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Biological and Methodological Variability in Retinal Nerve Fiber Layer OCT: The Framingham Heart Study

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Objective: To explore participant-level biological attributes and scan-level methodological attributes associated with retinal nerve fiber layer (RNFL) thickness variability in a population-based sample of elderly United States adults.

Design: Cross-sectional analysis using data from the Framingham Heart Study.

Participants: One thousand three hundred forty-seven eyes from 825 participants with ≥1 OCT scan and axial length data were included.

Methods: Three or more successive RNFL scans of each eye of each participant were obtained in a single session. Multivariable linear mixed models were employed to explore the associations between average RNFL thickness with participant-level biological attributes (age, gender, race, ethnicity, and axial length) and scan-level attributes (signal strength [SS]) as independent variables in the whole population as well as a subsample of adults with no self-reported history of glaucoma. Similar analyses were designed to assess methodological variability with average within-eye standard deviation (SD) for repeated scans as the dependent variable.

Main Outcomes Measures: (1) Biological variability: average RNFL thickness, and (2) methodological variability: average within-participant SD across repeated scans.

Results: Age (β = − 0.19 microns/year, [95% confidence interval {CI}: − 0.29, − 0.09]), female gender (β = +1.48 microns vs. male, [95% CI: 0.09, 2.86]), axial length (β = − 1.24 microns/mm of greater length, [95% CI: − 1.80, − 0.67]), and SS (β = +1.62 microns/1 unit greater SS, [95% CI: 1.16, 2.09]) were significantly associated with RNFL thickness, while race and ethnicity were not ($P > 0.05$). In analyses designed to assess methodological variability, higher RNFL thickness (β = +0.02 per micron increase, [95% CI: 0.01, 0.03]), and lower SS (β = +0.19 per 1 unit lower SS, [95% CI: 0.10, 0.27]) were significantly associated with greater RNFL variability. In adults with no self-reported history of glaucoma (n of eyes = 1165, n of participants = 712), female gender was not associated with RNFL, while African American race was associated with thicker RNFL (β = +4.65 microns vs. Whites, [95% CI: 1.28, 8.03]).

Conclusions: Retinal nerve fiber layer thickness is lower with older age, male gender, greater axial length, lower SS, and Whites (as compared with African Americans) without self-reported glaucoma. Measurement variability (SD) is higher with greater RNFL thickness and lower SS. Understanding these biological and methodological variations is important to aid in OCT interpretation.

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Glaucoma is a progressive disease in which accurate testing to identify the presence and severity of disease is a central element of management. Earlier diagnosis and methods to robustly monitor patients help guide treatment to prevent disease worsening.[1] Quantitative assessment of the optic nerve, the affected structure in glaucoma, can be performed by imaging the retinal nerve fiber layer (RNFL) thickness around the optic nerve. Understanding the variability of the RNFL thickness, both due to patient factors and measurement variation, is critical in facilitating accurate measurement and interpretation of test results (i.e., disease diagnosis and monitoring).[2]

In addition to optic nerve disease, RNFL thickness may also differ across individuals for other disease-unrelated

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reasons. For example, biological factors such as age, gender, and race have been associated with variations in RNFL thickness values.[3–6] Moreover, methodological variability such as equipment, operator experience, patient positioning, signal strength (SS), and other sources of measurement variability may also influence RNFL thickness readings.[7]

Previous studies have explored biological and methodological variability in OCT metrics. For instance, Nousome et al (2021) compared RNFL thickness across Black, Chinese, and Latin Americans using data from 3 population-based studies in California and found that Black Americans displayed the lowest RNFL thickness measures.[3] Other studies have assessed RNFL variability across repeated scans; however, estimates lacked generalizability or had limited sample size.[2,8,9] There are limited contemporary United States population-based studies that assessed features associated with both biological and methodological OCT variability in a large cohort.

Here, as part of OCT images obtained in participants of the Framingham Heart Study, we explore how RNFL thickness varied across individual characteristics (age, race, gender, and axial length) and across different scans within the same session as a result of scan-level features (SS and RNFL thickness). Additionally, we determined a minimum threshold that denotes a real change beyond measurement error (a confidence level of 95%) for all RNFL thickness measurements.

Methods

Study Design and Participants

In this observational study conducted from 2020 to 2022, participants from the Framingham Heart Study were examined.[10] The Framingham Heart Study is an ongoing prospective, longitudinal, population-based cohort study, and it has recently incorporated eye examinations, including 200 × 200 optic disc cube spectral-domain OCT, for its participants. The study included individuals from both the Offspring and the OMNI 1 cohort. The Offspring cohort includes offspring of the original participants, as well as their spouses, originally recruited in 1971. The OMNI 1 cohort includes multiethnic and racially diverse adults recruited in 1994 to account for the increasing diversity of the Framingham, Massachusetts community. Further details about sampling and participants' characteristics have been described previously.[10,11] The study protocol was approved by the Institutional Review Board of Boston University Medical Center, informed consent was obtained from all participants, and the study was conducted in accordance with the tenets of the Declaration of Helsinki.

