Normal Value Ranges for Central Retinal Thickness Asymmetry in Healthy Caucasian Adults Measured by SPECTRALIS SD-OCT Posterior Pole Asymmetry Analysis

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PURPOSE. To determine the normal variation in central retinal thickness asymmetry in healthy Caucasian adults using the posterior pole asymmetry analysis (PPAA) of a SPECTRALIS spectral-domain optical coherence tomography (SD-OCT) device.

METHODS. Healthy Caucasian individuals aged between 18 and 45 years with a visual acuity of minimum 20/20 and a spherical equivalent between −1.5 and +1.5 diopters were recruited. Retinal thickness and retinal nerve fiber layer thickness (RNFL) were measured using SPECTRALIS SD-OCT. Inter- and intraocular differences in central retinal thickness were calculated using the PPAA. The association between age, sex, and interocular asymmetry was evaluated by a linear model with Gaussian correlation structure.

RESULTS. A total of 105 individuals, 30 men and 75 women, were studied. The mean age ± SD was 28.8 ± 7.87 years. The grand mean interocular retinal thickness asymmetry was 5.6 μm (95% confidence interval [CI]: 4.6–6.5) and the grand mean intraocular retinal thickness asymmetry was 8.3 μm (95% CI: 6.8–9.9) in the right eye and 8.4 μm (95% CI: 6.7–10.0) in the left eye. The highest local asymmetries were found in the nasal corners of macula were the posterior pole thickness map overlaps the temporal vascular arches. A slight general age and sex effect on the mean interocular retinal thickness asymmetry was found to be respectively 0.04 μm/year (95% CI: 0.02–0.06 μm) and 0.54 μm (95% CI: 0.19–0.88 μm) for men compared with women.

CONCLUSIONS. Statistically significant physiological asymmetries in inter- and intraocular central retinal thickness exist. This must be considered when early signs of glaucoma or other pathologies are evaluated based on the retinal thickness asymmetry. (http://www.controlled-trials.com/isrctn/number, ISRCTN09017572.)

Keywords: posterior pole retinal thickness asymmetry analysis, normal value ranges for retinal thickness asymmetry, glaucoma posterior segment

Spectral-domain optical coherence tomography (SD-OCT) is considered one of the most promising image modalities in evaluating glaucoma, because of its ability to image both the optic nerve head, the retinal nerve fiber layer (RNFL), and the macula with high reliability, speed, and resolution.

Several previous studies have indicated the importance of retinal structural changes in glaucoma patients, due to the loss of retinal ganglion cells (RGCs) and nerve fibers. The functional visual field loss often cannot be detected until approximately 25% to 40% of retinal ganglion cells are lost; therefore, detection of glaucomatous changes may instead focus on early retinal structural changes in order to prevent irreversible blindness.

Circumpapillary RNFL thickness measurement is the primary structural investigation tool used in glaucoma diagnosis and management. Because RNFL and RGCs constitute 30% to 55% of the retinal thickness of the macula, the macular thickness has additionally been considered a valuable structural measurement in the diagnosis of glaucoma.

Investigations have indicated that the RNFL thickness is the more sensitive and specific measurement of the two for detecting glaucoma. In addition, the intereye RNFL asymmetry has also been proposed as a useful clinical measurement for assessing early glaucomatous damage.

In 2011, Heidelberg Engineering (SPECTRALIS SD-OCT, Heidelberg, Germany) customized the most recent retinal thickness protocol to obtain retinal thickness measurements of the central 20° of the posterior pole. The posterior pole retinal thickness map is a color-coded map that provides a mean retinal thickness value of an 8 × 8 grid centered on the foveal pit. The grid is positioned symmetrically to the fovea-disc axis. Each cell of the grid represents a square area of 3 × 3° of the posterior pole. Concurrently, the posterior pole asymmetry analysis protocol (PPAA) was created. This protocol compares retinal thickness measurements of the corresponding cells in...
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The retinal thickness map between the eyes and between the two hemispheres within each eye. The asymmetry map is displayed as a grayscale depiction of difference in thickness from 0 to 30 μm (see Fig. 1).

A few studies23–25 detected inter- and intraocular retinal thickness asymmetry in preperimetric glaucoma, and therefore concluded that retinal thickness asymmetry may be the first sign of glaucoma, and that the PPAA thus can be used in the early diagnosis and later follow-up of glaucoma.

