ANALYSIS OF AGE-RELATED CHOROIDAL LAYERS THINNING IN HEALTHY EYES USING SWEPT-SOURCE OPTICAL COHERENCE TOMOGRAPHY

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Purpose: To study the changes in the choroidal layers thickness with age in a healthy population using swept-source optical coherence tomography.

Methods: Retrospective data analysis of a subgroup of eyes from a previous single-center, prospective, cross-sectional, noninterventional study. One hundred and sixty-nine healthy eyes were evaluated using swept-source optical coherence tomography. Inclusion criteria were best-corrected visual acuity between 20/20 and 20/25, spherical equivalent between ±3 diopters, and no systemic or ocular diseases. Two independent investigators determined the macular horizontal choroidal thickness (CT) and the Haller’s layer thickness across a 9 mm line centered at the fovea. Subjects were divided into five age groups.

Results: Mean subfoveal choroidal thickness was 305.76 ± 80.59 μm (95% confidence interval: 294.85–319.33). Mean subfoveal thickness for Haller’s layer was 215.47 ± 67.70 μm (95% confidence interval: 207.30–227.86) and mean subfoveal thickness for choriocapillaris plus Sattler’s layer was 87.31 ± 40.40 μm (95% confidence interval: 83.38–95.65). No significant differences were found due to gender. Choroidal thickness profile was similar between groups with choroidal thickness and Haller’s layer thickness decreasing with age (P = 0.002).

Conclusion: Choroidal and Haller’s layer thickness profiles are similar between different age groups. Age-related choroidal thinning is mostly at the expense of Haller’s layer.

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The choroid is a highly vascular, pigmented tissue located between the retina and the sclera, responsible for the oxygenation and nourishment of the outer retina.1 Its study has been updated during the past few years due to breakthroughs in imaging technology. Previous testing such as fluorescein angiography and indocyanine green angiography provide valuable information about the choroid but are not able to do quantification or cross-sectional images.2–4 B scan ultrasonography and Doppler technology permit such cross-sectional imaging but with resolution that does not allow study of the individual layers. It was not until the development of the optical coherence tomography (OCT) that the “in vivo” study of the choroid was possible in a reproducible, quantifiable, and reliable fashion.5 The choroid’s posterior location and the light dispersion induced by the retinal pigment epithelium made it initially difficult to study the choroid in its full thickness through OCT. Enhanced-depth imaging OCT6 and more recently longer-wavelength, deep-penetration swept-source OCT (SS-OCT) provide high-resolution images of the choroid and the choroiscleral interface contour and shape.7–11

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The choroid plays a role in the development and progression of a wide variety of ocular diseases such as age-related macular degeneration,12–14 central serous chorioretinopathy,15,16 uveitis,17,18 and high myopia19 among others. Age-related thinning has been documented,20–28 as well as variations in the choriocapillaris layer.29 The “in vivo” anatomy of the healthy choroid has to be established to define its alterations.

Spaide stated that choroidal thinning is a manifestation of a loss of visible vessels and small-vessel disease (in patients with higher prevalence of glaucoma or age-related macular degeneration than similarly aged controls) that other authors call senile choroidal sclerosis with the findings of vascular sheathing and obliteration of choroidal channels.31 Histopathological findings show profound atrophy of the choroid with loss of small and medium vessels.20,31

This vascular sclerosis may even provide a potential explanation of the high prevalence of glaucoma in Spaide’s sample, with vascular sclerosis leading to a reduced blood supply to the prelaminar portion of the optic nerve referred as senile sclerotic glaucoma.20 Choriocapillaris loss has also been related to the production of vascular endothelial growth factor due to the presence of a deprived retinal pigment epithelium and outer retina, which may receive less oxygen and metabolites than needed.32

The purpose of this paper is to study the possible age-related thickness variations of different choroidal layers in the macular area.