Imaging and Quality Assessment

Axial length was measured using the commercially available IOL Master 500 (Carl Zeiss Meditec, Inc). Additionally, 200 × 200 Optic Disc Cube scans on a Cirrus 6000 were acquired for both eyes of each participant, with the right eye imaged first per protocol. An average of 4 OCT scans were acquired for each eye within the same session. The scans were taken in succession while the participant was seated, and the machine properly aligned. Image quality was assessed by 2 trained graders (A.S. and A.C.M.) not involved in image acquisition and masked to participant history and demographics. If differences in quality assessment were present, a senior grader (A.H.K.) arbitrated. The graders identified the single best scan for each eye from the acquired scans and graded the image as "Excellent," "Good," "Suboptimal," or "Not Acquired" based on the following criteria: (1) SS, (2) motion artifacts, (3) media opacities, (4) focus, and (5) decentration.

For the quantification of thickness values and further biological variability analyses, eyes with ≥1 OCT scan graded as "Excellent" or "Good" and available axial length data were included. The sample selection flow chart for our primary analysis is described in Fig S1 (available at www.ophthalmologyscience.org). In analyses designed to assess intrasession methodological variability across repeated OCT scans, eyes with <3 OCT scans were excluded (n = 197 eyes). Since only the single best scan from each eye was defined by the quality assessment, in the methodological variability analyses, we only included additional scans with a single strength ≥6 (n = 5018 scans), as previously defined.[12] Following manual review, certain included scans were still affected by motion artifacts, and hence, we excluded eyes outside the 0.25th and 99.75th percentiles for each sectoral (4 quadrants and 12 clock hours) RNFL thickness value; that is, scans that lie outside the 0.25th and 99.75th percentiles for any of the 16 different measurements were excluded (n = 214 scans). We manually reviewed a sample of included scans to confirm the robustness of our cutoff against any segmentation artifacts. Further, in a secondary analysis, we excluded adults who self-reported glaucoma to explore biological and methodological variability in a sample of adults without glaucoma. Throughout this paper, RNFL thickness is used interchangeably with average RNFL thickness, both of which refer to measured average RNFL thickness across all quadrants as reported by the OCT machine.

Statistical Analysis

Counts, proportions, means, and standard deviations (SDs) were used to summarize demographic and clinical characteristics. Pearson correlation coefficients were calculated to assess correlations between various OCT variables (average RNFL, temporal, superior, nasal, and inferior quadrants, average and vertical cup-to-disc ratio, disc area, disc diameter, rim area, and cup volume). Multivariable linear mixed models were employed to estimate the effect of age, gender, race, ethnicity, axial length, and SS as independent variables in models considering average RNFL thickness as the dependent variable, with participant-specific random effects to account for intereye correlation. The independent variables were identified based on clinical relevance and/or previous demonstration of impact on RNFL loss.[3]

Within-participant mean and SD for each measurement were calculated for each eye and averaged across the entire population. The intraclass correlation coefficients (ICC) and their corresponding 95% confidence intervals (CIs) were calculated to evaluate the level of agreement between scans using a 2-way random, single measures, absolute agreement approach. Further details about how ICC was calculated have been described previously.[13] A higher ICC value indicates that repeated measurements on the same participant show minimal fluctuations. In this study, an ICC value >0.9 was considered high, values between 0.8 and 0.9 were considered moderate, and values <0.8 were considered insufficient, as previously suggested.[14] The coefficient of variation (COV) for each measurement value was calculated by dividing the SD by the mean and expressing the result as a percentage. The COV was employed to capture the within-visit variability, where smaller values indicate lower variability and greater reproducibility. Measurements with a COV <10% were considered to have a good reproducibility, as previously defined.[9] Additionally, the smallest real difference (SRD) was calculated for

each measurement. The SRD represents the upper limit (95%) for the absolute differences between repeated measurements attributable to chance. It measures the smallest change that a method of measurement can reliably detect given the expected measurement error/variability. For example, an SRD value of 5 μm indicates that any observed change in RNFL thickness exceeding 5 μm is considered meaningful and not attributable to chance within the same session for each participant. Smallest real difference was calculated as: SRD = t(α, degrees of freedom [df]) * √(2 * variance), where t(α, df) represents the critical value of the t-distribution for a given significance level (α) and df. The overall SRD was calculated as Σ SRD/n_e, where n_e is the total number of eyes in the study with ≥3 scans (n_e = 1150).

To investigate factors associated with methodological variability, univariable mixed-effects models were utilized to identify relevant predictors, including participant-level features and scan-level features, with average within-participant RNFL SD as the dependent variable, and participant-specific random effects to account for intereye correlation. Further, a stepwise forward selection approach was utilized to identify the best-fit model with average RNFL SD as the dependent variable. Models were evaluated using Akaike information criterion and Bayesian information criterion.

As average RNFL and average RNFL SD may be intercorrelated with the other covariates, it can pose analytical challenges due to multicollinearity. We therefore assessed the multicollinearity between the variables, using variance inflation factors (a method of assessing multicollinearity by measuring the extent to which the variance of a regression coefficient increases when predictors are correlated) with a cutoff value of 2.[15,16] We planned to remove variables that showed evidence of multicollinearity.