Sullivan-Mee et al.24 used the PPAA to compare retinal thickness asymmetry in 50 healthy individuals and 50 subjects with early primary open-angle glaucoma over the age of 40. In their study, RNFL asymmetry and grand mean, superior, and inferior retinal thickness asymmetry were investigated. The results of this study indicated that macular thickness exhibits remarkable symmetry between paired eyes in normal individuals and suggested that retinal thickness asymmetry may be more capable of early identification of structural changes in glaucoma than either global RNFL thickness or RNFL thickness asymmetry.24

Although the PPAA may have an important role in the diagnosis of early glaucomatous changes, this new technique has not been investigated to its full extent. Currently we have no normative data regarding the physiological asymmetry in healthy individuals using the entire 64-cell PPAA protocol. This makes it difficult to distinguish between normal physiological asymmetries from asymmetries that may be early signs of glaucoma or other pathology.

In order to determine any subtle physiological asymmetry that may exist, our study investigated healthy adults aged younger than 45 years, comparing all 64 cells of the asymmetry grid in the PPAA. To our knowledge, this is the first study to present normal value ranges for asymmetry using the entire PPAA-protocol.

METHODS

Study Design

This study is an observational cross-sectional study. The study was carried out at the department of ophthalmology of Thy-Mors Hospital in Denmark, from March to July 2014. The study followed the tenets of the Declaration of Helsinki, and was approved by the ethics committee (Region Nordjylland, Denmark). The study was registered in the International Standard Randomized Controlled Trial Number Register (No. ISRCTN09017572). Informed consent was obtained from all participants after explaining the nature of the study.

Participants

We recruited 118 healthy Caucasian individuals aged between 18 and 45 years among the staff of Thy-Mors Hospital, relatives of patients, and from the local nursing school. Subjects with a spherical equivalent (SE) between −1.5 and +1.5 diopters (D), and a best corrected visual acuity of 20/20 or better in both eyes were enrolled. Individuals with a history of ophthalmic disease, including elevated ocular pressure, or chronic systemic illness, were excluded.

Data Collection

Retinal thickness and retinal RNFL measurements were obtained for both eyes of each subject by a single operator (JH) using the central 20° volume scan mode and the 12° circle scan mode of a SPECTRALIS SD-OCT device (software version 5.6b, viewing module version 5.6.8.0; Heidelberg Engineering).25 All scans were performed without pupil dilation using eye tracking software (TruTrack; Heidelberg Engineering).26 Subjects were instructed to fixate on the internal fixation target prior to each scan.

After data acquisition an independent operator (AGJ) evaluated each scan for correct segmentation and for morphological changes in the macula or RNFL thickness.

Participants with any detectable macular pathology or with one or more RNFL thickness segments measured out of normal limits were excluded.

The center of the fovea, and fovea-disc axis in the posterior pole retinal thickness map were manually adjusted. Posterior thickness values were calculated automatically for all 64 cells. In case of missing values for any of the 64 cells due to low valid pixel percentage, correct segmentation was reassessed. If no incorrect segmentation was found, the missing value was replaced by the average of the four nearest neighboring field values. Valid pixel percentage describes the percentage of the area of a cell where a valid thickness value is present. If it is under 50%, the value is not shown in the posterior pole thickness grid.

Grand mean retinal thickness was calculated as the mean thickness of all the 64 cells for the right and the left eye. Grand mean superior and inferior retinal hemisphere thickness were calculated as the mean thickness of the 32 cells located superior or inferior to the fovea-disc axis.

Statistical Analysis

All statistical analyses were conducted using statistical software (R, version 3.0.227; The R Foundation for Statistical Computing, Vienna, Austria).

Posterior pole asymmetry analysis data from each patient were exported as XML files and loaded into the R statistical program using the software’s XML-package.28

The mean inter- and intraocular retinal thickness asymmetries were calculated for all 64 cells of the posterior pole grid using the absolute differences between eyes and between superior and inferior hemispheres of the same eye, respectively. The grand mean absolute asymmetry across the 64 cells was calculated, and one-sided t-tests were conducted to examine which cells were significantly greater than the grand mean.

Both inter- and intraocular intraclass correlation coefficients (ICC) were calculated for each cell using the R software’s ICC package.29 The estimation of the ICC was based on the formula

$$ICC = \frac{\sigma^2 + \sigma^2_A}{\sigma^2 + \sigma^2_A + \sigma^2_R},$$

where $\sigma^2_A$ is the between-subject variation and $\sigma^2_R$ is the within-subject variation.

