500 μm from the fovea; T2, temporal 1,000 μm from the fovea; T3, temporal 1,500 μm from the fovea; T4, temporal 2,000 μm from the fovea; T5, temporal 2,500 μm from the fovea.

Intrasession repeatability of all choroidal thickness measurements in the T2D group is shown in Table 4. Intraclass correlation coefficients ranged from 0.981 (N2) to 1 (SF; $P < 0.001$). Coefficients of variation ranged from 0.17% (SF) to 1.79% (N2), and TRTV ranged from 0.64 μm (SF) to 6.29 μm (N2).

No significant difference ($P > 0.05$) was found in the intratest repeatability of any choroidal measurement between healthy controls and T2D patients.

Discussion

In recent years, investigations have begun to focus on the role played by the choroid in DR.[15] This increase in interest is related to the development of improvements in OCT, because of technology such as EDI, and more recently to SS-OCT. These advances have brought faster scanning speeds and a reduction in artefacts that allow for better visualization of this

Table 2. Intrasession Repeatability of Choroidal Thicknesses in the Whole Sample

	ICC	ICC, 95% Lower Bound	ICC, 95% Upper Bound	*P*	COV ± SD (%)	TRTV ± SD (μm)
SF	1	1	1	<0.001	0.18 ± 0.3	0.69 ± 1.2
N1	0.995	0.993	0.996	<0.001	0.96 ± 3.0	3.50 ± 11.2
N2	0.984	0.978	0.988	<0.001	1.79 ± 5.1	6.40 ± 19.6
N3	0.995	0.993	0.996	<0.001	1.39 ± 3.2	4.54 ± 10.6
N4	0.995	0.993	0.996	<0.001	1.45 ± 3.7	4.10 ± 10.5
N5	0.982	0.975	0.987	<0.001	2.75 ± 7.6	6.78 ± 18.3
T1	0.999	0.999	0.999	<0.001	0.76 ± 1.2	2.65 ± 3.7
T2	0.985	0.979	0.989	<0.001	1.21 ± 3.6	5.39 ± 17.1
T3	0.994	0.992	0.996	<0.001	1.12 ± 3.9	3.73 ± 10.3
T4	0.987	0.983	0.991	<0.001	1.51 ± 4.6	5.29 ± 14.6
T5	0.985	0.980	0.996	<0.001	1.43 ± 5.8	4.65 ± 16.1

N1, nasal 500 μm from the fovea; N2, nasal 1,000 μm from the fovea; N3, nasal 1,500 μm from the fovea; N4, nasal 2,000 μm from the fovea; N5, nasal 2,500 μm from the fovea; SD, standard deviation; T1, temporal 500 μm from the fovea; T2, temporal 1,000 μm from the fovea; T3, temporal 1,500 μm from the fovea; T4, temporal 2,000 μm from the fovea; T5, temporal 2,500 μm from the fovea.

Table 3. Intrasession Repeatability of Choroidal Thicknesses in Healthy Subjects

	ICC	ICC, 95% Lower Bound	ICC, 95% Upper Bound	*P*	COV ± SD (%)	TRTV ± SD (μm)
SF	1	1	1	<0.001	0.20 ± 0.4	0.76 ± 1.3
N1	0.999	0.999	1	<0.001	0.72 ± 1.0	2.89 ± 2.9
N2	0.986	0.977	0.991	<0.001	1.80 ± 4.9	6.53 ± 18.2
N3	0.990	0.985	0.994	<0.001	2.01 ± 4.6	6.85 ± 15.2
N4	0.991	0.985	0.994	<0.001	2.16 ± 5.3	6.25 ± 15.2
N5	0.965	0.944	0.979	<0.001	4.38 ± 11.0	11.12 ± 26.4
T1	0.998	0.957	0.999	<0.001	0.97 ± 1.6	3.40 ± 4.7
T2	0.968	0.950	0.981	<0.001	1.88 ± 5.2	8.74 ± 24.8
T3	0.988	0.981	0.993	<0.001	1.47 ± 5.7	4.78 ± 14.9
T4	0.974	0.959	0.984	<0.001	2.29 ± 6.7	8.07 ± 21.1
T5	0.970	0.952	0.982	<0.001	2.30 ± 8.8	7.81 ± 23.5