In sensitivity analyses, we assessed biological variability using axial length corrected RNFL instead of average RNFL. Axial length corrected RNFL was calculated as: axial length corrected RNFL = RNFL (machine exported) × axial length (biometry measured)/23.7 mm; where 23.7 mm is the axial length from a standard eye, as previously defined.[17] We also calculated axial length corrected RNFL using the Littman formula, as previously defined.[18] Further, we calculated the SRD for the higher RNFL eye and lower RNFL eye separately based on the higher and lower average RNFL thickness values, respectively. We also assessed methodological variability using average RNFL COV as the dependent variable instead of average within-participant RNFL SD. We reran the analyses in a normative sample of adults with no glaucoma (adults who by self-report had never been diagnosed with glaucoma) to explore how the associations with biological and methodological variability differ. Lastly, since diabetic retinopathy may also result in reductions in RNFL thickness values, we reran the analyses in a sample of adults with neither diabetic retinopathy (adults who self-reported that they have never been diagnosed with diabetic retinopathy) nor glaucoma. All *P* values were 2-sided but not adjusted for multiple analyses. Statistical significance level was defined at α = 0.05. All analyses were conducted using R software (R Foundation for Statistical Computing).

Results

One thousand three hundred forty-seven eyes from 825 participants were included in the study. The mean age of participants was 75.1 (±7.0), the majority were White (88.7%), female (59.6%), and non-Hispanic/Latino (96.0%); 73 participants (8.8%) self-reported glaucoma (Table 1). Figure 2 shows the distribution of RNFL thickness values across the study population, with a mean of 86.7 μm (±10.4). The average RNFL thickness statistically differed by gender (Fig S3, available at www.ophthalmologyscience.org), such that the mean (±SD) thickness was 85.33 ± 10.97 in males and 87.70 ± 9.93 in females ($P < 0.001$). Fig S4 (available at www.ophthalmologyscience.org) shows the measures of spread (median, range, and interquartile range) of clock hours across the study population, while Fig S5 (available at www.ophthalmologyscience.org) and Table S2 (available at www.ophthalmologyscience.org) show clock hours by gender, which showed significant differences between genders for clock hours 2, 6, 7, 8, 10, and 11 ($P < 0.05$).

Table 1. Demographics of Included Study Participants, Framingham Heart Study

Characteristic	
Number of participants (n of eyes)	825 (1347)
Eye, right/left (n)	683/664
Self-reported glaucoma, n of participants (n of eyes)	
Yes	73 (117)
No	712 (1165)
Age in years, mean (SD)	75.1 (7.0)
Gender, n (%)	
Female	492 (59.6)
Male	333 (40.4)
Race, n (%)	
White	732 (88.7)
African American	39 (4.7)
Other	54 (6.6)
Ethnicity, n (%)	
Hispanic or Latino	33 (4)
Not Hispanic or Latino	792 (96)

n = number; SD = standard deviation.

Figure 6 shows the correlations between OCT measurements that were statistically significant ($P < 0.05$). Average RNFL thickness was positively correlated with temporal, superior, nasal, and inferior quadrants, disc area, disc diameter, and rim area. Conversely, it was negatively correlated with average and vertical cup-to-disc ratio, and cup volume.

Participant-Level Biological Variability

Table 3 summarizes the association of participant specific biological attributes with RNFL thickness. Age (β = −0.19 μm per 1 year older, [95% CI: −0.29, −0.09]), female gender (β = +1.48 μm vs. males, [95% CI: 0.09, 2.86]), axial length (β = −1.24 μm per mm greater length, [95% CI: −1.80, −0.67]), and SS (β = +1.62 μm per 1 unit greater SS, [95% CI: 1.16, 2.09]) were significantly associated with average RNFL thickness, while race and ethnicity were not ($P > 0.05$). When exploring axial length corrected RNFL as the dependent variable, the relationship between axial length and RNFL changes, such that axial length (β = +2.37 μm per mm greater length, [95% CI: 1.80, 2.94]) was significantly and

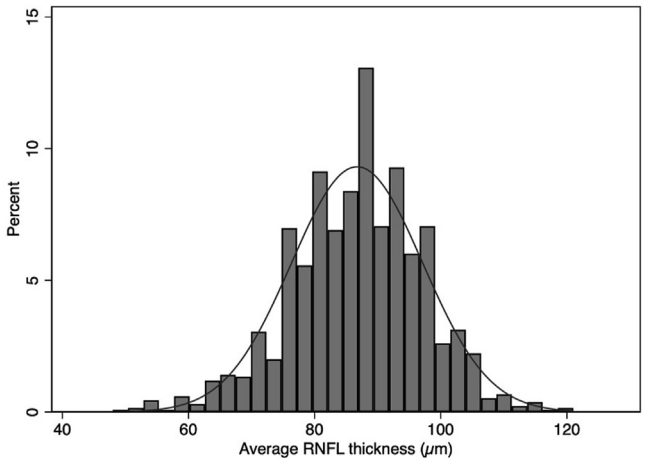

Figure 2. Distribution of average RNFL thickness values. RNFL = retinal nerve fiber layer.

positively associated with greater axial length corrected RNFL thickness. The remaining associations were largely unchanged (Table S4, available at www.ophthalmologyscience.org). Using the Littman formula to correct RNFL resulted in similar results for average RNFL and RNFL quadrants as well (Tables S5 and S6, available at www.ophthalmologyscience.org).