**Patients and Methods**

The data and images of a specific population subgroup from a previously published prospective study were analyzed.22,29,30 This study followed the tenets of the Declaration of Helsinki and the original study was approved by the institutional review board of Vissum Alicante. Data were collected from December 2011 to January 2013. Participation was offered to subjects, attending routine ocular examinations, who voluntarily agreed to participate, provided they met the inclusion criteria, with no limit of age and signed an informed consent. Inclusion criteria were best corrected visual acuity between 20/20 and 20/25, spherical equivalent between ±3 diopters (D) and no systemic or ocular diseases (other than cataract). Patients with prior history of any retinal pathology in either eye were not included. All examinations were obtained in the afternoon to avoid diurnal variations (between 16:00 and 20:00 hours).33

The macular area of all patients was studied with a SS-OCT system (Topcon Corp, Tokyo, Japan). The SS-OCT device used to image the full-thickness choroid and sclera is equipped with a tunable laser as a light source operated at 100,000 Hz A-scan repetition rate in the 1-μm wavelength region. A one-line scanning mode, which produces an OCT image that contains 1,024 axial scans with a scan length of 12 mm was used (averaging up to 96 B-scans). This sampling space in object space corresponds to 11.7 μm/pixel. Lateral resolution is set to be 20 μm with 24 mm axial eye length, whereas axial resolution is 8 μm in retina. Lateral and axial resolution is independent.
Acquisition time was 1 second that permitted high quality images even in subjects as young as 3 years old. A horizontal CT profile of the macula was manually created measuring CT (from the posterior edge of retinal pigment epithelium to the choroid/sclera junction) under the fovea using the prototype software. The outer aspect of the lamina fusca, rather than the outer limit of the choroidal vessels, was the landmark used to determine the most distal aspect of the choroid. Five further determinations were performed every 1,000 mm temporal (T1, T2, T3, T4, and T5) and 3 more nasal (N1, N2, and N3) to the fovea (Figure 1). Mean macular CT value was the average value of all nine determinations.

Following the methods proposed by Branchini et al for choroidal vasculature analysis, large choroidal vessels measuring 100 μm or more, and within the closest proximity to the locations of the CT measurement points were identified and the distance from their innermost edge to the choroid/sclera junction was calculated to obtain the Haller’s layer thickness (large choroidal vessels) (Figure 2). The measurements of this layer were subtracted from the total CT at the same nine locations to obtain the choriocapillaris plus the Sattler’s layer (medium choroidal vessels) thicknesses. As stated by these authors, current OCT devices do not have enough resolution to differentiate between these 2 layers yet, so they were analyzed together. Those locations in which the choroid was 100 μm or thinner were not taken into account for statistical analysis.

To analyze the possible evolution of the CT, the study group was divided into 5 sub-groups according to age distribution: 0 years to 10 years (n = 40), 11 years to 20 years (n = 25), 21 years to 40 years (n = 27), 41 years to 60 years (n = 38), and older than 60 years (n = 39). If both eyes of the same patient met the inclusion criteria, the eye with the highest image quality was used. An experienced technician determined refractive errors and best corrected visual acuity using an auto-refractometer (Nidek, Gamagori, Japan) that was later checked by a certified optometrist. Two independent observers determined the clear delineation of both choroidal limits (retinal pigment epithelium and choroido-scleral junction) and measured the thickness of the choroid and its layers. Results were expressed in mean ± standard deviation (SD).

### Statistical Analysis

Data obtained were statistically analyzed using a licensed version of SPSS 17.0 for Windows (SPSS, Chicago, IL). The inter-observer reproducibility was evaluated using intraclass correlation coefficient (ICC) for each variable measured (mean and 95% confidence interval), coefficient of variation between graders, and Bland Altman plots.

The average value of the two observers’ determinations was used for the rest of calculations. Kolmogorov–Smirnov test was applied for all data samples to check normality. Comparison between groups was performed using the Student t test when samples were normally distributed or Mann–Whitney test when parametric statistics were not possible. The level of significance used was P < 0.05. The homogeneity of variances was checked using the Levene test. For the comparison of several independent samples the Analysis of Variance (ANOVA) or Kruskal–Wallis test were used depending on whether normality could be assumed.

## Results

CT was manually measured in 169 eyes from 169 patients. Mean age was 33.5 ± 24.9 years (range 3–
Mean spherical equivalent was +0.10 ± 1.36 D (+3 to −3). Mean subfoveal CT was 305.76 ± 80.59 μm (95% confidence interval: 294.85–319.33). Mean subfoveal thickness for Haller’s layer was 215.47 ± 67.70 μm (95% confidence interval: 207.30–227.86) and mean subfoveal thickness for choriocapillaris plus Sattler’s layer was 87.31 ± 40.40 μm (95% confidence interval: 83.38–95.65).