N1, nasal 500 μm from the fovea; N2, nasal 1,000 μm from the fovea; N3, nasal 1,500 μm from the fovea; N4, nasal 2000 μm from the fovea; N5, nasal 2,500 μm from the fovea; SD, standard deviation; T1, temporal 500 μm from the fovea; T2, temporal 1,000 μm from the fovea; T3, temporal 1,500 μm from the fovea; T4, temporal 2,000 μm from the fovea; T5, temporal 2,500 μm from the fovea.

structure and therefore a better understanding of this vascular tissue.[11]

Knowledge of the behavior of the choroid in healthy subjects will elucidate the changes of the choroid in diabetic patients. Previous studies reported that the choroid undergoes significant changes throughout the day, with age and with axial length, in healthy individuals.[28–32] In diabetic patients, choroid thickness seems to decrease,[13–17] although other authors have reported the opposite finding (thicker choroid in diabetic patients).[12] Panphotocoagulation and antiangiogenic treatment may decrease choroidal thickness over the long-term[12,33–35] and choroidal thickness may predict the response to antiangiogenic treatment, where a greater thickness predicts a better anatomic and functional result after the injection.[20]

Overall, T2D patients presented with a thinner choroid than healthy subjects. In both groups, measurements revealed a similar pattern: the choroid was thickest in the SF location, followed by temporal and nasal measurements close to the SF area. The choroid was thinner in the temporal and nasal measurements far away from the SF area, and thinnest in the nasal choroid near the optic disc. Our results agree with previous reports by Regatieri et al, Querques et al, and Esmaeelpour et al that diabetic patients have a thinner choroid.[14–16]

Variability of measurements for any test may be critical for accurate diagnosis, follow-up, and assessment of the response to treatment. Repeatability of choroidal measurements can improve our understanding of the detection, progression, and response to treatment of DR, where the choroid may have an important role. To interpret these changes in choroidal thickness it is crucial to understand the test variability. We found that choroidal measurements acquired with SS-OCT had low variability (high ICCs and low COVs) for healthy and diabetic eyes.

To the best of our knowledge, this study is the first to report the intratest repeatability of 11 choroidal measurements with SS-OCT in a large population of healthy and diabetic patients. The results of

Table 4. Intrasession Repeatability of Choroidal Thicknesses in T2D Patients

	ICC	ICC, 95% Lower Bound	ICC, 95% Upper Bound	*P*	COV ± SD (%)	TRTV ± SD (μm)
SF	1	1	1	<0.001	0.17 ± 0.3	0.64 ± 1.2
N1	0.990	0.984	0.993	<0.001	1.17 ± 3.0	4.02 ± 15.0
N2	0.981	0.972	0.988	<0.001	1.79 ± 5.4	6.29 ± 20.9
N3	0.999	0.999	1	<0.001	0.86 ± 1.0	2.62 ± 2.9
N4	0.999	0.999	1	<0.001	0.85 ± 1.0	2.30 ± 2.4
N5	0.999	0.998	0.999	<0.001	1.39 ± 1.5	3.17 ± 3.6
T1	0.999	0.999	1	<0.001	0.59 ± 0.7	2.03 ± 2.3
T2	0.999	0.998	0.999	<0.001	0.66 ± 0.8	2.60 ± 3.5
T3	0.999	0.998	0.999	<0.001	0.84 ± 1.1	2.85 ± 3.4
T4	0.999	0.998	0.999	<0.001	0.86 ± 1.0	2.98 ± 3.3
T5	0.999	0.999	1	<0.001	0.71 ± 0.9	2.01 ± 2.2