Methodological Variability

One thousand one hundred fifty eyes from 730 participants with ≥3 scans were included in these analyses. Average RNFL demonstrated a high ICC value (ICC = 0.96, [95% CI: 0.96, 0.96]). Cup volume exhibited the highest ICC value (ICC = 0.98, [95% CI: 0.98, 0.99]), while clock hour 3 displayed the lowest ICC value (ICC = 0.80, [95% CI: 0.79, 0.82]). All OCT measurements showed good reproducibility with a COV <10% (Table S7, available at www.ophthalmologyscience.org). The SRD estimates were largely uniform across the OCT measurements. Average RNFL exhibited an SRD of ± 4.47 μm, with the lowest SRD observed for cup volume (±0.02). Smallest real difference values were largely unchanged when looking at higher and lower RNFL eye separately (Table S8, available at www.ophthalmologyscience.org).

When exploring methodological variability (Table 9), the univariable analyses showed that average RNFL (β = +0.02 μm, [95% CI: 0.01, 0.02]; per 1 μm increase) and SS (β = − 0.14 μm, [95% CI: − 0.23, − 0.06]; per 1 unit increase) were significantly associated with average RNFL within-participant SD, while the remaining variables were not ($P > 0.05$). In the multivariable model, average RNFL (β = +0.02 μm per 1 μm increase, [95% CI: 0.01, 0.03]) and SS (β = − 0.19 μm per 1 unit increase, [95% CI: − 0.27, − 0.10]) remained significantly associated with average RNFL SD. Similar

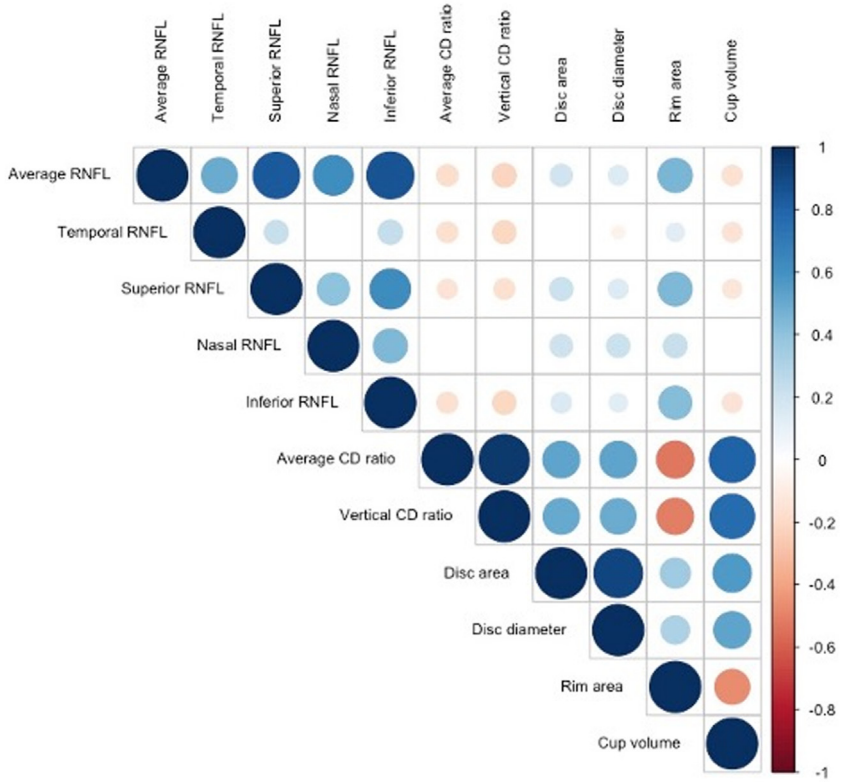

Figure 6. Correlation matrix—colored circle indicates significant difference; if not significant, left blank. CD = cup-to-disc ratio; RNFL = retinal nerve fiber layer.

Table 3. Multivariable Mixed-Effects Model Exploring the Effect of Age, Gender, Race, Ethnicity, Axial Length, and Signal Strength on Average RNFL Thickness

		Average RNFL (μm)		
Predictor	**Interval/Reference**	*Beta*	*95% CI*	*P Value*
Age	1 yr older	− 0.19	− 0.29, − 0.09	<0.001
Gender (female)	Male	1.48	0.09, 2.86	0.04
Race (African American)	White	1.71	− 1.43, 4.86	0.29
Race (other)	White	0.83	− 2.01, 3.67	0.57
Ethnicity (Hispanic or Latino)	Not Hispanic or Latino	3.24	− 0.32, 6.80	0.07
Axial length	1 mm increase	− 1.24	− 1.80, − 0.67	<0.001
Signal strength	1 unit increase	1.62	1.16, 2.09	<0.001

CI = confidence interval; RNFL = retinal nerve fiber layer.

analyses showed these 2 variables were associated with RNFL COV as well.