CT was less than 100 μm thick in 31 eyes at N3, 7 at N2 and 1 at N1. These cases were not taken into account to avoid any possible errors, and due to the large number of eyes that did not reach 100 μm at N3, this point was not taken into account for the statistical analysis in any of the patients. Mean macular horizontal CT was 278.24 ± 63.87 μm (95% confidence interval: 269.00–288.82). Mean macular horizontal Haller’s layer thickness was 191.93 ± 52.41 μm (95% confidence interval: 185.47–201.73) and mean macular horizontal choriocapillaris plus Sattler’s layer thickness was 83.72 ± 28.82 μm (95% confidence interval: 80.83–89.78) (Table 1). The horizontal choroidal layers’ profiles can be seen in Figure 3.

No statistically significant differences in choroidal, Haller, or choriocapillaris plus Sattler’s layers thickness were found in men compared with women (Figure 2). Both sexes showed similar profiles for all three measurements (Figure 4).

Mean subfoveal choroidal thickness of the different age study groups established decreased progressively when comparing the younger group with the group aged older than 60 years (P = 0.029; Kruskal–Wallis test). Mean subfoveal Haller’s thickness was 233.39 ± 49.47 μm in the 0 year to 10 years group, 235.51 ± 77.49 μm in the 11 years to 20 years group, and then progressively decreased to reach 190.32 ± 77.01 μm in the older than 60 years group (P = 0.063; Kruskal–Wallis test). Mean subfoveal choriocapillaris plus Sattler’s thickness did not show this trend towards a progressive thinning with age (P = 0.444; Kruskal–Wallis test) (Table 3).

Mean macular horizontal CT of the different age groups showed a statistically significant progressive reduction from the 0-year to 10 years old group to the older than 60 years old group (P < 0.001; Kruskal–Wallis test). Mean horizontal Haller’s layer thickness

![Fig. 4. Choroidal, Haller’s and choriocapillaris plus Sattler’s layer thickness in men compared with women (measurements in micrometers).](image)

<table>
<thead>
<tr>
<th>Table 2. Men vs. Women</th>
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<tbody>
<tr>
<td><strong>Men (N = 103)</strong></td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Spherical equivalent, D</td>
</tr>
<tr>
<td>Mean choroidal thickness, 95% CI</td>
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<tr>
<td></td>
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<tr>
<td>Mean Haller thickness, 95% CI</td>
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<tr>
<td>Mean Sattler thickness, 95% CI</td>
</tr>
</tbody>
</table>

Table 2. Men vs. Women

| **Men (N = 103)**      | **Women (N = 66)**     | **Comparison (Student t Test), P** |
|------------------------|
| **Age**                |                      |                                  |
| Spherical equivalent, D |                      |                                  |
| Mean choroidal thickness, 95% CI |                      |                                  |
| Mean Haller thickness, 95% CI |                      |                                  |
| Mean Sattler thickness, 95% CI |                      |                                  |

CI, confidence interval.
also decreased in a similar way from the younger group to the older than 60 years old group ($P = 0.002$; Kruskal–Wallis test). Mean horizontal choriocapillaris plus Sattler’s layer thickness did not show this trend towards a progressive thinning either from the 0 year to 10 years old group or the older than 60 years old group ($P = 0.109$; Kruskal–Wallis test) (Table 2).

CT profile was similar among the different age groups with both CT and Haller’s layer thickness decreasing with age ($P = 0.002$) (Figure 5). Choriocapillaris plus Sattler’s layer thickness showed an age-dependent reduction trend as well, but the difference was not statistically significant ($P = 0.109$). The choroid and Haller’s layer are thinnest in the most nasal aspect, they grow to reach their thickest levels at subfoveal locations and then they steadily decrease towards the temporal sector. On the other hand, the choriocapillaris plus Sattler’s layer was thinnest in the nasal side reaching its thickest point under the fovea; however, its thickness remained stable during the first 4,000 μm towards the temporal sectors and then slightly decreased at 5,000 μm from the fovea (Figure 5).