The association between age, sex, and interocular asymmetry was evaluated by a linear model with interocular asymmetry as the dependent variable and age and sex as the independent variables. A Gaussian correlation structure was applied to account for the spatial correlation in data. The model was fitted using the glm function30 of the statistical R software. To verify the robustness of the results of the linear model, a supplementary analysis of the age and sex effects was conducted fitting a generalized linear model based on the gamma distribution. The suitability of both models was evaluated by visual inspection of the residual plots.

The false discovery rate method31 was applied to adjust P values for multiple testing. Values of $P < 0.05$ were considered statistically significant.

Results

Out of 118 subjects in our investigation, we excluded six subjects due to macular drusen and seven subjects because one or more RNFL thickness segments were found to be out of normal limits, leaving 105 persons for further analysis. Out of
FIGURE 1. Posterior pole asymmetry analysis of SD-OCT. The color-coded pictures on the top show the posterior pole thickness map. The middle pictures show the interocular asymmetry analysis (right eye-left eye/left eye-right eye) and the intraocular asymmetry analysis (S/I/I/S), respectively. The intraocular hemisphere asymmetry analysis displays the asymmetry between the superior and inferior hemisphere. The fovea-disc axis is the horizontal symmetry line. Asymmetry is graded in a grayscale where darker gray indicates thinner retina and white indicates equal retinal thickness. The bottom pictures show the mean superior, total, and inferior retinal thickness. S, superior hemisphere; I, inferior hemisphere.
the remaining 105 participants, 30 were men (29%) and 75 were women (71%). The mean age ± SD was 28.8 ± 7.9 years.

Among a total of 13,440 retinal thickness values (105 persons × 2 eyes × 64 cells), 23 values were missing, due to a valid pixel percentage under 50%. This was considered acceptable because the missing values only amounted to 0.17% of the total values.

The grand mean interocular retinal thickness asymmetry was 5.6 µm (95% confidence interval [CI]: 4.6–6.5) and the grand mean intraocular thickness asymmetry was 8.3 µm (95% CI: 6.8–9.9) in the right eye and 8.4 µm (95% CI: 6.7–10.0) in the left eye.

The mean interocular asymmetry in retinal thickness among the 105 participants is shown in Figure 2 for all cells in the 64 cells grid; Figures 3A (right) and 3B (left) show the mean intraocular asymmetry in retinal thickness between the corresponding 32 cells of the hemispheres in the right and left eyes of the 105 participants.

Values of $P$ in the cells illustrate for which cell the asymmetry is significantly greater than the grand mean thickness asymmetry.

The inter- and intraocular retinal thickness asymmetries were found to increase toward the periphery of the macula, especially toward the nasal corners where the posterior pole thickness map overlaps the upper and lower temporal vascular arches.

The intraclass correlation coefficient for interocular asymmetry showed a greater variation between subjects than within subjects for all 64 cells in the asymmetry grid. The interocular ICC was highest in the central part of the macula with a maximum of 0.94 and decreased toward the periphery with a minimum of 0.76. See Supplementary Figure S1.

The intraclass correlation coefficient for intraocular hemisphere asymmetry showed a greater variation between subjects than within subjects for most of the 32 cells of both the right and the left eye. The intraocular ICC was largest in the central part of the macular with a maximum of 0.97 and decreased toward the periphery, especially toward the nasal corners where two cells in the right hemisphere and three cells in the left hemisphere had an ICC under 0.5. See Supplementary Figures S2A and S2B.

A statistically significant effect of age and sex on the general retinal thickness asymmetry was found, using a linear model.
with Gaussian correlation structure. The age effect was 0.04 μm/year (0.02–0.06 μm; P < 0.001). The 10-year age effect on the asymmetry was estimated to be 6.2% of the grand mean interocular retinal thickness asymmetry. The sex effect was 0.54 μm (0.19–0.88 μm) for men compared with women (P = 0.002).

The residual plot of the linear model showed some deviation from the Gaussian assumption; so to verify the robustness of the results, a generalized linear model based on a gamma distribution was fitted. This model supported the findings of significant age and sex effects on retinal thickness asymmetry. The residual plot of this model was acceptable.