In sensitivity analyses looking at adults with no glaucoma (n of participants = 712; n of eyes = 1165 — Table S10, available at www.ophthalmologyscience.org), the associations between RNFL and biological variability were similar (Table S11, available at www.ophthalmologyscience.org); that is, age, axial length, and SS showed similar magnitude of effects and significance. However, female gender (β = +0.88 μm

Table 9. Univariable and Multivariable Predictors of RNFL, SD, and COV

		RNFL SD (μm)		
Predictor (Univariable)	**Interval**	*Beta*	*95% CI*	*P Value*
Average RNFL	1 μm increase	0.02	0.01, 0.03	<0.001
Age	1 yr older	0.01	− 0.002, 0.02	0.11
Gender (female)	Male	− 0.12	− 0.28, 0.04	0.14
Race (African American)	White	− 0.15	− 0.53, 0.23	0.44
Race (other)	White	0.05	− 0.33, 0.42	0.81
Ethnicity (Hispanic or Latino)	Not Hispanic or Latino	− 0.18	− 0.60, 0.23	0.39
Axial length	1 mm increase	0.02	− 0.06, 0.09	0.69
Signal strength	1 unit increase	− 0.17	− 0.26, − 0.08	<0.001

		RNFL SD (μm)		
Predictor (multivariable)	**Interval**	*Beta*	*95% CI*	*P-value*
Average RNFL	1 μm increase	0.02	0.01, 0.03	<0.001
Signal strength	1 unit increase	− 0.19	− 0.27, − 0.10	<0.001

		RNFL COV (%)		
Predictor (univariable)	**Interval**	*Beta*	*95% CI*	*P-value*
Average RNFL	1 μm increase	0.02	0.02, 0.03	<0.001
Age	1 yr older	0.01	− 0.003, 0.03	0.11
Gender (female)	Male	− 0.14	− 0.33, 0.05	0.14
Race (African American)	White	− 0.19	− 0.62, 0.27	0.42
Race (other)	White	0.04	− 0.38, 0.48	0.85
Ethnicity (Hispanic or Latino)	Not Hispanic or Latino	− 0.18	− 0.69, 0.27	0.46
Axial length	1 mm increase	0.01	− 0.07, 0.11	0.79
Signal strength	1 unit increase	− 0.18	− 0.30, − 0.10	<0.001

		RNFL COV (%)		
Predictor (multivariable)	**Interval**	*Beta*	*95% CI*	*P-value*
Average RNFL	1 μm increase	0.02	0.01, 0.03	<0.001
Signal strength	1 unit increase	− 0.22	− 0.31, − 0.12	<0.001

CI = confidence interval; COV = coefficient of variation; RNFL = retinal nerve fiber layer; SD = standard deviation.
Multivariable mixed-effects models with a stepwise forward selection approach were utilized to identify relevant predictors. Models were evaluated using 2 criteria: Akaike's information criterion, and Bayesian information criterion. Multicollinearity was assessed using variance inflation factors with a cutoff of 2.

[95% CI: − 0.54, 2.29], vs. male) was not significantly associated with RNFL, while African American adults were associated with significantly thicker RNFL (β = +4.65 μm [95% CI: 1.28, 8.03], vs. White). Similarly, the associations between RNFL and methodological variability were largely unchanged (n of participants = 635; n of eyes = 998); that is, average RNFL and SS showed similar effects and significance levels (Table S12, available at www.ophthalmologyscience.org). Only 8 adults (9 eyes) had diabetic retinopathy. In analyses looking at adults without diabetic retinopathy and glaucoma, results were similar to those with no glaucoma; that is, age, race, axial length, and SS showed similar effects and significance levels on RNFL thickness, while average RNFL and SS showed similar magnitudes of effect and significance levels on RNFL SD/COV.

Discussion

Here, in one of the largest United States population-based studies including OCT imaging, we demonstrate thinner measured OCT RNFL thickness with older age, male gender, and longer axial length. Notably, African American race was also associated with greater RNFL thickness when excluding individuals who self-reported glaucoma. Also, when correcting RNFL thickness for axial length, the association was reversed (higher corrected RNFL thickness with axial length). We also evaluated same-session imaging variability, and found more variable RNFL thickness readings in eyes with greater RNFL thickness and scans with worse SS. Retinal nerve fiber layer thickness is a critical value in identifying optic neuropathy and is used daily in clinical practice. As clinical practice strives to define the elusive threshold between healthy aging and disease, it is essential to understand the reasons contributing to even subtle measurement variability.