**Correlation Analysis and Linear Regression Models**

The thickness reduction of the choroid, Haller’s, and Sattler’s layers correlated with age. This correlation was stronger for the whole choriocapillaris thickness and Haller’s layers and weaker for the choriocapillaris plus Sattler’s layer (Table 2). Multiple regression analysis models predicted are as follows:

1. Macular choroidal thickness (μm) = 295.187 \( -0.931 \times \text{Age (years)} \) \( r^2 = 0.130, P < 0.001 \)
2. Haller’s layer thickness (μm) = 207.029 \( -0.694 \times \text{Age (years)} \) \( r^2 = 0.149, P < 0.001 \)
3. Sattler’s layer thickness (μm) = 88.159 \( -0.237 \times \text{Age (years)} \) \( r^2 = 0.035, P = 0.005 \)

Mean CT was reduced by 0.93 μm per year. Mean Haller’s layer thickness was responsible for 0.69 μm, whereas Sattler’s layer was accountable for 0.24 μm per year, suggesting that Haller’s layer thinning was responsible for almost 75% of CT reduction with age.
Table 3. Age-related choroidal thickness variations

<table>
<thead>
<tr>
<th>Age Groups, Years</th>
<th>Mean SFT (mean ± SD), 95% CI</th>
<th>Mean HSFT (mean ± SD), 95% CI</th>
<th>Mean SSFT (mean ± SD), 95% CI</th>
<th>Mean CT (mean ± SD), 95% CI</th>
<th>Mean HT (mean ± SD), 95% CI</th>
<th>Mean ST (mean ± SD), 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to &lt;10</td>
<td>325.59 ± 55.71</td>
<td>233.39 ± 49.47</td>
<td>83.29 ± 34.15</td>
<td>304.04 ± 46.70</td>
<td>216.45 ± 39.44</td>
<td>87.79 ± 25.38</td>
</tr>
<tr>
<td>10 to &lt;20</td>
<td>325.83 ± 91.73</td>
<td>233.51 ± 47.86</td>
<td>88.65 ± 34.15</td>
<td>304.39 ± 46.70</td>
<td>210.05 ± 54.21</td>
<td>92.36 ± 30.86</td>
</tr>
<tr>
<td>20 to &lt;40</td>
<td>313.86 ± 81.10</td>
<td>228.61 ± 63.51</td>
<td>92.57 ± 34.15</td>
<td>277.93 ± 65.00</td>
<td>177.65 ± 47.64</td>
<td>92.36 ± 30.86</td>
</tr>
<tr>
<td>40 to &lt;60</td>
<td>295.80 ± 79.16</td>
<td>216.12 ± 41.07</td>
<td>103.39 ± 34.15</td>
<td>259.50 ± 62.16</td>
<td>171.83 ± 59.92</td>
<td>77.49 ± 22.94</td>
</tr>
<tr>
<td>≥60</td>
<td>273.46 ± 88.32</td>
<td>192.57 ± 54.88</td>
<td>111.39 ± 34.15</td>
<td>250.86 ± 64.09</td>
<td>170.33 ± 77.01</td>
<td>70.09 ± 38.56</td>
</tr>
</tbody>
</table>

If we take into account older than 40 years old patients only, Multiple regression analysis models predicted are as follows:

1. Macular choroidal thickness (μm) = 310.13 – 1.00 × Age (years) r² = 0.12, P = 0.023

This predicts a reduction of 1.00 micron per year.

Gender had no influence in mean CT or that of any of its layers. The influence of spherical equivalent on mean CT was not analyzed in this study as patients had to fit within the ±3D limit established as inclusion criteria.

Intraclass correlation coefficient in CT for the 2 independent observers varied between 0.961 and 0.978 for CT and between 0.706 and 0.787 for Haller’s layer thickness (Table 3). There were no statistically significant differences between variation coefficients obtained by each observer (P > 0.05; Wilcoxon test).

Discussion

The choroid has been proven to become thinner with age.20 In a previous study our research group evaluated the choroid of healthy individuals across a wide age span (3–95 years old) finding that macular CT profile in healthy population was similar among the different age groups, and that healthy choroid showed a trend to become thinner with age, particularly comparing adults older than 40 years with children and younger adults. These differences were not attributable to gender and greater age-related CT variations appeared in the temporal sectors.22 Analyzing our results, it can be stated that the choroid has a wide extent of normal thickness, with less than 5% of healthy population presenting CT thicker than 320 μm and no differences in CT between sexes.