**DISCUSSION**

Asymmetry parameters comparing retinal thickness in fellow eyes or hemispheres in the same subject have clinical
advantages compared with raw measurements. In contrast to raw measurements, asymmetry parameters are theoretically not biased by factors that affect the eyes symmetrically such as age, race, and sex.

Posterior pole retinal thickness asymmetry analysis was proposed to be a useful tool in detecting early glaucomatous changes.\textsuperscript{1,3}–\textsuperscript{5} However, to date there is sparse normative data available regarding physiological macular asymmetry in healthy subjects.

In our study, we examined physiological retinal thickness asymmetries in healthy adults using the entire 64 cell PPAA-protocol of a SPECTRALIS SD-OCT device. Our data show that statistically significant physiological asymmetries in intra- and interocular retinal thickness exist. In our dataset, the grand mean interocular retinal thickness asymmetry was 5.6 \(\mu \text{m} (95\% \text{CI}: 4.6–6.5)\) and the grand mean intraocular thickness asymmetry was 8.3 \(\mu \text{m} (95\% \text{CI}: 6.8–9.9)\) in the right eye and 8.4 \(\mu \text{m} (95\% \text{CI}: 6.7–10.0)\) in the left eye.

Sullivan-Mee et al.\textsuperscript{2} investigated retinal asymmetry in 50 subjects with early primary open-angle glaucoma (POAG) and 50 control subjects using the PPAA-protocol of the SPECTRALIS SD-OCT device. This study enrolled subjects aged over 40 years, and recorded the grand mean, superior and inferior macular thickness asymmetries, but not the difference in every single cell of the PPAA-protocol. The study found a grand mean interocular retinal thickness asymmetry of 2 \(\mu \text{m} (0.0–5.3)\) and a grand mean intracrucial hemisphere asymmetry of 2.6 \(\mu \text{m} (0.0–7.0)\) in the control group. In the PAOG group these values were 10 (0.0–27.0) and 8.0 \(\mu \text{m} (0.0–23.0)\), respectively. The fact that we in our study found a slightly higher inter- and intraocular retinal thickness asymmetry in healthy individuals may arise from either the smaller number of patients in the above study or from the differences in inclusion criteria.

Two other studies have previously investigated physiological interocular retinal thickness asymmetries using commercial OCT software (Cirrus OCT with Macular Cube 200 \(\times \) 200 protocol; Carl Zeiss Meditec, Dublin, CA, USA).\textsuperscript{3,25} Altemir et al.\textsuperscript{32} found 23.2 \(\mu \text{m}\) to be the interocular tolerance limit for average macular thickness asymmetry in 357 healthy children (mean age: 9 \(\pm\) 1.7 years), and Dalgliesh et al.\textsuperscript{33} found 8 \(\mu \text{m}\) to be the interocular tolerance limit of average macular thickness asymmetry in 1500 adolescent subjects (mean age: 17.3 \(\pm\) 0.51 years). A study measuring the central retinal thickness values using six different OCT instruments found similar results with the Cirrus HD-OCT and the SPECTRALIS OCT. This may arise from the similar image processing algorithms of the two OCT systems, both including the RPE in the retinal segmentation.\textsuperscript{2}

Optical coherence tomography software differences need to be addressed when selecting cutoff values for asymmetry analysis to detect early glaucomatous changes.\textsuperscript{30,33}

To evaluate local differences in asymmetry, we investigated asymmetry across the entire posterior pole thickness map. The results indicated that the intra- as well as the interocular retinal thickness asymmetry increases toward the periphery of the macula, especially toward the nasal corners where the 8 \(\times\) 8 grid in the posterior pole thickness map overlaps the upper and lower temporal vascular arches. Although ICC is relatively high for all cells, ICC shows the same trend, with higher within-subject variation toward the periphery. This marked asymmetry in the peripheral zones is supported by the study of Um et al.,\textsuperscript{22} where an increased interocular asymmetry in zones representing the inferior and superior periphery of the grid were observed in healthy controls, glaucoma suspects, and glaucoma patients using a modified PPAA protocol.

Furthermore, a study by Yamashita et al.\textsuperscript{34} found the intraocular symmetry in retinal thickness to be lowest in the peripheral nasal areas of macula. They found that the difference between the supranasal and infranasal retinal thickness and vein angle correlated significantly with the higher retinal thickness asymmetry in the peripheral nasal areas.