In this study, we found that measured RNFL thickness was lower with older age, approximately a decrease of 2 μm per decade. Previous studies have also found thinner RNFL measures with age, with values ranging from 1.5 to 3.8 μm per decade.[3,6,19–21] Of these studies, 3 were based in the United States, [3,6,19] 1 in the United Kingdom, [21] and 1 combined 8 European studies.[20] The effect of age on RNFL thickness across the 8 studies in the combined European study ranged from 0.9 to 9.1 μm per decade. These studies were conducted across multiple countries, using different OCT devices, and with varying average ages. The range for the United States-based studies was 1.5 to 2.5 μm per decade, which is more comparable to our findings. The average age for United States studies ranges from 47 to 60 years, which is significantly younger than our average of 75 years. The validation of these findings in the older adults studied here suggests that OCT thickness continues to change with age, even in older adults. Overall, these findings highlight the importance of considering population and device when assessing the effect of age on RNFL thickness, but there appears to be an overall consistent decline in RNFL thickness with age, as judged by United States population-based studies.

Like age, axial length is another factor that has consistently been found to have an association with measured RNFL thickness. We found that eyes with longer axial length had thinner measured RNFL thickness, approximately 1.25 thinner for every 1 mm increase in axial length. Previous work has similarly found a decrease in measured RNFL thickness with increasing axial length ranging from 1.2 to 2.0 μm per 1 mm increase in axial length.[3,19] However, axial length affects the measured RNFL due to differences in magnification.[21,22] We derived an axial length corrected RNFL thickness as has been done previously,[17] and using this corrected RNFL thickness, there was a positive correlation between RNFL thickness and axial length, approximately 2.4 μm thicker with each 1 mm increase in axial length. This reversal has been shown in other studies using various methods to correct for axial length.[22,23] Similarly, another study using GDxVCC (Carl Zeiss Meditec, Inc) instead of OCT, which partially corrects for axial length magnification, found a positive correlation.[21] We further reported on the relationship between axial length and corrected RNFL thickness in each quadrant separately. Corrected temporal RNFL thickness demonstrated the greatest increase with axial length, consistent with prior studies.[22] Retinal nerve fiber layer thickness is related to axial length due to multiple factors and is important to consider in any study, and also in clinical practice. While consideration should be given to correcting for axial length in both research and clinical practice, the reality is that this is rarely done, and thus reporting uncorrected RNFL and the factors which influence it remains important.

Beyond age and axial length, RNFL thickness has been shown to vary with gender and race. In this study we found that the RNFL thickness was greater in female participants, with approximately 1.5 μm thicker RNFL compared with male participants. This association is maintained even after accounting for axial length in the multivariable model and utilizing corrected RNFL. However, female gender was not significantly associated with average RNFL in a normative sample of adults who did not self-report glaucoma. These findings may be due to normal variation in RNFL or that glaucoma may be more prevalent in certain sexes (12.1% of males self-reported glaucoma, while 7.1% of females reported glaucoma). Further, we described the differences in each individual clock hour by gender. Though the clinical significance of these values is uncertain, we aimed to provide a clear description of the variables available in this population-based study. A few other population studies have shown thicker RNFL in female participants.[3,24,25] Further, our data did not find a significant effect of race, though the study had almost 90% White participants, limiting the ability to study this question. However, when looking at a sample of adults with no glaucoma, African American adults had thicker RNFL thickness values, agreeing with previous findings.[26] African American adults were more likely to have glaucoma in our study which likely masked the effect of race on RNFL in the full population sample,

which may have contained some disease. Previous work has shown that White participants had thinner RNFL than Hispanic, African, and Asian participants.[3,5,6] In a large sample of non-White patients, there was a significant difference from thickest to thinnest RNFL for Chinese, Latino, and Black American participants.[3] Overall there are differences in RNFL thickness in different populations which is important to consider, effects which may be most pronounced when looking at regional RNFL thickness.

The variability attributable to the OCT measurement itself, referred to as scan-level methodological variability, is important to study to determine the threshold for detection of change with a specific device. Previous studies have assessed this measurement variability in both healthy and glaucoma eyes.[9,27–33] The previous studies in healthy eyes had significantly fewer eyes, ranging from 50 to 100 eyes.[27,28,31–33] The methods to compare the repeated scans included measures of SD, COV, Bland–Altman plots, and ICCs. In our paper, we found high ICC (>0.9) in almost all parameters aside from nasal RNFL, some clock hours, and disc diameter, which were all still >0.8. The average within-participant SD for RNFL was 1.61 microns, though this was acquired during same-day, same-sitting measures, and likely would have been higher across days, or perhaps even if patients had to reposition themselves in the OCT. Coefficient of variation is another method to assess SD accounting for the mean. A lower COV indicates better reproducibility, with <10% being a measure of good reproducibility. All the values had COV ≤5%, and RNFL was the lowest at 1.87%. These values are well within range from the previous reports with ICC of 0.97 to 0.99 and COV of 1.2 to 1.7.