SS-OCT (1,040 nm) was chosen to perform this study due to its reported precision and accuracy, permitting determination of full choroidal thickness in up to 100% of the eyes in previous papers.11,21 Other devices were not so reliable while determining the limit between the sclera and the choroid.24,35,36 CT was successfully determined in 100% of the eyes in this study. Esmaeelpour et al used a spectral domain optical coherence tomography (840 nm) with a raster protocol image consisting of 512 A-scans and 512 B-scans across a 36° × 36° field to automatically analyze the choroidal layers of 45 healthy eyes with a mean age of 44 years (ranging from 23 to 84 years old), although using a different method.37 Mean CT through the areas analyzed was 228 μm and the thickness of the Haller’s layer and Sattler’s layer + choriocapillaris were 141 μm and 87 μm, respectively.37
Adhi et al27 studied 24 eyes of 24 patients with a mean age of 64 years. CT measurements were manually performed using the caliper provided by the spectral domain optical coherence tomography device and the choroid-scleral limit was well delimited in 79% of the eyes. The imaging protocol used consisted of one-line rasters centered at the fovea. Subfoveal CT was 276 μm, subfoveal Haller’s layer thickness was 224 μm, and subfoveal Sattler’s layer + choriocapillaris was 52 μm.27

Using the same method, Branchini et al34 analyzed the choroidal layers of 42 eyes of 42 patients with a mean age of 52 years, locating the choroid-sclera limit in 92% of them. They manually measured CT and its layers at the subfoveal location using 2 more measurement points 750 μm away from the fovea in both the nasal and the temporal sectors. They found the subfoveal CT of their sample to be of 256 μm. Haller’s layer and Sattler’s layer + choriocapillaris thickness were 204 and 53 μm, respectively.34

In the present study, 169 eyes of 169 patients were analyzed using a SS-OCT device, being the largest sample analyzing choroidal layers to the best of our knowledge. The mean age was lower that other studies mostly at the expense of Haller’s layer. There were 2 cases with choroidal sclerosis whose retinoscopy showed well-demarcated areas of choroidal sclerosis that are probably in relation with atrophic age-related macular degeneration, whereas our study analyzes healthy patients. This age-related choroidal thinning could be a key factor to establish the role of the choroid in retinal pathology.

The limitations of this study are the facts that it is retrospective, the choriocapillaris could not be differentiated from Sattler’s layer due to the limitations in imaging technology and that despite the high intraclass correlation coefficient (Table 4), the measures for all layers were manually determined.

According to our results, CT and Haller’s layer grow progressively thinner as patients grow older and their profiles are similar between different groups of age. Age-related choroidal thinning seems to be mostly at the expense of Haller’s layer. There were no differences due to gender.

Key words: choroidal layers, Sattler, Haller, swept-source OCT, choroidal thickness, age-related, eye, imaging, choroid.

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Author contributions: Conception and design of the work/project: J. M. Ruiz-Moreno; Acquisition of data:

<table>
<thead>
<tr>
<th>Table 4. Correlation analysis</th>
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<tbody>
<tr>
<td>ICC (Choroid)</td>
</tr>
<tr>
<td>N3 0.967 (0.956–0.976)</td>
</tr>
<tr>
<td>N2 0.978 (0.970–0.984)</td>
</tr>
<tr>
<td>N1 0.972 (0.962–0.979)</td>
</tr>
<tr>
<td>SF 0.977 (0.970–0.983)</td>
</tr>
<tr>
<td>T1 0.977 (0.969–0.983)</td>
</tr>
<tr>
<td>T2 0.978 (0.970–0.984)</td>
</tr>
<tr>
<td>T3 0.972 (0.962–0.979)</td>
</tr>
<tr>
<td>T4 0.964 (0.952–0.973)</td>
</tr>
<tr>
<td>T5 0.961 (0.948–0.971)</td>
</tr>
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</table>

Mean and 95% CI.

 ICC, intraclass correlation coefficient.
J. Ruiz-Medrano and I. Flores-Moreno; Conceptualization of the manuscript and review and synthesis of the literature: J. Ruiz-Medrano and J. M. Ruiz-Moreno; Analysis and interpretation of data: J. Ruiz-Medrano, I. Flores-Moreno, J. A. Montero, and J. M. Ruiz-Moreno; Critical review and revision of the manuscript: J. Ruiz-Medrano, I. Flores-Moreno, J. A. Montero, J. S. Duker, J. García-Feijóo, and J. M. Ruiz-Moreno; Drafting of the manuscript: J. Ruiz-Medrano; Final approval of the version to be published: J. Ruiz-Medrano, I. Flores-Moreno, J. A. Montero, J. S. Duker, J. García-Feijóo, and J. M. Ruiz-Moreno; Providing language help: J. S. Duker, J. García-Feijóo, and J. A. Montero.

Detail has been removed from this case description/these case descriptions to ensure anonymity. The editors and reviewers have seen the detailed information available and are satisfied that the information backs up the case the authors are making.

References