The segmentation algorithm in the SPECTRALIS SD-OCT device includes the retinal vessels in the retinal thickness measurements.\textsuperscript{25,26} Therefore, it is important to take the effect of the retinal vessels on the retinal thickness into account. A new segmentation algorithm that subtracts the thickness of the vessels from the retinal thickness measurement is necessary, if accurate retinal thickness and retinal thickness asymmetry measurements are needed.

The central 20° area assessed by the PPAA corresponds closely with the 24-2 visual field test. A recent report\textsuperscript{21} showed a pointwise relationship between visual field sensitivity (VFS) and macular thickness determined by the SPECTRALIS SD-OCT device using a modified 16-cell posterior pole thickness grid. The study revealed that VFS and mean retinal thickness showed the strongest correlation centrally, and weakening correlation toward the peripheral cells of the grid. This finding supports that the PPAA of peripheral cells—that are influenced by the vascular structures—may have a lower value in glaucoma diagnostics.

The posterior pole asymmetry analysis evaluates retinal asymmetry by measuring all layers of the macula, thus symmetry/asymmetry is biased by other layers than the RNFL and the RGC layer. A modified PPAA software assessing asymmetry only regarding the RNFL-RGC complex could help us to take full advantage of this technology in the diagnostic of glaucoma.

We found a slight statistically significant average age and sex effect on retinal thickness asymmetry of 0.04 \(\mu \text{m/year} (0.02–0.06 \mu \text{m})\) and 0.54 \(\mu \text{m} (0.19–0.88 \mu \text{m})\), respectively, for men compared with women. However, in evaluating these results, it must be taken into account, that our study is slanted toward younger women. The magnitude of the age and sex effect on asymmetry should be considered with caution, since the residual plots of the linear model showed slight deviation from the Gaussian assumption. However, the findings of significant age and sex effects were supported by the more complicated and less interpretable generalized linear model based on the gamma distribution.

A handful of studies have investigated the relation between age and sex and retinal thickness using the SD-OCT.\textsuperscript{35}–\textsuperscript{39} Two of these publications found greater foveal thickness among men\textsuperscript{56} and a third one showed a significant increase in central foveal subfield thickness with age.\textsuperscript{59} To the best of our knowledge, there has been no other investigation that assessed the possible effect of age and sex on posterior pole asymmetry to date.

Our study has certain limitations. We enrolled only healthy adults aged between 18 and 45 years, to eliminate any structural change of the retina due to any age-related macular pathology, primarily AMD. Additionally, subtle changes in the refractive media might affect data acquisition and automated segmentation with age.\textsuperscript{40} Most patients in ophthalmology, however, are aged older than 45 years and are often troubled by more than one ocular disease.

Our study is also limited by our choice to only enroll individuals with a refractive error between \(-1.5\) and \(+1.5\)D (SE) in order to eliminate the effect of anisometropia or high ametropia. Therefore, our normal values only reflect retinal thickness asymmetry in the near emmetropic and the isometropic. A recent study by Kim et al.\textsuperscript{41} investigating healthy Korean eyes (RE: \(-3.97 \pm 2.84\) D) showed that retinal thickness correlated positively with RE with regional variations within the 8 \(\times\) 8 posterior pole grid.\textsuperscript{41}

Finally, the PPAA analysis does not take the anatomical symmetric placement of the arcuate nerve fibers around the temporal raphe into account. Instead the grid is placed
they found the angle between the temporal raphe and the subjects using adaptive optics scanning laser ophthalmoscope, Vorum

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Acknowledgments

Vorum

Correlates better with the anatomical symmetry of the nasal fibers, than the symmetry of the temporal fibers.

As a consequence hereof, the PPAA causes incorrect pairing of regions in the hemisphere asymmetry analysis. For the time being the manufacturer is analyzing the problem. However, at present it is not possible to place the asymmetry grid around the anatomical symmetry line.

In conclusion, statistically significant physiological asymmetries in inter- and intraocular central retinal thickness were found in healthy Caucasians when using the PPAA protocol of a SPECTRALIS SD-OCT device. Normal variation, as presented in this article, must be considered when early signs of glaucoma or other pathologies are evaluated based on the retinal thickness asymmetry.

It is important to take into consideration that the PPAA protocol includes the retinal vessels in the thickness measurements, and that the protocol alignment is not completely in accordance with the anatomical symmetry of the retinal nerve fibers. These limitations of the PPAA-protocol lead to increased variability of the asymmetry measurements across the asymmetry grid.

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References