The measurement variability was higher for thicker RNFL measurement and decreased SS. The effect was relatively modest at 0.02 and 0.19 microns for each increase in RNFL (per 1 micron) and decreased SS (per 1 unit), respectively. This would suggest that for a similar SS, normal patients may have greater variability than glaucoma patients because of higher RNFL values. Notably, only scans with an SS of ≥6 were included, and the degree of additional variability may well be nonlinear across the range of possible SSs.[34] However, other studies using measures like ICC and COV have shown higher variability for glaucoma patients compared with those without glaucoma, suggesting another process causing increased variability among patients with glaucoma.[33] That decreasing SS is associated with increasing variability is not surprising and was found in the only other large population study of variability, although this was in glaucoma patients and using a different device than the Cirrus OCT used in our study.[35]

Another important measure was calculating the amount of change in RNFL that would constitute a significant change. In our paper we have noted this as SRD which is dependent on the average variance across participants. Other papers report similar values described as test-retest or reproducibility constants, which are not all necessarily comparable. The SRD for the OCT parameters are shown (Table S2) and found to be about 4.5 microns for RNFL thickness, which is slightly larger than 3.5 microns which was the only other study to assess this value for normal patients.[33] The SRD increased for all the quadrants except for the temporal quadrant. This cutoff does not necessarily apply to population level data but is likely a useful cutoff for repeated measurements in an individual subject data. Though there is good consistency in RNFL thickness measurement, test-retest variability can obfuscate real change. Understanding and incorporating this measurement variability into assessments will be critical for longitudinal assessment. Moreover, as described above, this value likely represents a minimum value for real change, while actual values in daily practice (or research) may be greater when individuals are imaged on different days, or if they have disease (i.e., glaucoma).

There are some limitations with this work, namely the limited number of non-White participants available in the Framingham community. As such, we had limited ability to assess race/ethnicity or further elucidate the relationship between biological and methodological variability by race/ethnicity groups. Although there are limited number of minorities in the study because of the demographics of the underlying Framingham population, our study provides unique and important data on elderly White adults that is not available from other studies in the United States. Another limitation is the lack of clinical eye examinations to assess for ocular disease, so we cannot explicitly exclude the possibility that some participants had occult or prevalent optic nerve disease. We also did not classify the presence or extent of peripapillary atrophy, which may affect RNFL thickness measurements. Also, all OCT measurements were acquired on a single day, limiting our ability to look at across-visit variability.

This study is one of the largest United States-based population studies to assess OCT RNFL and shows that the measured RNFL thickness decreases with increasing age, male gender, axial length, lower SS, and Whites as compared with African Americans without self-reported glaucoma. Within the same day and the same eye, a difference of <5 microns in average RNFL is unlikely to represent real change from one scan to the next, and this threshold would decrease with lower RNFL thickness and higher SS. Understanding this biological and methodological variation is important to determine real change in RNFL.

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Abbreviations and Acronyms:
CI = confidence interval; **COV** = coefficient of variation; **ICC** = intraclass correlation coefficient; **RNFL** = retinal nerve fiber layer; **SD** = standard deviation; **SRD** = smallest real difference; **SS** = signal strength.

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References

1. Swaminathan SS, Jammal AA, Berchuck SI, Medeiros FA. Rapid initial OCT RNFL thinning is predictive of faster visual field loss during extended follow-up in glaucoma. *Am J Ophthalmol*. 2021;229:100–107.
2. Budenz DL, Fredette MJ, Feuer WJ, Anderson DR. Reproducibility of peripapillary retinal nerve fiber thickness measurements with stratus OCT in glaucomatous eyes. *Ophthalmology*. 2008;115:661–666.e4.
3. Nousome D, Mckean-Cowdin R, Richter GM, et al. Retinal nerve fiber layer thickness in healthy eyes of Black, Chinese, and Latino Americans. *Ophthalmology*. 2021;128:1005–1015.
4. Sung KR, Wollstein G, Bilonick RA, et al. Effects of age on optical coherence tomography measurements of healthy retinal nerve fiber layer, macula, and optic nerve head. *Ophthalmology*. 2009;116:1119–1124.
5. Knight OJ. Effect of race, age, and axial length on optic nerve head parameters and retinal nerve fiber layer thickness measured by cirrus HD-OCT. *Arch Ophthalmol*. 2012;130:312.
6. Alasil T, Wang K, Keane PA, et al. Analysis of normal retinal nerve fiber layer thickness by age, sex, and race using spectral domain optical coherence tomography. *J Glaucoma*. 2013;22:532–541.
7. Vizzeri G, Bowd C, Medeiros FA, et al. Effect of signal strength and improper alignment on the variability of stratus optical coherence tomography retinal nerve fiber layer thickness measurements. *Am J Ophthalmol*. 2009;148:249–255.e1.
8. Wu JH, Moghimi S, Walker E, et al. Clinical factors associated with long-term OCT variability in glaucoma. *Am J Ophthalmol*. 2023;255:98–106.
9. Mwanza JC, Chang RT, Budenz DL, et al. Reproducibility of peripapillary retinal nerve fiber layer thickness and optic nerve head parameters measured with cirrus HD-OCT in glaucomatous eyes. *Invest Ophthalmol Vis Sci*. 2010;51:5724.
10. Andersson C, Johnson AD, Benjamin EJ, et al. 70-year legacy of the Framingham Heart study. *Nat Rev Cardiol*. 2019;16:687–698.
11. Lara FR, Scruton AL, Pinheiro A, et al. Aging, prevalence and risk factors of MRI-visible enlarged perivascular spaces. *Aging*. 2022;14:6844–6858.
12. Sung KR, Kim DY, Park SB, Kook MS. Comparison of retinal nerve fiber layer thickness measured by cirrus HD and stratus