N1, nasal 500 μm from the fovea; N2, nasal 1,000 μm from the fovea; N3, nasal 1,500 μm from fovea; N4, nasal 2,000 μm from the fovea; N5, nasal 2,500 μm from the fovea; SD, standard deviation; T1, temporal 500 μm from the fovea; T2, temporal 1,000 μm from the fovea; T3, temporal 1,500 μm from the fovea; T4, temporal 2,000 μm from the fovea; T5, temporal 2,500 μm from the fovea.

intraobserver repeatability were excellent. We obtained ICCs values close to one in all choroidal locations in the whole sample and in both healthy and diabetic groups.

In the healthy group, choroidal thickness at N5 (2,500 μm from the fovea) exhibited the worst repeatability values. This may be due to the presence of the optic nerve in the nasal zone, which could make measuring more difficult, and also to the decrease in choroidal thickness peripherally. In T2D patients, choroidal thickness at N2 (1,000 μm from the fovea) showed the worst repeatability values. Although no significant differences were found in the intratest repeatability between healthy controls and T2D patients, diabetics tended to have better repeatability values than healthy controls. This could be due to the thinner choroidal thicknesses in diabetics, which would decrease the range of change of the same variable.

Previous studies evaluated the reproducibility of choroidal thickness measurements using SD-OCT, especially in healthy and young populations[21–25,36,37]; few of these studies, however, were based on SS-OCT technology.[38,39] Shao et al[25] studied the intraobserver reproducibility of 21 healthy eyes (mean age, 63.1 ± 10.6 years) with EDI SD-OCT. They scanned 10 times with 1-minute breaks between each examination. They found an ICC of 1 ($P < 0.001$) and a mean COV of 0.85 ± 1.48%. Mansuri et al[39] studied intraobserver reproducibility with SS-OCT in 54 eyes of 27 healthy subjects (mean age, 36.6 ± 10.4 years) with 4 different scanning protocols, one of which was the same as ours, a 12-mm horizontal line centered on the fovea in undilated patients. Each scan protocol was repeated three times consecutively on the same visit, similar to our study, and the ICC was 0.93 (95% CI: 0.91–0.95). Sim et al[37] studied reproducibility in a cohort of 51 eyes of 51 patients with T2D (mean age: 60.1 ± 13.6 years) using SD-OCT, a manual segmentation made by 2 different graders, and calculation of mean choroidal thicknesses within Early Treatment Diabetic Retinopathy Study (ETDRS) areas. They found an ICC of 0.97 (95% CI: 0.94–0.99). Our results confirmed the low variability of choroidal thickness measurements acquired with SD-OCT and SS-OCT.

A strength of our study is that this was a prospective study of 11 choroidal measurements with SS-OCT in a large sample, including healthy and T2D patients. Another strength was that we used automatic detection and segmentation software to delineate choroidal structures, which theoretically allows for a more accurate and objective analysis, although errors of segmentation had to be manually corrected in 10% to 20% of images. A limitation of this study was that we did not perform vertical or radial scans and, consequently, our results are based only on the horizontal axis measurements. Another limitation is that the T2D group was characterized by different stages of DR, which limits any conclusion on choroidal differences between the two groups. In addition, clinicians should take into account that only good-quality scans were included in the statistical analysis, which might have influenced the upper and lower limits, as real-world practice includes patients with cataracts, poor fixation, and larger refractive errors. Further studies using SS-OCT are needed to elucidate the differences in choroidal thickness between diabetic and healthy eyes.

In conclusion, intrasession repeatability of choroidal thickness measurements in healthy and T2D patients obtained with SS-OCT was excellent. Clinicians must take into account the repeatability of every parameter to differentiate normal variability from significant clinical changes.

Key words: choroid, choroidal thickness, diabetic retinopathy, repeatability, swept-source OCT.

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