optical coherence tomography. *Ophthalmology*. 2009;116:1264–1270.e1.
13. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J Chiropr Med*. 2016;15:155–163.
14. Vaz S, Falkmer T, Passmore AE, et al. The case for using the repeatability coefficient when calculating test–retest reliability. *PLoS One*. 2013;8:e73990.
15. Akinwande MO, Dikko HG, Samson A. Variance inflation factor: as a condition for the inclusion of suppressor variable(s) in regression analysis. *Open J Stat*. 2015;05:754–767.
16. Craney TA, Surles JG. Model-dependent variance inflation factor cutoff values. *Qual Eng*. 2002;14:391–403.
17. Pan-Doh N, Guo X, Arsiwala-Scheppach LT, et al. Associations of midlife and late-life blood pressure status with late-life retinal OCT measures. *Transl Vis Sci Technol*. 2023;12:3.
18. Savini G, Barboni P, Parisi V, Carbonelli M. The influence of axial length on retinal nerve fibre layer thickness and optic-disc size measurements by spectral-domain OCT. *Br J Ophthalmol*. 2012;96:57–61.
19. Budenz DL, Anderson DR, Varma R, et al. Determinants of normal retinal nerve fiber layer thickness measured by stratus OCT. *Ophthalmology*. 2007;114:1046–1052.
20. Mauschitz MM, Bonnemaijer PWM, Diers K, et al. Systemic and ocular determinants of peripapillary retinal nerve fiber layer thickness measurements in the European eye epidemiology (E3) population. *Ophthalmology*. 2018;125:1526–1536.
21. Khawaja AP, Chan MPY, Garway-Heath DF, et al. Associations with retinal nerve fiber layer measures in the EPIC-norfolk eye study. *Invest Ophthalmol Vis Sci*. 2013;54:5028.
22. Kang SH, Hong SW, Im SK, et al. Effect of myopia on the thickness of the retinal nerve fiber layer measured by cirrus HD optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2010;51:4075–4083.
23. Hirasawa K, Shoji N, Yoshii Y, Haraguchi S. Determination of axial length requiring adjustment of measured circumpapillary retinal nerve fiber layer thickness for ocular magnification. *PLoS One*. 2014;9.
24. Li D, Rauscher FG, Choi EY, et al. Sex-specific differences in circumpapillary retinal nerve fiber layer thickness. *Ophthalmology*. 2020;127:357–368.
25. Wang YX, Pan Z, Zhao L, et al. Retinal nerve fiber layer thickness. The Beijing Eye study 2011. *PLoS One*. 2013;8:2–9.
26. Racette L. Differences in visual function and optic nerve structure between healthy eyes of blacks and Whites. *Arch Ophthalmol*. 2005;123:1547.
27. Savini G, Carbonelli M, Parisi V, Barboni P. Repeatability of optic nerve head parameters measured by spectral-domain OCT in healthy eyes. *Ophthalmic Surg Laser Imag*. 2011;42:209–215.
28. Lee SH, Kim SH, Kim TW, et al. Reproducibility of retinal nerve fiber thickness measurements using the test-retest function of spectral OCT/SLO in normal and glaucomatous eyes. *J Glaucoma*. 2010;19:637–642.
29. Araie M. Test-retest variability in structural parameters measured with glaucoma imaging devices. *Jpn J Ophthalmol*. 2013;57:1–24.
30. Zhang X, Iverson SM, Tan O, Huang D. Effect of signal intensity on measurement of ganglion cell complex and retinal nerve fiber layer scans in fourier-domain optical coherence tomography. *Transl Vis Sci Technol*. 2015;4:7.
31. Wadhwani M, Bali SJ, Satyapal R, et al. Test-retest variability of retinal nerve fiber layer thickness and macular ganglion cell-inner plexiform layer thickness measurements using spectral-domain optical coherence tomography. *J Glaucoma*. 2015;24:e109–e115.
32. Yang B, Ye C, Yu M, et al. Optic disc imaging with spectral-domain optical coherence tomography: variability and agreement study with heidelberg retinal tomograph. *Ophthalmology*. 2012;119:1852–1857.
33. Budenz DL, Chang RT, Huang X, et al. Reproducibility of retinal nerve fiber thickness measurements using the stratus OCT in normal and glaucomatous eyes. *Invest Ophthalmol Vis Sci*. 2005;46:2440.
34. Yohannan J, Cheng M, Da J, et al. Evidence-based criteria for determining peripapillary OCT reliability. *Ophthalmology*. 2020;127:167–176.
35. Garas A, Vargha P, Holló G. Reproducibility of retinal nerve fiber layer and macular thickness measurement with the RTVue-100 optical coherence tomograph. *Ophthalmology*. 2010;117:738–746.